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PROCEEDINGS

**The International Allied Health Student Conference (IAHSC) 2022
2nd Edition**

“Health Innovation for Strengthening Global Health”



Indonesia, 14-15 September 2022



www.stikesmitrakeluarga.ac.id



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STIKes Mitra Keluarga



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Mitra Press

PROCEEDINGS

THE INTERNATIONAL ALLIED HEALTH STUDENT CONFERENCE 2022

2nd Edition

“Health Innovation for Strengthening Global Health”

SEPTEMBER 14-15, 2022

BEKASI, INDONESIA

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Welcome Message from the Organising Chair



Greetings to all, on behalf of the organizing committee, I am delighted to welcome you to the 2nd International Allied Health Student Conference (IAHSC) 2022 from September 14-15 2022. I hope you will enjoy our two days of virtual experiences on the theme “Health Innovation For Strengthening Global Health”.

In the past decade, Indonesian Higher Education has become a center for the development of science and technology. As written in Indonesian Law no.12 of 2012 about Higher Education that higher education has an academic responsibility to the community through the implementation of the Tri Darma of Higher Education which includes education, research, and community engagement. Higher Education need to pay attention to the quality of the implementation of the Tri Darma that is carried out by the academic communities of each university. The academic community as a scientist is tasked with developing a branch of science and/or technology through scientific research and disseminating it. As individuals or groups, the academic community is obliged to write articles/ textbooks published by universities and/or scientific publications as a source of learning, for the development of academic culture and writing activities for other academicians and members of society.

The lack of a forum that can accommodate the various ideas of the academic community in the form of research results and community engagement results is one of the reasons for the not optimal scientific output produced by students. Based on this, the team of the Center for Research & Community Engagement (P3M) STIKes Mitra Keluarga aims to organize the 1st international Allied Health Student Conference (IAHSC) which can accommodate the results of scientific results from the health academics in particular.

As the organizing chair, I humbly acknowledge the dedication and commitment of many people who have worked together in making this event a success. Most of all, I appreciate the participants and our distinguished speakers for enriching this conference with your presence. Thank you very much.

Best Wishes,

Noor Andryan Ilsan Ph.D

Organizing Chairperson The 2nd IAHSC 2022

Welcome Message from the Head of Center of Research & Community Engagement

Dear Colleagues,

On behalf of Center of Research and Community Engagement STIKes Mitra Keluarga, it is our great pleasure to welcome you to the 2nd International Allied Health Students Conference (IAHSC) 2022 that will be held in virtual meeting from 14-15 September 2022. The aims of this conference are to delivery output of scientific research from student researcher and lecturer in the field of health.



Nowadays the coronavirus pandemic has caused many problems in the world especially in health problem, economi, etc. All over the world we are seeing the devastating impact of the invisible killer. Unfortunately technology can help lecture, student and researcher sharing knowledge from virtual. The sharing of knowledge is just as important as receiving it because without sharing knowledge has no value. In this Occasion we like to say thank you for the presenter who have submitted their article and for the participant who join in this conference. We also like to say thanks to keynote speaker and speaker. In this conference there are four areas of health will be present : nutrition, phamacist, nursing and technology laboratory of medic. There will be many result of research that can be listened and watched in this event which will certainly add our knowledge and insight.

It is an honor to our colleagues that has been able to join this event, we are hope this event can give information that useful for the public and this annual can trigger collaboration between higher education institution, especially in the field of research.

We welcome all of you to this meeting where you can share knowledge, friendship and leave an unforgettable memory in your life.

Kind Regards,

Afrinia Eka Sari, S.TP., M.Si.

Head of Center of Research and Community Engagement STIKes Mitra Keluarga

Welcome Message from the Head of STIKes Mitra Keluarga



It is my great pleasure to welcome you to the 2nd International Allied Health Student Conference. In these unprecedented times, nothing is more important to us than the health and safety of our members, families and the communities we serve. While we regret that the COVID-19 pandemic prevent us from holding the conference in Bekasi, Indonesia, we are excited about the opportunities of holding an innovative virtual conference. For our first conference, holding it virtually will

be a great opportunity for us to explore “Health Innovation For Strengthening Global Health” as well as using digital and social media during pandemic to reach a wide audience across the world.

I believe this conference will be continuously serves as a meeting platform for researchers, academicians, health practitioners and students from around the world. I also credit this conference to be one of the media to discuss and share current topics in scientific development, especially in the area of Nursing Science, Nutrition Science, Medical Laboratory Technology and Pharmaceutical science. It presents a remarkable opportunity to exchange knowledge in many aspects and to create and renew a global network of colleagues. Our committees are already at work to find ways to create an exceptional virtual experience like no other through this challenging period.

Furthermore, I would also like to thank all the authors, keynote speakers and panellists for making great effort of sharing innovative minds and research results, and to all the participants, academic and industry communities.

Last but not least, I would like to express many sincere and special thanks to the IAHS 2022 Organizing Committee for their devotion, vision, courage, passion and constant effort to make this conference a success.

We are looking forward to warmly welcome you to the IAHS 2022 Conference.

Best Regards,

Dr. Susi Hartati, S.Kp., M.Kep., Sp.Kep.An.

Head of STIKes Mitra Keluarga

THE BOARDS

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- 1) Head of STIKes Mitra Keluarga, Dr. Susi Hartati, S.Kp., M.Kep., Sp.Kep.An.

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- 7) Elda Mariyani (Department of Nursing 3rd grade)**
- 8) Salsabila (Department of Medical Laboratory Technology 3rd grade)**
- 9) Devy Mirnawaty (Department of Nutrition 1st grade)**

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Sekolah Tinggi Ilmu Kesehatan
MITRA KELUARGA

Kampus Merdeka
BERKUALITAS BERKEMAJUAN

THE 2ND INTERNATIONAL ALLIED HEALTH STUDENT CONFERENCE (IAHSC) 2022

The Presentation includes the results of reviewing articles, original research, community engagement the area of :
Nursing Sciences, Science of Nutrition, Medical Laboratory Technology, Pharmaceutical Sciences



Ir. Budi Gunadi Sadikin, CHFC, CLU

Indonesian Ministry of Health

*to be confirmed

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Research Center of Applied Microbiology, BRIN



Dr. Susi Hartati, S.Kp., M.Kep., Sp.Kep.An

Head of STIKes Mitra Keluarga



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College of Biomedical Engineering, Taipei Medical University



Prof. Sirichai Adisakwattana

Dept. of Nutrition and Dietetics, Faculty of Allied Health Sciences, Chulalongkorn University



Prof. Abu Syed Md. Mosaddek

Dept. of Pharmacology, Uttara Adhunik Medical College, Bangladesh



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Dept. of Nursing, University of Indonesia



Dr. Siti Helmyati, DCN, M.Kes

Dept. of Nutrition, Gadjah Mada University



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Dept. of Chemistry, Institut Teknologi Bandung



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Reza Anindita, M.Si

Dept. of Pharmacy, STIKes Mitra Keluarga



Maulin Inggroini, M.Si

Dept. of Medical Laboratory Technology, STIKes Mitra Keluarga

Important Dates

- Abstract Submission : April 1- August 30, 2022
- Abstract Acceptance Notification : September 1, 2022
- Full Paper Submission : September 1-October 1, 2022
- Oral Video Submission : September 1-13, 2022

Payment Method

Account Name : Yayasan Pendidikan Mitra Keluarga-
STIKes Mitra Keluarga
Account Number : 157-000-1621-662
Bank : Bank Mandiri Cabang Bekasi
Jakasampurna

Registration Fee

External Attendees

- Early-Bird (until June 30th, 2022)
Audience = Rp 100.000
Oral-Presenter = Rp 200.000
- Normal Price
Audience = Rp 125.000
Oral-Presenter = Rp 250.000

International Attendees

- Audience = \$ 25
- Oral-Presenter = \$ 25

Internal Attendees

- Early-Bird (until June 30th, 2022)
Audience = Rp 50.000
Oral-Presenter = Rp 150.000
- Normal Price
Audience = Rp 75.000
Oral-Presenter = Rp 175.000



Wednesday - Thursday
(September 14 & 15th 2022)



Day 1: 08.00 AM - 04.00 PM (GMT+07)
Day 2: 08.30 AM - 04.30 PM (GMT+07)



Virtual Meeting by Zoom



Registration Info

<http://scievent.stikesmitrakeluarga.ac.id>



DPP PPNI, IAI, PERSAGI



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ABOUT ORGANIZER

SEKOLAH TINGGI ILMU KESEHATAN (STIKES) MITRA KELUARGA

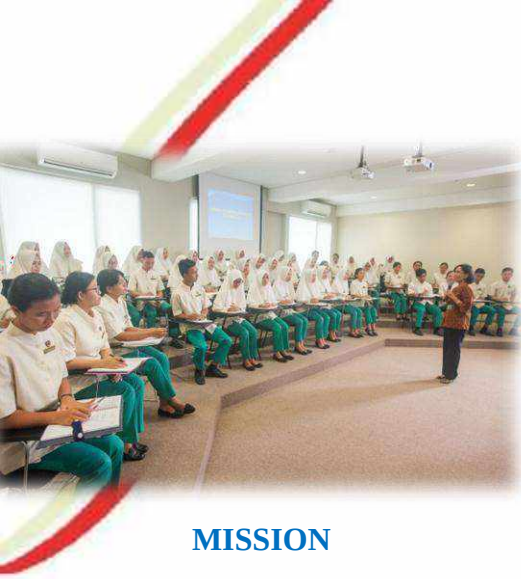
Mitra Keluarga Education Foundation is under the auspices of the Mitra Keluarga Hospital Group which has a hospitals network spread across DKI Jakarta, West Java,

Central Java, and East Java. To meet the needs of health workers in the Mitra Keluarga Hospital Group, in 1993 the Mitra Keluarga Education Foundation proposed the establishment of higher education in the health sector to the Ministry of Health with a license to provide a Diploma III Nursing education called the Mitra Keluarga Nursing Academy (AKMK).

In 2014, AKMK has changed its form to the School of Health Sciences (STIKes) which organizes 3 study programs, namely: Nursing Study Diploma Program, Health Analyst Diploma, and Nutrition Science Bachelor Degree. In further development, STIKes obtained a permit for the addition of a Bachelor of Pharmacy study program in 2016, and in 2017 obtained a permit to administer a Bachelor of Nursing + Nursing Profession (Ners) study program. So that until now there are 6 (six) study programs owned by STIKes Mitra Keluarga.

The values and culture of the work environment that are applied in the form of actions and behavior of every STIKes Mitra Keluarga human resource are Honesty, Integrity, Tolerance, Scientific, Innovative, Responsibility, and Obedience to the Principles. STIKes Mitra Keluarga has a motto: “The Best Health Education for Your Bright Future”.





VISION AND MISSION

VISION

Becoming an innovative higher education institution in health at the National level by 2035.

MISSION

1. Organizing professional education governance
2. Organizing cooperation that supports the implementation of the Tri Dharma of Higher Education.
3. Organizing health education based on science, technology and new innovation.
4. Organizing innovative and sustainable research for the development of science and technology.
5. Organizing community service based on the results of research and innovation in the health sector as an effort to improve public health status.

DEPARTMENTS

STIKes Mitra Keluarga owns 4 Departments, namely:

1. Department of Nursing
 - a. Nursing Diploma
 - b. Bachelor of Nursing
 - c. Nursing Profession (Ners)
2. Department of Nutrition
3. Department of Pharmacy
4. Department of Medical Laboratory Technology



Mengenal PROGRAM STUDI

D3 Teknologi
Laboratorium Medis

STIKes Mitra Keluarga



Kampus
Mendia
INDONESIA JAYA

Prodi D3 TLM STIKes Mitra Keluarga
unggul dalam pemeriksaan penyakit
infeksi berbasis imun

FASILITAS LABORATORIUM

- Laboratorium Media dan Reagen
- Laboratorium Analisis Instrumen
- IV Admixture
- Laboratorium Kimia
- Laboratorium Hematologi, Kimia Klinik
- Laboratorium Imunoserologi
- Laboratorium Plebotomi, Bioanalisis
- Laboratorium Bakteriologi, Mikologi, Parasitologi, Sitohistoteknologi, dan Mikrobiologi

LAHAN KERJA

1. Laboratorium Rumah Sakit
2. Puskesmas
3. Laboratorium kesehatan daerah
4. Laboratorium klinik
5. Badan Narkotika Nasional (BNN).
6. dll





**Kampus
Merdeka**
INDONESIA JAYA

Mengenal PROGRAM STUDI S1 FARMASI

STIKes MITRA KELUARGA

KEUNGGULAN

Prodi S1 Farmasi STIKes Mitra Keluarga
unggul dalam Farmasi Klinis dan
fasilitas yang memadai dan terkini

LAHAN KERJA

- Rumah Sakit
- Puskesmas
- Apotek
- Industri Farmasi
- Industri Obat Tradisional
- Industri Obat Veteriner
- Industri Kosmetik
- Industri Makanan - Minuman
- Pedagang Besar Farmasi
- Pedagang Besar Bahan Baku Farmasi
- Marketing (Medical Representative/Detailer)
- Pemerintahan (BPOM, Departemen Kesehatan, BNN, LIPI, BPPT)
- Asuransi Kesehatan, BPJS
- Perguruan Tinggi Negeri dan Swasta

FASILITAS LABORATORIUM

1. laboratorium teknologi farmasi
2. Laboratorium Farmakognosi
3. Laboratorium Simulasi Farmasi Rumah Sakit
4. Laboratorium Farmasetika
5. Laboratorium Farmakologi
6. Laboratorium Kimia Farmasi
7. IV Admixture
8. Laboratorium Mikrobiologi
9. Simulasi Farmakoterapi



Yuk! Tunggu Apa Lagi?

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KEUNGGULAN

**PRODI S1 GIZI STIKES MITRA KELUARGA UNGGUL
DALAM PENERAPAN INOVASI PANGAN DAN GIZI**

LAHAN KERJA

1. RUMAH SAKIT
2. PUSKESMAS
3. KLINIK GIZI
4. INSTITUSI PENYELENGGARAAN MAKANAN (CATERING, ASRAMA, PANTI, DLL)
5. INDUSTRI PANGAN
6. LEMBAGA PEMERINTAHAN (KEMENTERIAN KESEHATAN, KEMENTERIAN PERTANIAN, BADAN PENGAWAS OBAT DAN MAKANAN/BPOM, DAN BADAN NARKOTIKA NASIONAL/BNN)
7. LSM/NGO

FASILITAS LABORATORIUM

- LABORATORIUM KULINER, TEKNOLOGI PANGAN, DAN DIETETIK
- LABORATORIUM PENILAIAN GIZI DAN KONSULTASI GIZI
- LABORATORIUM KIMIA
- LABORATORIUM MIKROBIOLOGI



**MENGENAL
PROGRAM STUDI
S1 GIZI
STIKES MITRA KELUARGA**





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Merdeka**
INDONESIA JAYA

MENGENAL PRODI S1 KEPERAWATAN STIKES MITRA KELUARGA

KEUNGGULAN

Program Studi S1 Keperawatan STIKes Mitra Keluarga menyiapkan lulusan sarjana keperawatan yang unggul dan berdaya saing nasional dalam penatalaksanaan Penyakit Tidak Menular (PTM).

LAHAN KERJA

1. Rumah Sakit
2. Puskesmas
3. Pusat Pelayanan Kesehatan Masyarakat
4. Lembaga Pemerintahan (Kementerian Kesehatan, Badan Narkotika Nasional/BNN)
5. LSM/NGO.

FASILITAS LABORATORIUM

- Laboratorium Keperawatan Jiwa dan Keperawatan Komunitas
- Laboratorium Keperawatan Anak
- Laboratorium Keperawatan Maternitas
- Laboratorium Keperawatan Gawat Darurat
- Laboratorium Keperawatan Medikal Bedah
- Laboratorium Keperawatan Dasar



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Proceeding 2nd International Allied Health Student Conference

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THE EXPERIENCES OF PARENTS IN FAMILY CENTERED CARE FOR CHILDREN IN HOSPITAL: A LITERATURE REVIEW

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Abstract

Introduction: Hospitalization is a condition that causes anxiety in children and parents. Most parents experience mild stress (68.0%) while children experience anxiety disorders (60-80%). Pediatric nurses to provide further care in facilitating optimizing Family Centered Care (FCC) through the provision of health education and ongoing assistance from initial hospital admission to recovery from discharge to reduce the impact of the hospital on children in hospital. This article aims to identify and discover the family's experience of family centered care for children who have been hospitalized.

Method: A literature review using PRISMA by collecting the results of scientific publications in the period 2014 to 2022 obtained from PubMed, ProQuest, Science Direct and secondary searches. Then the articles were screened according to the inclusion criteria.

Results: In this literature review, 4 clinical studies were identified that matched the criteria for research on family-centered care in children. There are 3 qualitative studies and 1 study using a cross-sectional study.

Conclusion: Parents have obstacles in the application of family centered care. Family centered care can improve parental skills and reduce parental anxiety in caring for children in hospital.

Key words : *Family Centered Care; Hospitalization; Parents; Experiences*

INTRODUCTION

Hospitalization is a condition that is experienced by children, from infants to teenagers. According to Wong in Timiyatun & Hariyanti (2018) the impact of hospitalization is feeling hopeless, causing protest reactions, being uncooperative and depressed. Wong said the impact of hospitalization on children arises from separation, loss of control and pain. Hospitalization has a negative impact on children which causes anxiety. The cause of children experiencing anxiety and stress is influenced by several factors, namely from health workers who accompany during treatment and the new environment.

Hospitalization also has an impact on parents who experience the same anxiety as experienced by children. Parents feel anxious because they think about the safety of their children and see that their children are equipped with many tools and invasive measures are being carried out. In general, the parent's response to the hospitalization of a child includes: distrust, anger, guilt, fear, anxiety, stress, and frustration. This is supported by research Idris & Hartati (2016) which found that the stress level of parents mostly experienced mild stress (57.5%) and a small portion of severe stress (42.5%). The prevalence of anxiety disorders that occur in children while in hospital ranges from 60-80% of the general population (Juwita, 2019).

Pediatric nurses as a profession whose job is to provide nursing care to children have a very important role in facilitating parents to optimize Family Centered Care (FCC) through the provision of health education and ongoing assistance from the beginning of hospital admission to preparation for returning home (Yugistiyowati, 2018). According to the American Academy of Pediatrics (2012) in Suza (2016) FCC is the most important thing in child hospitalization which is based on collaboration between children, parents, doctors, nurses, and other professionals in clinical care based on planning, providing interventions and evaluating health services. FCC aims to maintain and strengthen the role of families and emotional bonds with children (Festini, 2014).

FCC can improve clinical decision making based on better information and collaborative processes,

increase follow-up when a treatment plan is developed together with families, be more efficient and effective in the care process, improve communication between health team members and increase professional satisfaction (Suza, 2016). In this regard, the development of the FCC is an important key to reducing the impact of hospitalization on children in hospitals. Therefore, this article aims to identify and evaluate family experiences with FCC in children who have been hospitalized.

METHOD

The rules for making this review article are based on the 2009 PRISMA checklist (Moher et al., 2009).

Search Strategy

The search strategy was carried out by searching for several studies found through the database, namely the PubMed, ProQuest, Science Direct and secondary searches. This strategy uses a combination of title/abstract terms. The research questions were designed using the PICO principles. The PICO review questions consist of Population (Inpatient OR pediatric hospitalization), Intervention (Family centered care OR FCC), Comparison (None), Outcomes (Parental experience AND family centered care outcomes).

Inclusion Criteria

The selected studies were published from 2014 to 2022. The year 2015 was chosen as the starting point for the literature search for expansion because the search in the previous year published articles on family centered care in children aged 0-12 years undergoing hospitalization with a qualitative study by Watts et al (2014). The study search was conducted on June 12, 2022. In addition to the database, a manual search was conducted to find studies relevant to the keyword articles. Studies were eligible if they met the inclusion criteria are all types of research studies, published in 2014-2022, published in full text and in English, and studies of family centered care of various diseases experienced by hospitalized children.

Data Extraction and Quality Assessment

Extraction includes: researcher, year, place of study, method, objectives, participants, outcome measurement, and study results. Meanwhile, to assess the quality of each, the authors criticize qualitative article studies using the tools of the Critical Appraisal Skills Program (CASP) Qualitative research (Critical Appraisal Skills Program, 2013) while cross sectional article studies use tools from the Center for Evidence-Based Management (CEBM, 2014).

Study Characteristics

The total search results for articles from several databases are 408 articles. Then screened into 159 articles based on the inclusion criteria. The number of articles that match the research questions and inclusion criteria is 4 articles. In this literature review, 4 clinical studies were identified that matched the criteria for research on family centered care in children. There are 3 qualitative studies (Finlayson et al., 2014; Moore et al., 2015; Serlachius et al., 2018) and 1 study using a cross-sectional study (Shimizu, 2018). The four articles were not intervening and constitute an article based on the experience of mothers who had children who were hospitalized. 3 research articles were conducted in the NICU, and 1 was conducted in the pediatric ward. All studies have a FCC approach and are not interventioned.

Experience

In qualitative research, there are several different themes. According to research conducted by Serlachius et al (2018) in Auckland New Zealand, the identified themes center on the challenges parents feel with FCC from admission to hospital discharge. Three main themes were identified, namely powerlessness, hierarchy between parents and staff and the peripheral role of fathers. The first theme (helplessness) focuses on factors in the NICU that contribute to parents feeling powerless in their role as caregivers. Parents feel frustrated because they are not allowed to read files or medical records about their babies and are not close to their babies. The second key theme describes the hierarchy or gap between parents and NICU staff. This gap is often exacerbated by the busy NICU environment and limited access to the NICU. The third theme is the role of the peripheral father. Father finds it difficult to carry out his

role as a second person after mother and the main support because it is controlled by the environment in the NICU.

RESULTS

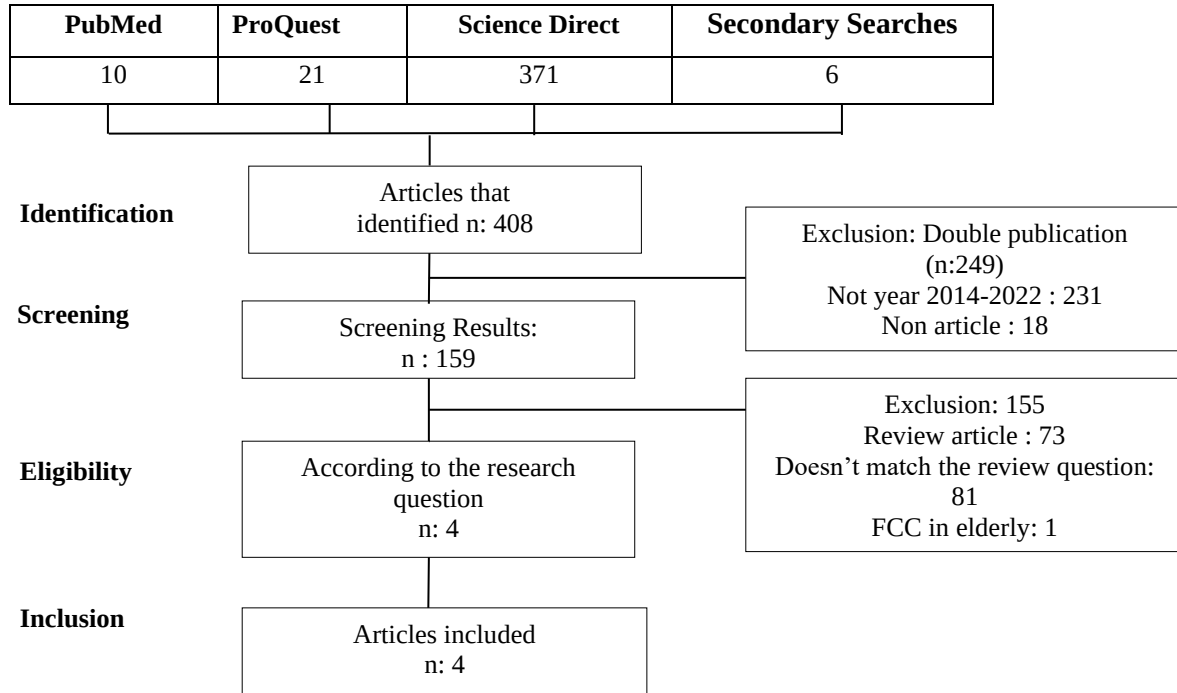


Figure 1. Search Algorithm

Table 1. Sintesis Grid

No	Researcher, City	Method	Aim	Partisipant	Outcome measurment	Outcome
1	Serlachius, et al (2018), New Zealand's.	Qualitative research design: Interviews during hospital admission and discharge	to examine the experiences of parents from the FCC during hospital admission and discharge, their perceptions of NICU staff, how informatio	83 parents namely 63 mothers and 20 fathers of premature babies in the National Women's Health NICU.	Parental and infant characteristics were assessed in the initial questionnaire or extracted from clinical records. The questions are not asking about the FCC but more about treatment in general. Interviews were hand-recorded by J Hames (verb recording) and written directly after each interview. The decision not to record audio was to	Three main themes (and sub-themes) were identified: powerlessness, hierarchy between parents and staff and the peripheral role of fathers. The challenges related to FCC reported by parents upon admission and discharge represent some of the main barriers parents still face in terms of being fully involved in the care of their infant in the NICU.

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		n received and their suggestions for improving care in the NICU.		allow participants to freely provide feedback on their experience in the NICU. Qualitative data analysis using theoretical thematic analysis (deductive approach).	
2	Shimizu & Cross Mori (2018), Japan.	Cross sectional study to evaluate maternal perceptions of FCC in hospitalized preterm infants.	98 mothers whose babies are being cared for in the NICU.	using two standard revisions questionnaire in Japanese: Measure of Process of Care in the NICU (Neo-MPOC 20), Enabling Practice Scale in the NICU (Neo-EPS), and the author developed Mother and Infant Questionnaire.	Maternal perceptions of FCC with hospitalized preterm infants were better promoted. As many as 48.9% of mothers visited their babies comfortably and 40.8% were able to make contact. Mothers' perceptions were almost always positive, but indicated a lack of opportunity to share information.
3	Moore, M et al (2015), Washington.	Qualitative method with semi-structured interview explores the experience of families undergoing pediatric Trauma Brain Injury (TBI) care to identify the specific factors and barriers associated with FCC.	Mothers of children under 18 years who were treated for TBI in the past 5 years. Sampling using purposive sampling. The number of participants is 15 people.	Content analysis was used to code the transcribed interviews and develop a family-centered care model.	The parental experience is that all parents spend a lot of time at the bedside during their child's hospitalization, each having opportunities to interact and communicate with staff. Three main themes emerged: 1) thorough, timely, compassionate communication, 2) capacity building for families, providers, and facilities, and 3) coordination of care transitions. Thorough and timely communication can reduce family anxiety.
4	Finlayson et al (2014), UK.	Qualitative To explore maternal perceptions of FCC in the neonatal intensive care unit (NICU) in	12 mothers from 3 NICU in the UK were obtained using individual interviews.	A central global theme supported by a number of organizing themes was developed to reflect the views of mothers and their experiences with the FCC.	This finding obtained a global theme, namely finding my place which consists of six, namely mothering in limbo, deference to the experts, anxious surveillance, muted relations, power struggle, consistently inconsistent. Mother

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UK.

experiences a limiting role in the NICU.

Table 2. CASP Qualitatif

No	Appraisal Checklist	Serlachius et al (2018)	Moree et al (2015)	Finlayson et al (2014)
1	Aims of the research	yes	yes	yes
2	Methodology appropriate	yes	yes	yes
3	Research design appropriate	yes	yes	yes
4	Recruitment strategy appropriate	yes	yes	yes
5	Data collected in a way that addressed the research issue	yes	yes	yes
6	Relationship between researcher and participants been adequately considered	yes	yes	yes
7	Ethical issues been taken into consideration	yes	yes	yes
8	The data analysis sufficiently rigorous	yes	yes	yes
9	A clear statement of findings	yes	yes	yes
10	Valuable	yes	yes	yes

Table 3. Cross Sectional

No	Appraisal Checklist	Shimizu (2018)
1	Did the study address a clearly focused question / issue?	Yes
2	Is the research method (study design) appropriate for answering the research question?	Yes
3	Is the method of selection of the subjects (employees, teams, divisions, organizations) clearly described	Yes
4	Could the way the sample was obtained introduce (selection) bias?	Yes
5	Was the sample of subjects representative with regard to the population to which the findings will be referred?	Yes
6	Was the sample size based on pre-study considerations of statistical power?	Yes
7	Was a satisfactory response rate achieved	Yes
8	Are the measurements (questionnaires) likely to be valid and reliable?	Yes

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9	Was the statistical significance assessed?	Yes
10	Are confidence intervals given for the main results	Yes
11	Could there be confounding factors that haven't been accounted for?	No
12	Can the results be applied to your organization?	Yes

According to research by Moore et al (2015) in Washington, three main themes emerged, the first theme was thorough, timely communication, the second theme was capacity building for families, providers, and facilities, and the third theme was coordination of care transitions. The parental experience with the FCC is that all parents spend a lot of time at the bedside during their child's hospital stay, each having ample opportunities to interact and communicate with staff. In addition, staff's thorough and timely communication to families reduces family anxiety.

Meanwhile, according to research by Finlayson et al (2014) in the UK, the global theme is finding my place which consists of six, namely mothering in limbo, deference to the experts, anxious surveillance, muted relations, power struggle, consistently inconsistent. This finding found that the mother experienced a state of liminality and was very sensitive to power struggles, awkward relationships and inconsistencies in care. To try to maintain their balance and protect their babies, they form harmonious relationships with healthcare professionals and stay under close supervision.

In a cross-sectional study by Shimizu (2018) in Tokyo Japan, mothers' perceptions of FCC with hospitalized premature babies were promoted better. As many as 48.9% of mothers visited their babies comfortably and 40.8% were able to make contact. Mothers' perceptions were almost always positive, but indicated a lack of opportunity to share information.

DISCUSSION

This review article focuses on parental experiences of family centered care for parents. Parents' experience of family-centered support with hospitalized children promoted better collaboration between mothers and nurses to acquire parenting craft skills. The more mothers involved in the care of their hospitalized child, the lower their risk of depression, the greater their mother's role identity and confidence in caring for their child.

Mothers who are involved in the FCC have a better level of comfort because they are beside their children during treatment and improve communication with health care staff in the room. This can also reduce parental anxiety, especially in the NICU. Mother's perception of family-centered support with hospitalized preterm infants promoted better collaboration between mothers and nurses to gain parenting craft skills Shimizu (2018).

However, from some of these experiences, parents still face significant barriers to the FCC. Challenges related to FCC reported by parents upon admission and discharge are some of the main barriers parents still face in terms of being fully involved in their child's care. Parents need the same amount of support during their hospital stay. Parents need more information and feel like getting involved in their baby's care as this helps them feel more in control and less anxious in a highly stressful and uncertain environment. Therefore, it is necessary to support parents in FCC services to improve outcomes such as parental skills in caring for their children during hospitalization to returning home, reducing anxiety and improving communication with health care staff at the hospital.

CONCLUSION

Parents have obstacles in the application of family centered care. Family centered care can improve parental skills and reduce parental anxiety in caring for children in hospital. Family-centered implementation may be a turning point to support parents more effectively in assisting in the care or improvement of children's health.

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THE RELATIONSHIP OF EDUCATION LEVEL AND SOURCES OF INFORMATION WITH FAMILY KNOWLEDGE ABOUT THE RISK OF DEMENTIA IN THE ELDERLY IN PADURENAN

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ABSTRACT

Background: The increasing elderly population can cause various changes that have an impact on health problems, one of which is the decline in cognitive function, namely the occurrence of dementia. Family knowledge about dementia shows that the term dementia is still foreign to hear. A person's knowledge is influenced by the information he gets. From the lack of information obtained, the family does not have adequate knowledge about dementia. One of the knowledge possessed by someone comes from education.

Purpose: This study was to determine the relationship between the level of education and sources of information with family knowledge about the risk of dementia in the elderly in the Padurenan Village, Bekasi. This research has received a letter of ethics no. EC.052/KEPK/stkbs/IV/2022.

Method: by using a probability sampling technique totaling 107 respondents, a cross sectional approach with predetermined inclusion and exclusion criteria. Results: there was no relationship between education level and knowledge level with p value > 0.005 (p: 0.131) and there was no relationship between information sources and knowledge level with p value > 0.005 (p: 0.370).

Conclusion: Someone with a low level of education does not mean absolutely low knowledge and someone with a high level of education does not mean absolutely good knowledge. Media that is right on target will facilitate the achievement of goals. The success of an activity using the media can be measured by assessing the level of effectiveness of the media used by the community.

Key words: Dementia, family, education

INTRODUCTION

Elderly is someone who has entered the age of 60 years and over who experiences anatomical, physiological, and biochemical changes in the body that affect the function and ability of the body as a whole (Setiyorini et al., 2018). Elderly is an age group in humans who have the final stages of life. This group which is categorized as the elderly will experience a process called the aging process (WHO, 2015).

Indonesia is said to be entering a period of aging population, where there is an increase in life expectancy followed by an increase in the number of elderly people. In Indonesia, there is an increase in the number of elderly people from 18 million people (7.56%) in 2010, to 25.9 million people (9.7%) in 2019, and it can be expected to continue to increase where in 2035 it will be 48, 2 million people (15.77%). An increase in the number of elderly people in the future can have a positive or negative impact. It will have a positive impact if the elderly population is in a healthy, active and productive state. On the other hand, it will be a negative impact if there is an increase in the elderly population who has health problems because it will become a burden (Kemenkes RI, 2017).

This increasing elderly population causes various changes that have an impact on health problems, one of which is cognitive function, namely the occurrence of dementia (Tamher, 2019). This decline in cognitive function causes the elderly to need the help of others in their activities. So in this case the family has a very important role in the care of elderly people with dementia who live at home. Dementia in the elderly is a condition of general and progressive cognitive impairment related to the physical health of the elderly. The task of family health as a support system is very important for people

with dementia to be able to carry out daily activities (Nugroho, 2011). Dementia is one of the mental emotional disorders that often occur in the elderly in addition to depression and anxiety (WHO, 2013). It is also stated that dementia is a collection of symptoms as a manifestation of structural brain damage. The collection of symptoms includes loss or decline in intellectual function (including the functions of thinking, orientation, understanding, arithmetic, learning capacity, language and judgment) and memory that is so severe that it causes daily dysfunction. Structural brain damage can be acute or chronic, but is more often chronic and the most common form of dementia is Alzheimer's disease, accounting for about 60-70% of cases (WHO, 2012).

Overall, 78.7% of dementia patients have Alzheimer's, and 43.9% require long-term care (Igarashi et al., 2020). Families who care for people with dementia will experience an increased burden. Health policies related to dementia need to be considered not only for patients, but also for families of people with dementia to improve their quality of life (Igarashi et al., 2020). To optimize family involvement, it is important to adopt a family-centered approach and provide training and mentoring. Making a comprehensive personal plan with family caregivers and offering them guidance can help them cope with their uncertainty and remove barriers to more involvement with care programs aimed at improving the quality of life of their relatives. Also recommended is training for staff to improve communication with family caregivers (Tasserone-Dries et al., 2021). So we need supporting factors that influence family knowledge of dementia. The first is in the form of strategic research that will build knowledge and understanding of family members about the previous course of the disease to and after admission to long-term care; and secondly in the form of practice development for elderly care staff to promote open, collaborative, and sustainable dialogue about dementia (Andrews et al., 2017). So that a good coping mechanism is needed, the coping mechanisms carried out by the family while caring for the elderly with dementia can be in the form of; 1. Treatment efforts are made to heal the couple. 2. Activities undertaken to avoid boredom. 3. Resources owned by the family (Pradana et al., 2021).

From the results of research on family knowledge about dementia, it shows that the term dementia is still unfamiliar to the participants, all participants say that dementia is better known as senile dementia or the Dayak language "Ngalilu" which is a disease that is easy to forget. The term is less popular even though the disease is experienced by the elderly. A person's knowledge is influenced by the information he gets, this condition is experienced because of the lack of information obtained, the family does not have adequate knowledge about dementia (Missesa, 2017). Education will affect family readiness. One of the knowledge possessed by someone comes from education. It was stated that the aspect of knowledge is a very important domain for the formation of a person's behavior where the higher a person's level of knowledge will be able to influence the mindset and attitude towards something this will affect behavior change. The behavior and emotional conditions of individuals with the elderly are often a form of communication about their unmet needs because of their reduced communication skills (Sembiring & EA, 2019). When the family can maintain a calm attitude, the family can encourage calm in the elderly and try to validate the feelings or intentions that the elderly want to convey. When an elderly person makes a mistake, the thing for the family to do is to remain calm, not to blame, and not to argue about what is right or wrong (Berryhill & Jones, 2012). Higher levels of education in early life are associated with significant reductions in both prevalence and incidence of dementia (Meng & D'arcy, 2012).

Based on the above background, the authors are interested in taking the title "Factors that Influence Family Knowledge of the Risk of Elderly Dementia in Padurenan Village in 2022". From this description, the researcher has a general goal, namely to find out the factors that influence family knowledge about the risk of dementia in the elderly in Padurenan Village. Padurenan, 3) Analyzing the relationship between sources of information obtained by the family and family knowledge about the risk of dementia in the elderly in Padurenan Village, 4) Analyzing the relationship between family education level and family knowledge about the risk of dementia in the elderly in Padurenan Village.

METHOD

The research design used in this research is quantitative with a cross sectional approach. A quantitative approach is an approach taken if the data collected is in the form of quantitative data or other types of data that can be quantified and processed using statistical techniques (Muri, 2014). In this study to calculate the number of samples using the Slovin formula. by using simple random sampling technique. The sample in this study was 107 respondents. The sampling technique uses probability sampling or random sampling. This research was conducted in the Padurenan Village, Bekasi.

The data collection technique used to collect data is by using a questionnaire sheet about the assessment of family knowledge factors about the risk of dementia in the elderly, which was collected by interview method. The data obtained will be analyzed using the SPSS (Statistical Package Social Science) program. By analyzing the univariate variables of each independent variable and dependent variable. As well as analyzing bivariate variables using the chi square test.

RESULTS

Based on the research, the following results were obtained:

A. Interpretation of Univariate Analysis Results:

Table 5.1
Characteristics of respondents

Characteristics	Category	Total	Percentage
Age	26-35	46	43.0
	36-45	54	50.5
	46-55	7	6.5
Total		107	100
Work	Working	54	50,5
	Doesn't work	53	49,5
Total		107	100
Experience	Have ever cared for the elderly with dementia (senile)	28	26.2
	Never take care elderly dementia (senile)	79	73.8
Total		107	100
Level education	Higher education	28	26.2
	Low education	79	73.8
Total		107	100
Resources	Conventional media	57	53.3
	Social media	50	46.7
Total		107	100
Knowledge level	High knowledge	60	56.1
	Low knowledge	47	43.9
Total		107	100

Table 5.1 shows that most of the respondents in the age group are in the age range of 36-45 years with a total of 54 respondents (50%). In working families, there are 54 respondents (50.5%). In families who never cared for the elderly with dementia as many as 79 respondents (73.8%). In families with low levels of education as many as 79 respondents (73.8%). In the families who obtain sources of information from conventional media amounted to 57 respondents (53.3%). And in families with high knowledge as many as 60 respondents (56.1%).

B. Interpretation of research results Bivariate analysis

1. The relationship between education level and family knowledge about the risk of dementia in the elderly can be seen in the following table:

Table 5.2

The relationship between education level and knowledge about the risk of dementia in the community in the work area of Padurenan Village, Bekasi City in 2022 (n = 107).

Level education	Family knowledge about the risk of dementia in the elderly				Total		OR (95% CI	P value
	High knowledge		Low knowledge					
	n	%	n	%	n	%	2,257 (0,77	0,131
High	23	82,1	5	17,9	28	100	0-6,612	
Low	53	67,1	26	32,9	79	100	),	
Total	76	71,0	31	29,0	107	100		

Based on table 5.2 above, it can be seen that the level of knowledge of respondents at the higher education level at most had a high level of knowledge as many as 23 respondents (82.1%) while those with low knowledge were 5 respondents (17.9%) and in the low education group the most many have a high level of knowledge there are 53 respondents (67.1%) and those who have low education are 26 respondents (32.9%).

From the results of the chi square statistical test, it was found that the p value was 0.131 ($p > 0.05$), this indicates that there is no relationship between the level of education and the level of knowledge about the risk of dementia in the community in the work area of Padurenan Bekasi Village in 2022. Based on the results of the odd ratio obtained a value of 2,257 (95% CI 0.770-6.612), it can be concluded that respondents with a low level of family education have a chance of 2,257 times having low knowledge compared to respondents with a high level of education.

2. The relationship between sources of information and family knowledge about the risk of dementia in the elderly can be seen in the following table:

Table 5.3 :

The relationship of information sources with knowledge about the risk of dementia in the community in the work area of Padurenan Village, Bekasi City in 2022 (n = 107).

Resources	Family knowledge about the risk of dementia in the elderly		Total	OR (95% CI)	P value
	High knowledge	Low knowledge			

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	n	%	n	%	n	%	1,467	0,370
Media konvensional	44	74,6	15	25,4	59	100	(0,634-	
Media sosial	32	66,7	16	33,3	48	100	3.393)	
Jumlah	36	71,0	31	29,0	107	100		

Based on table 5.3 above, it can be seen that the most conventional media have a high level of knowledge, 44 respondents (74.6%) while 15 respondents have low knowledge (25.4%). And respondents' sources of information originating from social media mostly have a low level of knowledge of 32 respondents (66.7%), while high knowledge are 16 respondents (33.3%).

From the results of the chi square statistical test, the p value is 0.370 ($p > 0.05$), this indicates that there is no relationship between the source of information and the level of knowledge about the risk of dementia in the community in the work area of Padurenan Bekasi Village in 2022. Based on the results of the odd ratio obtained a value of 1.467 (95% CI 0.634-3.393), it can be concluded that respondents who get information sources from conventional media have 1,467 times the chance to have high knowledge compared to respondents who get information sources from social media.

DISCUSSION

A. Univariate Analysis

1. Overview of Respondents' Characteristics Based on Education Level

Education is an attempt to develop one's personality and abilities in order to understand something. Education affects the learning process, the higher a person's education, the easier it will be for that person to receive information. Knowledge is very closely related to education where it is hoped that someone with higher education will have more extensive knowledge (Mubarak, 2011). The frequency distribution of respondents based on education level shows that most families with low education levels are 79 respondents (73.8%). In families that have a higher education level as many as 28 respondents (26.2%), the results of this study indicate that most of the respondents are in families with low education levels.

According to the researchers, respondents in this study were more likely to be in families with low levels of education, possibly influenced by many things, including the following: low income of the population, as a result, parents are unable to send their children to higher education levels, lack of awareness of the population regarding the importance of education, educational facilities and infrastructure are still inadequate, the number of educational facilities is not balanced with the population of school age (Umang et al., 2018). Based on the results of the analysis, the education participation rate in Bekasi City is relatively low. The world of education in Bekasi City, West Java, is considered to have to be improved given the low education participation. Not a few people complete education only at the junior high school level. The level of education awareness in Bekasi City is still in the stage of development towards a better direction, compared to the past, now there has been a lot of progress, awareness has begun to grow, and has increased even though it must continue to be encouraged but has not yet reached university (Mayolus Fajar D, 2016). Education can affect a person's perspective on the new information he receives. So it can be said that the higher the level of education, the easier it is for someone to receive the information he gets (Taufik, 2019).

2. Characteristics of Respondents Based on Information Sources

Someone who has more sources of information will have broader knowledge. In general, the easier it is to obtain information, the faster one acquires new knowledge (Mubarak, 2011). The frequency distribution of respondents based on education level shows that most families get information from conventional media as many as 57 respondents (53.3%). In families who received information from social media as many as 50 respondents (46.7%), the results of this study showed that most of the respondents were families who received information from conventional media.

According to the researcher, respondents in this study were more likely to have families receiving information from conventional media and some sources of information from social media, possibly because conventional media routinely cite blogs as sources, and vice versa. Blogs mostly rely on conventional media to obtain information. Now the existence of conventional media and social media influence each other, even benefit each other (Meteti, 2011). Supported by the results of Yeni's research (2015), it is known that the source of information comes from conventional media (41.2%), but other sources (28.9%).

Conventional media provides learning regardless of age, as long as the individual can access and read the news. A data from Mark Plus Insight, an organization engaged in research on internet technology says that in 2011, the number of internet users in Indonesia reached 55 million users (Triwijanarko et al., 2013). Information originating from social media is highly inaccurate. Social

media users very easily edit and forward the news according to its purpose. Therefore, you should not fully trust the information obtained from social media. Social media users must confirm the news that has been accessed from the social media with trusted media (Juditha, 2019).

In social life, information is something that is very important for individuals in today's modern era. In fact, information has become a basic need that must be met. The role of information and communication technology in everyday life cannot be separated, the ease of use is very helpful (Pexels et al., 2021). The information obtained can come from conventional media and social media. Conventional communication media are communication media that have been discovered before new media. Conventional media are commonly used to send or receive messages and information to the wider community. Therefore, conventional media is often also referred to as mass communication media. Mass communication media or mass media are media that are capable of disseminating messages or information to the public or audience (mass) simultaneously with a relatively wide reach and in a relatively short time. (Pexel et al., 2021). Meanwhile, social media can be understood as a digital platform that provides facilities to carry out social activities for each user. Some activities that can be done on social media, for example, are communicating or interacting to providing information or content in the form of writing, photos and videos. Various information in the shared content can be open to all users for a full 24 hours (Blog, 2021).

3. Characteristics of Respondents Based on Knowledge Levels About Dementia Risk in the Elderly

Knowledge is the result of knowing and this occurs after someone has sensed an object. Sensing occurs through the human senses, namely, the senses of hearing, sight, smell, feeling and touch. Some human knowledge is obtained through the eyes and ears (Notoatmodjo, 2012). Knowledge is the result of knowing and this happens to a person after sensing a certain object. Sensing occurs through the five human senses, namely the senses of sight, hearing, digestion, and taste and touch. Most human knowledge is acquired through the eyes and ears. This means that knowledge or cognitive is a very important domain factor for the formation of one's actions. The knowledge stage in the cognitive domain has 6 stages, namely: knowing, understanding, application, analysis, synthesis, and evaluation (Notoatmodjo, 2011). The frequency distribution of respondents based on education level shows that most families with a high level of knowledge are 60 respondents (56.1%). In families who have a low level of knowledge as many as 47 respondents (43.9%). The results of this study indicate that most of the respondents are in families with a high level of knowledge. This study is in line with research conducted by Harahap, (2018) regarding the relationship between the level of knowledge about dementia and primary prevention of dementia in the elderly at the Tegal Sari Health Center. %).

According to the researcher, the respondents in this study were mostly in families with a high level of knowledge, possibly because the knowledge possessed by the respondents was obtained apart from education and could also come from experience. The respondent's experience in caring for the elderly will affect the respondent's level of knowledge (Narayani & Kartinah, 2017). The number of respondents who have good knowledge is caused by the respondents working. This is understandable, because when they work they can exchange information with their co-workers or their relations. A merchant's family for example, he can get information and experience from buyers as well as from other merchants (Hasanah et al., 2013).

Good knowledge of family caregivers about the lives of their relatives before dementia can be

useful in providing daily care centered on the elderly at risk of dementia (UH Graneheim, A. Johansson, 2014). Elderly care requires a true understanding by the caring family and setting the right goals using modified methods as well as care and support accompanied by adequate support so that the elderly will be able to achieve quality of life. This is in line with the expectations of caregivers for quality elderly care (Prabasari et al., 2017).

B. Bivariate Analysis

1. Characteristics of Respondents Based on the Relationship between Education level and knowledge about the risk of dementia in the elderly.

A person's knowledge as well as family knowledge will be influenced by the level of education, the higher the education, the more the family's knowledge about dementia care will increase in order to provide good care and in accordance with the needs and conditions of people with dementia, in order to reduce the burden on the family. adequate, family support is also very important to improve the quality of life of dementia patients, so families must have extensive education and knowledge about how to care for dementia patients (Oki et al., 2019).

The results of the analysis of the relationship between the level of education and the level of family knowledge about the risk of dementia in the elderly found that respondents with low levels of education had high knowledge of 53 respondents (67.1%). The respondents with higher education level have high knowledge as many as 23 respondents (82.1%). The results of the Chi Square statistical test showed that the p value was 0.185 ($p > 0.05$), it can be said that H_0 failed to be rejected, which means that there is no relationship between education level and knowledge about the risk of dementia in the community in the work area of Padurenan Bekasi Village in 2022. OR (odd ratio) value of 2.257 (95% CI 0.770-6.612), it can be concluded that respondents with a low level of family education are 2,257 times less likely to have low knowledge than respondents with a high level of education.

This is in accordance with the research conducted by Khasanah & Ardiansyah, (2012) which obtained from the study that there was no relationship between education level and the incidence of dementia in the elderly at the Senja Cerah Paniki Elderly Support Center, Manado District. The results are the same as the research conducted by Khairani (2016) which shows that there is no significant relationship between education and family knowledge about the elderly with dementia.. The results of Setiawan et al.(2014) research also stated that there was no relationship between education level and the incidence of dementia in the elderly at the Senja Cerah Paniki Elderly Support Center, Mapanget District, Manado..

According to the researcher's assumption, by looking at the results of data processing, it shows that there is no relationship between education level and family knowledge about the risk of dementia in the elderly. This may illustrate that knowledge is not absolutely obtained from formal education, but can be obtained from non-formal education. Someone with a low level of education does not mean absolutely low knowledge and someone with a high level of education does not mean absolutely good knowledge (Arrasily & Dewi, 2016). Information obtained from both formal and non-formal education can have a short-term impact (Immediate Impact) resulting in a change or increase in knowledge. Advances in technology will provide various kinds of mass media that can affect public knowledge about new information. As a means of communication, various forms of mass media such as television, radio, newspapers, magazines and others, have a major influence on the formation of public opinion and trust (Notoatmodjo, 2011). Knowledge possessed by respondents apart from education can also come from experience. The respondent's experience in caring for the elderly will affect the respondent's level of knowledge about the risk of dementia (Narayani & Kartinah, 2017).

With low education and high knowledge, this is supported by the increasing number of people who open themselves to seek the widest possible information in an effort to fulfill the level of information in order to increase self-knowledge, one of which is participating in activities such as posbindu, visiting other sources of information by reading. (Yuda, 2022). Information obtained from various sources will affect a person's level of knowledge. Someone who gets a lot of information then he tends to have broad knowledge. The more people read, knowledge will be better than just hearing or seeing (Notoatmodjo, 2011). In addition to things that can affect information input from the community or even colleagues, so that it will have an impact on the knowledge they have This is because work makes the intensity of individual interactions with other individuals wider, so that individual exposure to information is also greater (Yeni, 2015).

2. Description of Respondents Characteristics Based on the Relationship of Information Sources with Knowledge About Dementia Risk in the Elderly

Efforts that can be made to increase knowledge are with sources of information that can be obtained from health education. Health education has several objectives, including the achievement of behavioral changes for individuals, families and communities in fostering and maintaining healthy behavior and a healthy environment as well as an active role in efforts to achieve optimal health status. In addition, it can form healthy behavior in individuals, families and mentally and socially so that it can reduce morbidity and mortality (Pakpahan et al., 2021).

The results of the analysis of the relationship between sources of information and the level of family knowledge about the risk of dementia in the elderly found that respondents who received information from conventional media had high knowledge of 44 respondents (74.6%). The respondents who received information from social media had high knowledge as many as 32 respondents (66.71%). The results of the Chi Square statistical test showed a p value of 0.370 ($p > 0.05$), it can be said that H_0 failed to be rejected, which means that there is no relationship between sources of information and knowledge about the risk of dementia in the community in the work area of Padurenan Bekasi Village in 2022. The results of the OR (odd ratio) obtained a p value of 1.467 (95% CI 0.634-3.393), it can be concluded that respondents who get information sources from conventional media are 1.467 times more likely to have high knowledge than respondents who get information sources from social media.

According to the researcher's assumption, by looking at the results of data processing, it shows that the source of information has no relationship with the level of family knowledge about the risk of dementia in the elderly. concerned with through conversations, interviews, discussions, seminars, and others. The resource persons are of course people who are considered experts in their fields, such as religious leaders, teachers, and scientists (Ardhana & Kusuma, 2012). Information obtained from someone depends on several things, namely: accurate, meaning that the information must be free from misleading errors. Accurate also means it must clearly reflect the intent. Just in time means that the information coming to the recipient should not be hampered. Relevant, meaning that the information has benefits for the user (Mahdalena, 2014). In their research by Sembiring & EA (2019) that there are still most families do not seek information because they consider dementia as a normal condition due to the aging process and despite the worsening condition they do not seek health services.

Sources of information can be in the form of print media or electronic media, such as television, radio, computers, newspapers, books, and magazines. Someone who has easy access to information will get knowledge faster. Advances in technology can affect people's knowledge about new innovations that can have an impact so as to produce changes or increase knowledge

(Lukoschek et al., 2013). However, there are also those who read more and more respondents do not necessarily understand and can understand more clearly or have not yet reached the stage of understanding (Sidik, 2015).

Overcoming knowledge deficits is one of the keys to improving future care and health for people with Alzheimer's (Smith et al., 2014). Increased knowledge will happen to everyone based on the experience experienced. And newly acquired information can be a substitute for previously acquired knowledge or refinement of previous information (Noverina, 2011).

This study differs from research conducted in rural America by providing education to people at risk of Alzheimer's aged 50 years and over. Education is given once a week for 90 minutes per session. The results of this study stated that there was an increase in knowledge about Alzheimer's after being given education with p value = 0.03 which means there is a relationship between the source of information and the level of knowledge (Blocker, 2020). Same result as Subdari et al (2020) in the intervention group which showed that the booklet media with the question and answer lecture method increased family knowledge more than the question and answer lecture method with a p value of <0.001 . So there is a significant effect of providing health information using booklet media and the question and answer lecture method compared to only the question and answer lecture.

According to the researcher, the difference in research results is likely to be influenced by education which is closely related to a person's perspective or knowledge in perceiving something, and age also has an effect, the more age, the level of maturity and strength of a person will be better in thinking and working, so that increasing age a person can effect on the increase in knowledge gained and easy to accept the material given to him (Kholid, 2014). In addition to education and age, the respondent's experience in caring for the elderly can affect the respondent's level of knowledge about the risk of dementia (Narayani P & Kartinah, 2009). So that families living with older family members with dementia will have a higher level of knowledge(HJ Park, 2011). Similarly, working experience in work relevant to dementia can be considered as a way to find relevant information and increase knowledge (Allen et al., 2020).

So it can be concluded that there are differences in the results of research on sources of information with family knowledge about the risk of dementia in the elderly, which can be influenced by several factors, namely education, age, experience and occupation. The type of information source obtained is more effective from direct information sources such as education and counseling using several media such as leaflets and booklets than information sources obtained from conventional media and social media. The public will feel interested in the news discussed by the media if the news is indeed important for them. Thus, the mass media are no longer fully in control of people's mindsets, but are intelligently able to sort and select information that is indeed good and deserves them (Allen et al., 2020). According to Fachry & Pertamasari (2011) the right media on target will facilitate the achievement of goals. The success of an activity using the media can be measured by assessing the level of effectiveness of the media used by the community.

Respondents who have been exposed to information about the risk of dementia in the elderly, either obtained from health services or information from print/electronic media/social media, have higher knowledge than those who have not received information (Kaid et al., 2021). Like what Alzheimer's Indonesia (ALZI) did, which made a social campaign with #Do not Understand With senile to build public awareness. Senile is no longer a common thing because it can be one of the signs or symptoms of dementia. #Don't Understand With Pikun is a grabber in the message

because the slogan can attract people's attention and is implemented into several programs. For example, using a strategy by making health education in the form of talk shows on the media, such as Kompas TV, Radio Kosmonita, She, and other (Immanuel, 2021).

CONCLUSION

Based on the results of the research and discussion of the research entitled the relationship between education level and sources of information with family knowledge about the risk of dementia in the elderly in the Kelurahan Padurenan Bekasi, the following conclusions can be drawn:

1. The description of respondents based on age shows the majority of late adulthood (36-45 years) as many as 54 respondents (50.5%), the majority working as many as 54 respondents (50.5%), never caring for the elderly with dementia as many as 79 respondents (73.8%), had low education as many as 79 respondents (73.8%). sources of conventional media information as many as 57 respondents (53.3%).
2. The description of respondents based on the level of knowledge shows that most of the respondents are in families with a high level of knowledge as many as 60 respondents (56.1%).
3. There is no relationship between education level and family knowledge about the risk of dementia in the elderly with $p\text{-value } 0.131 > \alpha (0.05)$. The odd ratio value was 2.257 (95% CI 0.770-6.612), which means that respondents with a low level of family education had a chance of 2,257 times having low knowledge compared to respondents with a higher education level.
4. There is no relationship between sources of information and family knowledge about the risk of dementia with $p\text{-value } 0.370 > \alpha (0.05)$. the odd ratio value was 1.467 (95% CI 0.634-3.393), ie respondents who received information from conventional media had 1.467 times the chance to have high knowledge compared to respondents who received information from social media.

IMPLICATION

Implication is a consequence or direct result of the findings of a scientific research. The results of this study are about the factors that influence family knowledge about the risk of dementia in the elderly in the willayah of Padurenan Village, Bekasi. Based on the results of the study, it is known that the source of information variable has a significant influence on the level of family knowledge about the risk of dementia in the elderly. While the education level variable does not have a significant effect on the level of family knowledge about the risk of dementia in the elderly. A research that has been conducted in the Kelurahan Padurenan Bekasi, the conclusions drawn certainly have implications in the fields of government institutions, health service institutions and educational institutions. In this regard, the implications are as follows:

1. Government Institutions
It can open the eyes of the Bekasi City government that dementia cases are urgent cases. The needs of the elderly for health services needed by caregivers are those related to finance, the needs of the elderly for health services are free of charge, so special services for the elderly and health education are needed. and adequate sources of information. Thus, it is hoped that the Bekasi City government can make policies regarding the handling of elderly dementia.
2. Health care institutions
Special services are needed for the elderly and their families for health education needs. Where the health services needed by the elderly and their families are affordable health services and regular home visits as well as efforts to increase family independence with health education and the provision of wider information.
3. Educational Institutions
It is hoped that with the results of this research, educational institutions can make further planning programs for this research.

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THE EFFECTIVENESS OF CABBAGE COMPRESSES AGAINST MASTITIS REDUCTION IN POST PARTUM MOTHERS IN BEKASI PRIVATE HOSPITALS 2022

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ABSTRACT

INTRODUCTION: Pain due to breast swelling can make the feeling of discomfort and painful not only in the mother but also in the baby. Breastfeeding problems generally occur in the first 2 weeks of puerperium, which occurs postpartum, one of which is breast swelling caused by uneven milk production because the baby does not breastfeed enough to his mother. So that the rest of the milk is collected in the duct system which results in swelling and breast milk dams. This study found out the effectiveness of cabbage compresses against reducing mastitis in post-partum mothers in hospitals. Private Bekasi.

METHOD: is quantitative with a pre-experimental method research design with a one group pre test-post test design approach, the object of the study is the effectiveness of cabbage compresses against mastitis reduction in post partum mothers. Data analysis using univariate and bivariate with wilcoxon and mannwhitney tests. The average post partum maternal milk dam before cabbage compresses with an average of 53.00 after cabbage was given with an average of 22.75 with a p-value of 0.0001.

RESULTS: There is an effectiveness of cabbage compresses against mastitis reduction in post partum mothers in the hospital. Private Bekasi.

CONCLUSION: Of health workers in order to provide counseling and good information about the benefits of nonpharmacological treatment in dealing with the problem of mastitis reduction in post partum mothers, especially for primiparous mothers where the mother has not understood correctly about how to breastfeed both primiparous mothers to avoid the occurrence of breast milk dams.

Key words : Compress cabbage leaves, Postpartum, Mastitis.

INTRODUCTION

Adaptation of physiology and psychology in the postpartum period some problems may occur. Such as breast swelling that causes discomfort and pain, not only in the mother but also in the baby. Mothers will experience some changes in physiology and psychology in the *postpartum* period. One of the physiological changes that occur is a change in the breast to prepare for the breastfeeding or lactation process. (Beautiful et al., 2019)

Breastfeeding problems generally occur in the first two weeks of the puerperium, at this time the role of the nurse is very necessary so that breastfeeding problems can be addressed immediately. (T. Sari et al., 2017). The role of nurses in the puerperium includes helping mothers in caring for postnatal breasts regularly to avoid static flow of breast milk (breast milk). Provide counseling for mothers on how to prevent, recognize the signs and symptoms of breast swelling, and carry out nursing care management to prevent complications towards mastitis. (Hasanah, Hardiani, & Susumaningrum, 2017).

Based on the World Health Organization estimates that more than 1.4 million women experience breast dams (WHO, 2010). According to basic health research (RISKESDAS, 2018) breastfeeding at the age of 0-1 months is 45.5%, 2-3 months is 38%, and 4-5 months is 31%. This rare breastfeeding is what causes problems in breastfeeding as well as swollen breasts. According to data from indonesia's demographic and health survey in 2015, it was stated that there were puerperal mothers who experienced breast milk dams as many as (37.12%) puerperal mothers (SDKI, 2015).

Based on research (Juliani & Nurrahmaton, 2020) the characteristics of puerperal mothers with

breast swelling in Sariningsih Hospital are mostly aged 20-35 years (96.2%), based on education, most of them are educated in high school / vocational school (57.7%), based on the parity of most primipara mothers (42.3%), based on occupation most are working mothers (57.7%).

Breast swelling occurs almost 90% in mothers who have given birth for the first time, this incident occurs on the second to the fourth day after giving birth. The breasts begin to feel full and hard, causing pain. In this first week, if the mother does not get information on how to deal with her breasts, it can cause the mother to stop giving her milk. Swelling is actually physiological but can continue to be more severe, namely into mastitis and breast abscesses. (Hasanah, Hardiani, & Susumaningrum, 2017) . One way to smoothen the breastfeeding process is by doing breast care regularly. Other studies have shown that breast care such as massage, warm and cold compresses, and cabbage leaf compresses can facilitate breast milk production. This breast treatment can excrete breast milk without reducing breast milk production. (Komala Sari & Nelda Putri, 2020).

Success in breastfeeding needs to be addressed and considered because U.S.I dams if not handled properly can often continue towards mastitis. Thetreatment of swollen breast talaksanan can be done independently by the mother, namely by compressing cabbage leaves. Compresses of cold cabbage leaves (*Brassica Oleracea Var.Capitata*) are shown to decrease breast swelling in post partum mothers.(Juliani & Nurrahmaton, 2020)

METHOD

This type of research uses quasi-experimental, which is a research design that is used to find causal relationships. The type of design used is a parallel design in two groups, namely arranged two groups and the implementation in that group is carried out in parallel and simultaneously. The study was conducted for 2 months.

The research location is at the Bekasi Private Hospital Jl. Jendral Ahmad Yani, Kayuringin Jaya, Bekasi City, West Java. The study population was a puerperal mother at the Bekasi Private Hospital from March.-April 2022 as many as 80 people. The sample was divided into 2 groups, namely 40 respondents of the intervention group and 40 respondents of the control group. The intervention is carried out 3 times every day (morning, afternoon, evening) for 30 minutes. The action is carried out until the swelling of the breast is reduced or a maximum of 3 days.

RESULTS

Table 5. 1
Mann Whitney Test Of Breast Size Before Intervention In The Intervention Group And Post Partum Maternal Control Group In Hospital. Private Bekasi 2022

Variable name	No.	Group Name	Median	Average ranking	P-value
Breast Size	1	Intervention Group	43,00	57,83	0,0001
	2	Control Group	38,00	23,18	

Based on table 5. 1 it is known that there are differences in breast size in 2 groups that will be compared at the beginning before the intervention, breast size in the intervention group averaged (57.83) then in the control group (23.18) with a p-value of 0.0001 (p-value < α 0.05.)

Table 5. 2
Mann Whitney Test Of Breast Milk Dam After Intervention In Respondent Group And Post Partum Mother Control Group In Hospital. Private Bekasi 2022

Variable name	No.	Group Name	Median	Average ranking	P-value

asi dam	1	Intervention Group	1(Smooth)	22,75	0,0001
	2	Control Group	2(Slight Changes in the breasts)	58,25	

Based on table 5. 2 it is known that there are differences in the intervention and control groups, the two variables get a p-value of 0.0001 (p-value < α 0.05) meaning that there is a significant difference in breast milk after the intervention. The median of the intervention group's breast milk dam is a difference of 1 meaning that there is a change in the breast and the median in the control group is a difference in the 2nd difference, which means there is a difference in the breast.

Table 5.3
Mann Whitney Breast Size Test After an intervention in kelompok respondents and the Post Partum Maternal Control group in the HOSPITAL. Private Bekasi 2022

Variable name	No.	Group Name	Median	Average ranking	P-value
Breast Size	1	Intervention Group	40,00	58,15	0,0001
	2	Control Group	35,00	22,85	

Based on table 5. 3 it can be seen that there were differences in the intervention and control groups, In the breast size variables the intervention group was obtained Median 40.00 medium in the control group Median 35.00 with a p-value of 0.0001 (p-value < α 0.05) meaning that there was a significant difference in breast size after the intervention.

DISCUSSION

The results of statistical tests showed the effectiveness of cabbage leaf compresses against mastitis reduction in post partum mothers compared to breast treatment alone to reduce the decrease in mastitis in post partum mothers. The attribution of respondents based on the age of the majority aged 26 to 35 years with a total of 48 people. This Hal is in line with the results of the research (Yunita, 2021) found that the age of the most people who experience mastitis aged 18 to 35 years is 15 people (50%).

The characteristic of respondents that affect breast swelling is the paritas status, which is mostly multipara, as many as 58 people. This is in line with the results of the study (Hasanah, Hardiani, Susumaningrum, et al., 2017) found that the parity status of those who experienced multipara mastitis is alah ibu as many as 39 people (68.4%) Other studies also stated the parity status of respondents of multipara mothers as many as 12 people (60%). (Hasibuan et al., n.d.) because there are more multipara respondents. According to the concept that based on parity status generally those who experience mastitis are primiparous mothers, so the results of this study have nothing in common with the concept. The assumption of respondents with multipara status experienced a lot of breast swelling because babies born in caesarea and spontaneous sections were not treated with their babies so that mothers could not breastfeed at any time. The results of the study of 80 respondents of post partum sectio caesarea and post partum spontaneous mothers who experienced breast swelling before and after being given cabbage compresses were obtained p-value of 0.0001 (<0.005) which means that there is a significant difference in the scale of breast swelling between before and after the leaf compress intervention was carried out cabbage. From the results of the study, breast size was obtained before the intervention with a median of 43.00 and after the intervention with a median of 40.00 with an average ranking before the procedure of 57.83 and after the action of 58.15 with a p-value of 0.0001. Researchers used wilcoxon tests on the breast size of the intervention group before the action was performed with a median of 43cm and after the

action with a median of 40cm with an IQR of 3.5cm, in the breast size control group before the action was performed with a median of 38 cm and after the action a median of 35 cm with an IQR of 3cm. the results of this study in line with (Astuti & Anggarawati, 2019) which explained that cabbage leaf compresses with breast care are very effective for relieving breast pain and swelling compared to early breast care and regular treatment in post partum mothers with a p-value value of 0.0001. And the study (Rohmah et al., 2019) said from the results of breast size studies obtained a p-value value of 0.0001 meaning that there was a significant difference after the administration of cabbage leaf compresses was effective in reducing the scale of breast swelling in post partum mothers. According to (Astuti & Anggarawati, 2019) Cabbage compress is a method of doing compresses using cabbage leaves with the aim of reducing breast swelling. From the results of the study obtained in the intervention group of breast milk dams before the intervention with media 6 (breasts felt very hard) with an average rating of 53.00 and after the intervention median 1 (smooth) with an average rating of 22.75 and p-value of 0.0001. This study is in line with research (Komala Sari & Nelda Putri, 2020) that the average breast swelling in puerperal mothers before being given cabbage leaf compresses and breast care is with the lowest breast swelling scale of 5 and height 6. Based on the results of the study, there were differences in the scale of breast swelling after breast treatment using the mann whitney test, the whitney man test obtained the results of p values = 0.0001 where the p< value was 0.05 with the conclusion that there was a difference in breast swelling in puerperal mothers between the intervention group and the control group after breast care was carried out on the intervention group. According to the results of the study showed that early breast treatments and cabbage compresses are considered effective for removing breast swelling which has softened the breasts and reduced the level of swelling.(V. K. Sari et al., 2020)

CONCLUSION

There is a difference in breast size after cabbage leaf compresses are given in reducing mastitis in post partum mothers at Bekasi Private Hospital, there are changes in breast milk dams before and after cabbage leaf compresses and breast care for post partum mothers in hospitals. Private Bekasi.

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HIGH LEVEL OF PHYSICAL ACTIVITY IN NURSING PERSONNEL AT RSI FATIMAH CILACAP

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ABSTRACT

Introduction : Research related to stroke risk factors recommends educating the public to reduce stroke risk factors by increasing physical activity. Regular physical activity can improve physical, mental function and maintain movement and independence of the elderly. Physical activity is very effective in preventing stroke and reducing mortality. Some people are still lacking in physical activity because they still do not understand the benefits of physical activity. Stroke risk factors can be prevented by lifestyle modification. People who meet the 5 criteria for a healthy lifestyle, namely not smoking, carrying out regular physical activity for more than 30 minutes every day, consuming healthy nutrition, and having a Body Mass Index (BMI) of less than 25 kg/m² can reduce the risk of stroke by 80% than people who are not in the above criteria. Research on physical activity in nurses shows that the level of activity of nurse clinicians is higher than that of nurse managers. Nurses are able to become agents of change to help patients live a healthy lifestyle. Nurses are able to influence the increase in physical activity.

Purpose: This research purpose is to examine and provide information about the level of physical activity in nursing staff.

Method: The study in 147 nursing staff and using analyze observational analytic with the Global Physical Activity Questionnaire. The analysis of the results was carried out using a description method that produced a percentage.

Results: The results of the activity level questionnaire obtained the results of a high level of physical activity 82.3%, a medium level of 14.3% and a low level of 3.4%. There was correlation with age and physical activity level ($p=0.031$).

Conclusion: The conclusion of the study nursing staff in RSI Fatimah Cilacap have a high level of physical activity.

Key words : stroke; physical activity; nursing staff

INTRODUCTION

The World Health Organization (WHO) estimates the global health data for stroke as reason second death and third inability adapt activity life. There are 16.9 million new case incidence of stroke and there are 33 million stroke patients who are still live around the world in 2010, more than half be in a country with income low until medium. Estimation final from *The Global Burden of Disease, Injuries, and Risk Factors Study* (GBD, 2015) revealed that there is shift trend from easy disease transmitted, maternal and problems nutrition switch to disease that is not infectious like strokes (Katan & Luft 2018) . Based on results Research Basic Health (Riskesdas, 2018) prevalence data obtained stroke in Indonesia is 10.9 per mile population with incidence the highest was in East Kalimantan Province at 14.7 per mile and the lowest was in Papua Province at 4.1 per mile (Boehme et al. 2017). Stroke prevention requires management factors major stroke risk cover hypertension, hyperlipidemia, diabetes mellitus and the use of tobacco, as well treatment with antithrombotic (Guzik & Bushnell 2017) . Physical activities very effective for prevent stroke and reduce number death. Some people still not enough in physical activity because still not yet understand benefit from physical activity (Jeong et al. 2017) . Study about physical activity on nurses show that level activity nurse clinician more high compared with nurse manager (Jirathananuwat & Pongpirul 2017) . Nurse capable becomes change of agent who help patient in under style healthy life. Nurse capable to give influence physical activity enhancement. However, research about this not yet conducted more (Richards 2016) .

Study related factor stroke risk recommend existence education to society to reduce factor risk of stroke with increase physical activity. Regular physical activity could increase the body function physically, mental and caring movement as well as independence elderly (Blake et al. 2017) . Nursing staff as *role models* should give good example _ in lower factor risk of stroke to public (Richards 2016) . Study cohort in lower risk of stroke against nurse have risk low compared group no nurse , but should for prevent stroke nurse must to take physical activity in order to life healthy and prosperous (Liao et al. 2018) . This study's aim to provide information about level physical activity on nursing staff. Hypothesis in study this is " How the profile level physical activity on nursing staff at RSI Fatimah Cilacap?"

METHOD

Type study is quantitative descriptive with *cross sectional* approach. The problem formulas descriptive is something formula related problems with question to existence variable independent good only on one variable or more. The research method is use questionnaire survey. Study carried out at RSI Fatimah Cilacap in the month of June until July 2021. Population study this is nursing staff both contract and permanent employee in RSI Fatimah Cilacap. Researcher use technique *purposive sampling* to get sample appropriate research with destination research. Respondents who will made sample study must fulfill criteria inclusion and exclusion. The sample amount there are 147 nursing staff. Study variable is level of physical activity in nursing staff at RSI Fatimah Cilacap. Data collection is carried out use questionnaire *Global Physical Activity Questionnaire* (GPAQ). Method data analysis using analysis univariate using SPSS.

Information worthy ethics obtained from KEPK Stikes Muhammadiyah Gombong with number 239.6/II.3.AU/F/KEPK/V/2021.

RESULTS

Demographic data respondent part big women, diploma education and employees permanent in accordance Table 1, while the average age respondents 33.4 years. Research results show part big nursing staff have level physical activity high in Table 2. There were meaningful relationship by statistic ($p=0.031$) between age and level physical activity RSI Fatimah Cilacap nursing staff in Table 3.

Table 1. Demographic Data Respondent

aracteristics Respondent	ount	centage (%)
pe sex		
Man		.7
Woman	99	.3
ucation Level		
S1		.7
D3	3	.3
mployee Status		
Permanent	5	.5
Contract		.5
Age		
17-25 years old		.4
26-35 years old		.6
36-45 years old		.9
46-55 years old		.1

Table 2. Activity Level Physical Nursing Personnel

ET's	requency	centage (%)
ET's > 3000	1	3

MET's 600-3000	.3
MET's < 600	.

Table 3. Analysis of Age and Activity Level Physique

	Activity Level Physique			Value
	Low	Currently	High	
Age	25			
	35			31
	45			

DISCUSSION

Results of research conducted to nursing staff show that level physical activity nursing staff in category high the total MET's more from 3000 minutes in a week. This is supported by activity physique nursing staff on -site nursing work including intensity currently as much as 60.5%. Physical activity nursing staff dominant in the work and transportation domains, while activity physique recreational in category low. This possibility caused because a pandemic that affects physical activity. Someone who reduces activity his physical activity have high risk to experience disturbance *mood*, because it's an activity program physique must implemented, although there is application *social distancing* for prevent deployment *Severe Acute Respiratory Syndrome Coronavirus 2* (Puccinelli et al. 2021). Physical activity benefits in prevent disease through effect protect system immunity body. Adaptation in a time of pandemic also done in fulfill needs in physical activity can be conducted is make schedule sport for prevent or resolve condition activity less physical (Nieman & Wentz 2019) . Activity level low in nursing staff from the recreational domain could upgraded with method making schedule by routine sport on the work, accordance guide in activity physical.

Total time used for physical activity for one week and intensity physical activity multiplied raised number of MET's. WHO recommendations in physical activity that is during one week, including activity work, transportation and recreation in adults at least fulfill one among the 3 conditions this that is physical activity with intensity currently for 150 minutes, or physical activity intensity high for 75 minutes, or combination among activity medium and high achieve a minimum of MET's 600 minutes (Natalucci et al. 2020) . A study state that activity regular safe for health and for the frail elderly as well as risk of caught disease cardiovascular and metabolic risk disease will decrease with activity regular physical from intensity low to high (McPhee et al. 2016). Activities physical decrease stroke risk. Physical activities in medium and high level relate with subtraction incidence of stroke, both ischemic and bleeding stroke. Man reach drop highest stroke risk if to do physical activity intensity currently until high, while woman already get benefit maximum with intensity low like walk (Howard & McDonnell 2016) . Someone who just to do physical activity medium-high 1-2 times in a week could reduce 16% risk have a stroke than people who don't take physical activity medium-high (Jeong et al, 2017) .

Study about physical activity this in line with study to woman age 25 – 54 years in Bogor City shows part big woman tend physical activity level high with MET's value of work domain of 4,271.69 minutes every week, transport domain of 1,058 minutes every week and recreational domain of 181.23 minutes every week (Nainggolan et al. 2019) . This different with research conducted by student medicine at university Brawijaya show that level activity physique they part big in category low 60%. Study it also generates that no there is significant influence among age, type gender, condition the place stay, activity organization / committee and level knowledge to physical activity with level activity physical student (Riskawati et al. 2018) .

CONCLUSION

Physical activity level at RSI Fatimah nursing in high category. Activities physical done by some nursing staff more than 3000 minutes in a week consist of activity work, transportation and activities

recreational.

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SUPPORT FROM HEALTHCARE PROFESSIONALS FOR COUPLES/PARTNERS USING ASSISTED REPRODUCTIVE TECHNOLOGY IN JAPAN: A LITERATURE REVIEW

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Abstract

Introduction: Assisted reproductive technology (ART) is being used for infertility by an increasing number of couples in Japan. The family care/caring theory proposed by Hohashi (2015) encompasses these factors. In Hohashi's family care/caring model, family support consists of support in the family internal environment; family system unit; family external environment; and family chrono environment. This study sought to clarify the support provided by health professionals to couples/partners undergoing ART based on the support vectors of the family care/caring model.

Method: Articles on Ichushi Web, a Japanese medical literature database, were searched applying keywords "assisted reproductive technology", "infertility", "family", "couple", "nursing", "care", and "support." Family support was extracted from 12 articles, coded into subcategories and categories, and classified in accordance with the targeted support vector of family care/caring.

Results: A total of 21 categories were identified. The main categories were: support for the family system unit, such as "Nurses coordinate the couple's relationship"; support for the family internal environment, such as "Nurses maintain close contacts with the women"; support for the family external environment, including such categories as "Healthcare professionals should provide opportunities for peer support" in micro-system support, and "Nurses should encourage doctors to relate to women" in macro-system support. Family chrono environment support included "Nurses keep in continuous contact with women".

Conclusion: The support was systematically organized according to the support vector of family care/caring theory. It will be necessary to consider direct and indirect support for men in the future. Although support for the supra-system was not considered, support will be needed considering couples' diversity of culture, religion, national characteristics, and regional characteristics of couples in Japan, whose population is largely ethnically homogenous, and many do not practice a religion.

Key words: assisted reproductive technology, family care/caring theory, family support, infertility, literature review

EDUCATION EFFECTIVENESS OF BRAIDED METHODS WITH MEDIA OF KLOP KB ON KNOWLEDGE, ATTITUDE, AND BEHAVIOR OF KB ACCEPTORS IN THE MATERNITY WARD AT PRIVATE HOSPITAL IN BEKASI 2022

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ABSTRACT

Background: Family Planning (KB) is a pillar of world health development to improve maternal and child health. Contraceptive services are a priority as an effort to reduce maternal and infant mortality rates.

The purpose of this study was to determine the effectiveness of the BRAIDED method with KB KB media on the knowledge, attitudes, and behavior of family planning acceptors in the maternity care ward of the Bekasi 2022 Private Hospital.

The research method used a quasi-experimental (quasi-experimental) with one pretest-posttest design with a total sampling technique of 52 people. The analysis used using univariate and bivariate tests. The research was conducted by distributing questionnaires in one meeting in the maternity care room at the Bekasi 2022 Private Hospital.

Results: Based on the research, the majority of family planning acceptors were aged 31-35 years, as many as 20 (38.5%) respondents, with tertiary education, as many as 36 (69.2%) respondents, with parity of more than 2 children, namely as many as 47 (90.4%) respondents, 39 (75%), and 39 (75%). The results of the study based on the Wilcxon test obtained a significant value with a p-value of $0.000 < \alpha 0.05$ which means that there is an increase in knowledge, there is a change in attitude, and there is a change in the behavior of family planning acceptors after the BRAIDED method education with KB KLOP media on knowledge, attitudes, and behavior of family planning acceptors in the maternity care ward of the Bekasi 2022 Private Hospital.

Conclusion: there is an effectiveness of the BRAIDED method with KB KLOP media on the knowledge, attitudes, and behavior of family planning acceptors in the maternity care room at the Bekasi 2022 Private Hospital.

Key words: BRAIDED method, KB acceptor, KLOP KB

SYNERGISTIC POTENTIAL OF THE COMBINATION MENGKUDU (*MORINDA CITRIFOLIA* L.) AND KELOR LEAVES (*MORINGA OLEIFERA*) POWDER AS FUNCTIONAL FOOD RICH IN FLAVONOIDS AND FIBER: ANALYSIS AND SYSTEMATIC LITERATURE REVIEW

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Abstract

Introduction: Mengkudu and kelor leaves have the potential as herbal plants that can be used for disease therapy because they have high flavonoids and fiber and can have a synergistic effect. But studies using a combination of the two have not been conducted. The purpose of this study is to determine the content of flavonoids and fiber on mengkudu and kelor leaves powder to determine the synergistic potential and compare it with previous studies in a systematic literature review

Method: The materials used are mengkudu powder and kelor leaves powder obtained from the drying process, flavonoid content analysis test using the UV-Vis Spectrophotometry method, and the fiber test using the multi-enzyme method or according to the AOAC guidelines and carried out a systematic literature review.

Results: The two ingredients have their advantages and disadvantages. Where kelor leaves powder has a flavonoid content (2.49 g /100 grams) 3.7 times greater than the mengkudu powder, which is only 0.67 g /100g. Mengkudu powder has the advantage of its fiber content of 26.61 g /100 grams and 1.4 times higher when compared to kelor leaves powder which is only 19.86 g /100 grams. A systematic literature review showed the highest fiber content in mengkudu in powdered extracts, which was 48.55 to 49.26 %, while flavonoids in fermented mengkudu juice were 36.73 mg /100 ml. The highest fiber and flavonoid content in dried kelor leaves or powder is a dietary fiber of 24.97 g /100 g DW and flavonoids are 21.21%.

Conclusion: Combining mengkudu and kelor leaves powder can be used as a way to create complementary products that are rich in flavonoids as well as fiber and cause the expected synergistic effect. Previous research that used the type of processing was powdered was closest to the results of the analysis of this research.

Key words : Fiber, Flavonoid, Kelor, Mengkudu, Powder.

INTRODUCTION

Humans have long utilized plant biodiversity for a variety of purposes. One of the most prominent tropical fruits and vegetables, namely *Morinda citrifolia* or can be known as mengkudu and kelor leaves (Souza & Marcos, 2021). Mengkudu is often used as a complementary and alternative therapy in different countries due to its high antioxidant content and its various benefits that are good for health. Many people use mengkudu as a traditional medicine because it can act as an antibacterial, antitumor, antioxidant, anthelmintic, analgesic, anti-inflammatory, increase immunity, and is even proven to help control gastritis, skin diseases, menstrual disorders, respiratory infections, fever, diabetes, hyper cholesterol, hypertension, cardiovascular diseases, and dyslipidemia (Ali, Kenganora, & Manjula, 2016; Souza & Marcos, 2021). Juices and extracts of mengkudu contain phytochemical compounds that are good for health, such as amino acids, flavonoids, anthraquinones, fatty acids, sterols, terpenoids, lignans, and others (Ali et al., 2016).

Kelor or *Moringa oleifera* is a very abundant plant in Indonesia, where the leaves of this plant have various types of nutrients that are good for health, such as minerals (iron, calcium, and sodium), vitamins C, E, beta carotene, as well as antioxidants (flavonoids, phenolics, glucosinolates, saponins and the presence of fiber isothiocyanates containing fiber ((Putra et al., 2021). Mengkudu and kelor leaves have a very high water content, so the shelf life is concise and limited. A drying method is needed to turn fresh

mengkudu into powder to extend the material's shelf life. Bending or twisting mengkudu into powder can also be used to remove rancid odors due to the content of capric acid and kaproat (Marbun & Rahayuni, 2020; Sudibyo & Hutajulu, 2016). While kelor leaves powder is produced through drying young Moringa leaves can use in an oven or sunlight kelor leaves powder has a longer shelf life than fresh and some better nutrients (Marbun & Rahayuni, 2020).

Mengkudu dan kelor leaves powder contains flavonoids and fiber, which are suitable for health. Flavonoids are one of the most promising phytochemical compounds used as antioxidants and therapeutics for cancer diseases, pathogenic bacterial infections, inflammations, cardiovascular dysfunction, and prevent free radicals. (Arifin & Ibrahim, 2018). Both ingredients contain flavonoids and fiber, which are suitable for health. Flavonoids are one of the most promising phytochemical compounds used as antioxidants and therapeutics for cancer diseases, pathogenic bacterial infections, inflammations, cardiovascular dysfunction, and prevention of free radicals. (T. yang Wang, Li, & Bi, 2018). More than 9000 flavonoids are known to be spread and found in various parts of the world, where these compounds can be found in all types of green plants or plants. The need for flavonoids is very diverse, is around 20 mg to 500 mg. Flavonoids are found in plants that have red, yellow, blue, orange, and purple pigments both at the root, flower, stem, and leaves (Arifin & Ibrahim, 2018).

Dietary fiber is a group of complex carbohydrates that cannot be hydrolyzed by human enzymes so that they cannot be absorbed and digested by the human body. Fiber consists of soluble fiber such as pectin, gom, fructans, and some resistant starches and water-insoluble fibers such as lignin, and cellulose, which can be beneficial for health, accelerate gastric emptying and increase the number of feces to facilitate bowel movements. However, fiber also correlates with the risk of diabetes, metabolic syndrome, inflammation, obesity, atherosclerosis, and heart disease (Ghada A. Soliman, 2019). Based on the description above, we can see that the mengkudu and kelor leaves powder to contain fiber and flavonoids, which can potentially cause synergistic effects if combined and can be developed as a therapy for the control of a disease. However, a deeper exploration of the flavonoid and fiber content of the two ingredients is needed to ascertain the possibility of a potential synergistic effect. Therefore, researchers are interested in testing the range of flavonoids and fiber in the mengkudu and kelor leaves powder. To see the content of the two phytochemical compounds owned through laboratory tests and conduct a systematic literature review to compare the test results with previous studies with various types of preparations.

METHOD

Analysis of Flavonoid and Fiber Content

Materials used in the study were mengkudu, and kelor leaves powder obtained from buying at the herbal shop in the east Porwokerto area, Banyumas-Central Java. The powder is 100% original without additional ingredients; the product already has a distribution permit, ethanol, 5% AlCl₃ solution, 95% alcohol, aquades, antifoam agent, and 5% acetic acid.

The tools used in the analysis test are scales, erlenmeyer, filter paper, spatula, claws, stove, test tube, desiccator, measuring cup, and spectrophotometer. The flavonoid and fiber content analysis test was carried out at the Chem-Mix Pratama Bantul-Yogyakarta Laboratory in September-October 2021.

Analysis of Flavonoid Content

The Flavonoid content analysis test uses the UV-Vis spectrophotometry method. With several stages,

- a. Determining the Maximum Wavelength
Take 1 ml of 100 ppm quercetin solution added at 1 ml of AlCl₃ 10% and 8 ml of 5% acetic acid. Read wavelengths using a UV-Vis Spectrophotometer wavelength of 400-500 nm.
- b. Determining The Operating Time
Take 1 ml of 100 ppm quercetin solution added at 1 ml of AlCl₃ 10% and 8 ml of 5%

acetic acid. Read wavelengths using a UV-Vis Spectrophotometer wavelength of 400-500 nm.

c. Determining the Quercetin Standard Curve

They made a series of solutions using quercetin ranging from 40, 60, 80, 100, 120, and 140 ppm. Each series of 1 ml is included in 1 ml of AlCl₃ 10% and 8 ml of 5% acetic acid. Let it stand according to the maximum time of the results of determining the operating time. Read with a spectrophotometer at a total length of waves

d. Determining Total Flavonoid Levels

A solution of mengkudu powder and kelor leaves powder is taken as much as 1 ml and added with 1 ml of AlCl₃ 10% and 8 ml of 5% acetic acid. Let stand at the optimal time and read absorbance at maximum wavelength using a spectrophotometer, carried out twice. (Fatahillah Pasaribu, Wiboworini, & Retna Kartikasari, 2021; Ramadhani, Hati, Lukitasari, & Jusman, 2020)

Analysis of Fiber Content

The fiber content analysis test using the multienzyme method was carried out utilizing sample powder weighed as much as 0.5 grams and taken in Erlenmeyer with 50 ml of phosphate pH seven. Buffer added, 0.1 ml of alpha-amylase enzyme heat it in a 100 °C temperature water affirmation for 30 minutes by stirring occasionally. Cool the sample and add 20 ml of distilled water and 5 ml of HCL 1N, 1 ml of 1% pepsin enzyme, and reheat for 30 minutes. After that, remove the Erlenmeyer tube and add the sample with 5 ml of NaOH 1N, the enzyme beta-amylase 0.1 ml, then close the Erlenmeyer tube and incubate in a water affirmer for 1 hour, after which strain with constant paper. The sample was washed with 1 x 10 ml of ethanol and 2 x 10 ml of acetone, dried in a 105°C oven for one night, dried in a desiccator, and weighed the final weight (insoluble dietary fiber). The filtrate is set in volume to 100 ml, and the ethanol is 95% warm by 400 ml. the filtrate is precipitated for 1 hour. After that, it is filtered with ash-free paper, washed with 2 x 10 ml of ethanol and 2 x 10 ml of acetone, dried in an oven at a temperature of 105°C, and again put a desiccator input and weighed the final weight (soluble fiber). Total dietary fiber is insoluble dietary fiber + soluble dietary fiber.

Systematic Literature Review

This research uses Systematic literature review studies, which is a process or activity of collecting data from various sources such as journals and comparing the results of one study with another. The systematic literature review provides a comprehensive overview of multiple sources related to themes/topics and methods and conducts synthesis to strengthen the knowledge base (Paul & Criado, 2020). The article used in this study comes from a Google scholar database with Inclusion Criteria 1) Displaying the results of the examination of Flavonoids and mengkudu fibers or kelor leaves 2) Fiber tests using proximate, multienzyme methods, acid-base hydrolysis, or according to AOAC and Flavonoid guidelines with spectrophotometric tests or Solid-phase microextraction coupled with gas chromatography-mass spectrometry (SPME-GC-MS) 3) Article published in the last 5 years (2018-2022) 4) Using English or Indonesia 4) Full Text 5) Can be accessed for free. Exclusion criteria 1) not displaying flavonoid or fiber levels, 2) Research with animals or humans, 3) Incomplete or non-free articles, 4) Review Articles, 5) Adding other ingredients to mengkudu powder or kelor leaves powder.

The literature search used keywords such as "Flavonoid", "Dietary fiber", "Morinda citrifolia", and "Flavonoid", "Dietary fiber", and "Moringa Oleifera". The research process was carried out comprehensively by going through 5 stages, including comparing, contrasting, criticizing, synthesizing, and summarizing. The method of reviewing and selecting studies is carried out based on the guidance of PRISMA, which is an instrument based on evidence for the manufacture of systematic reports or meta-analysis (Moher, Liberati, Tetzlaff, & Altman, 2009).

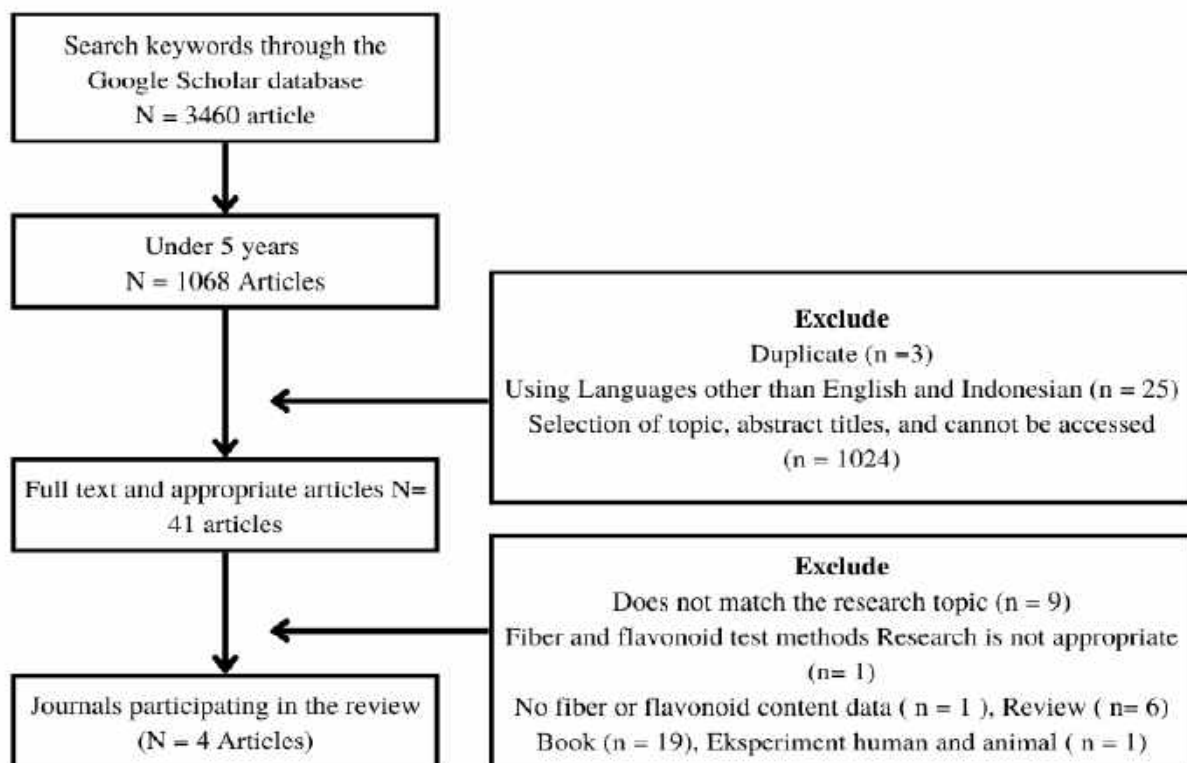


Figure 1. PRISMA Flow Chart *Morinda Citrifolia*

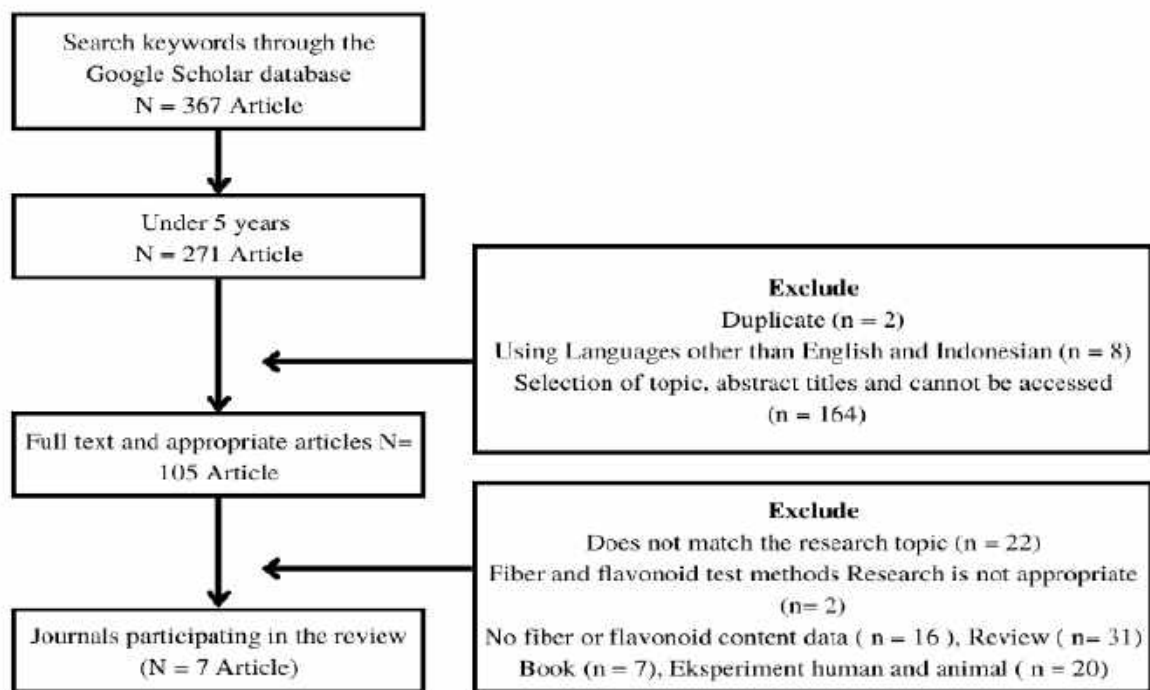


Figure 2. PRISMA Flow Chart *Moringa Oleifera*

RESULTS

Analysis of Flavonoid and fiber Content in mengkudu Powder and Kelor leaves powder

The study's results tested the content of flavonoids and fibers in the mengkudu powder, and kelor leaves powder is presented in tables 1 and 2.

Table 1. Analysis of Flavonoid and fiber Content in Mengkudu Powder

Sample	Test		Result	
	Test 1 (g/100 g)	Test 2 (g/100 g)	(g/100 g)	%
Fiber	26,62	26,71	26,67	26,67
Flavonoid	0,66	0,67	0,67	0,67

Table 2. Analysis of Flavonoid and fiber Content in Kelor Leaves Powder

Bahan	Test		Result	
	Test 1 (g/100 g)	Test 2 (g/100 g)	(g/100 g)	%
Fiber	19,94	19,78	19,86	19,86
Flavonoid	2,49	2,49	2,49	2,49

Systematic Literature Review

After making a selection using PRISMA principles in the Google Scholar database, the article that best suits this research was obtained, which is presented in tables 3 and 4

Table 3. Mengkudu (*Morinda Citrifolia* L) Literature Search Results

Author/Years/ Country	Title	Sample Type	Method	Results
Saah and David /2021/Ghana	Phytochemical, Proximate, and Vitamin C Content in <i>Morinda citrifolia</i> (Noni)	<i>Morinda citrifolia</i> , L Extracts	Fiber : Proximate analysis	Crude Fiber 4.49 %
Wang Z, Rong D, Ruili Y, Kun C, Congfa Li and Wu Li/2021/China	Changes in Phenols, Polysaccharides and Volatile Profiles of Noni (<i>Morinda citrifolia</i> L.) Juice during Fermentation	<i>Morinda Citrifolia</i> , L Fresh Juice and Fermentation	Flavonoid : Solid- phase microextraction coupled with gas chromatography– mass spectrometry (SPME-GC-MS)	Flavonoid Fresh Juice = 26, 07 mg/100ml Flavonoid Fermentation Juice = 36,73 mg/100 ml
Nguyen CT, Tuyen CK, Hoang TH, Duong HQ , Tien TN, Dinh HD and Dang XC/2020/Vietnam	Phytochemistry, nutrient, mineral, and antioxidant activities of two species <i>Morinda</i> L. grown in three provinces in Vietnam	<i>Morinda citrifolia</i> Extracts Powder	Fiber : Enzymatic- Gravimetric-Liquid Chromatography (AOAC)	Fiber Extracts Powder = 48.55 to 49,26 %
Oly-Alawuba and Amaka AI/2019/Nigeria	Evaluation of Nutrient, Antinutrient and Phytochemical Properties of Noni Fruit (<i>Morinda citrifolia</i>) Concentrate, Pulp, and Seed	<i>Morinda citrifolia</i> Juice Extract / konsentrat	Fiber : Proximate Analysis	Crude Fiber = 1.95 %

Table 4. Kelor (*Moringa Oleifera*) Literature Search Results

Proceeding 2nd International Allied Health Student Conference

Author/Years/Country	Title	Sample Type	Method	Results
Solichah E, Ade CI, Devry P, Dewi D, Wawan A, Woro S and Aini H/2022/Indonesia	Evaluation of physicochemical, nutritional, and organoleptic properties of nuggets based on moringa (<i>Moringa oleifera</i>) leaves and giant catfish (<i>Arius thalassinus</i>)	<i>Moringa oleifera</i> Extract	Flavonoid : UV-Vis spectrophotometer	Flavonoid 2.53 mg/100 g
Penalver R, Lorena MZ, Jose ML, Gaspar R and Gema N/2022/Spain	Nutritional and Antioxidant Properties of <i>Moringa oleifera</i> Leaves in Functional Foods	<i>Moringa oleifera</i> leaves dried	Fiber : Procedure 985.29 of the Fiber : AOAC	Dietary Fiber 24.97 g/100 g DW
Siskawardani DD, Sri W and Khwunta K/2021/Indonesia	The Antioxidant Activity of Kelor (<i>Moringa oleifera</i> Lam.) Leaves Based on Drying Method	<i>Moringa oleifera</i> powder	Flavonoid : Spectrophotometer Fiber : AOAC (Enzymatic-Gravimetric-Liquid Chromatography)	Fiber 3.09 % Flavonoid Cabin dryer (50°C) 1.41 mg/g Oven (50°C) 1.82 mg/g
Murdiasa PY, I Putu S and Putu TI/2021/Indonesia	Pengaruh Penambahan Puree Daun Kelor (<i>Moringa oleifera</i>) Terhadap Karakteristik Siomay Ayam	Puree <i>Moringa oleifera</i>	Fiber : Acid-base hydrolysis method	Crude Fiber 6.40%
Ola-Adedoyin A, Samuel OE, Ifreke ML, and Kayode OO/ 2021/ Nigeria	Effect of Different Blanching Treatments on the Nutritional Composition, Phytochemical Contents and Antioxidant Activity of Dried <i>Moringa oleifera</i> Lam. Leaf Flour	<i>Moringa oleifera</i> powder	Flavonoid : UV-Vis spectrophotometer Fiber : Proximate analysis	Crude Fiber Steam blanching 29,31 %, Boil water blanching 17.74 % on 100°C 3 min unblanching 23.87% Flavonoid Steam blanching 51.77 mg/100g, Boil water blanching 51.73 mg/100 g on 100°C 3 min unblanching 56.93 mg/100 gram
Rane MA, Marsianus P and Catootjie LN/2021/ Indonesia	Evaluasi Komposisi Nutrisi Tepung Daun Kelor dari Lokasi yang Berbeda di Nusa Tenggara Timur, Indonesia	<i>Moringa oleifera</i> powder	Fiber : AOAC (2005)	Crude Fiber 96.63 to 107.30 g/kg
Wickramasinghe YWH, Indira W and Isuru W/ 2020/ Sri Langka	Effect of Steam Blanching, Dehydration Temperature & Time, on the Sensory and Nutritional Properties of a Herbal Tea Developed from <i>Moringa oleifera</i> Leaves	<i>Moringa leaves</i> Dried	Flavonoid : UV-Vis spectrophotometer Fiber : Proximate analysis	Crude Fiber Blanching 3 min 7.05% unblanching 8.03% Flavonoid Blanching 3 min 21.21%

DISCUSSION

Analysis of Flavonoids And Fibers With The Resulting Synergistic Potential

The Mengkudu powder (*Morinda citrifolia* L.) is a nutritious food containing carbohydrates, proteins, vitamins B, and essential minerals. The powder includes several vitamins, including vitamins A, C, niacin, thiamin, and riboflavin, and several minerals, namely iron, calcium, sodium, and potassium. Phytochemical compounds in mengkudu are terpenes, alizarin, Anthraquinone, caproic acid, caprylic acid, and alkaloids. The powder also has a high bioactive compound where antioxidant activity is 60,473 mg/ml, and flavonoid content is 7.2 mg QE/g (Irawan, Yusmarini, & Hamzah, 2017). Kelor leaves powder is a food ingredient. That is familiar to us to consume and can usually be processed into various, such as additional baking ingredients, drinking brews, nuggets, and others, the fiber content in kelor powder is also high, namely crude fiber 3.67%. In addition, the characteristics of the nutritional content in kelor leaf powder are 20% (w/b), water content 6.64%, ash content 11.67%, fat content 6.74%, protein content 23.37%, carbohydrates 51.59%, calories 342.31 kcal/kg, iron (Fe) 177.74 ppm, calcium (Ca) 16350.58 ppm, sodium (Na) 1206.54 ppm and phosphorus (P₂O₅) of 290.65 mg / 100 g .

Dietary fiber has been linked to the management of non-communicable diseases and metabolic syndromes (Kurniawati & Fitriyya, 2018). The two powders tested, namely mengkudu and kelor leaves, have different advantages and limitations and have the potential to cause synergistic effects when combined, namely, which will complement each other to create products rich in flavonoids as well as fiber. Almost 70% of the people in the world use plants as herbs that can help to treat and maintain health. Several plants are consumed singly or combined. When plants are combined, the effects caused will be even more complex because it allows for interactions between compounds. The expected interaction is to take advantage of synergistic or additional therapeutics. Combinations of herbs or plants that are potentially good for health are necessary for the treatment of critical, chronic, and complex illnesses (Haryanti, Sholikhah, & Widiyastuti, 2019). Kelor leaf powder has a flavonoid content of 2.49 g / 100 grams which means it is 3.7 times greater than the powder of only 0.67 g / 100 grams. However, the powder has a twist on its fiber content of 26.61 g / 100 grams and is 1.4 times higher when compared to kelor leaves which are only 19.86 g/100 grams.

The combination of the two, rich in flavonoids and fiber, can be used as functional food, which is helpful as a disease therapy and maintains a healthy body. Antioxidants play a role in neutralizing free radicals effectively to reduce the damage caused. Flavonoids are substances that can act as antioxidants that can inhibit oxidation reactions from ROS by binding to free radicals so that oxidative stress can decrease. one of them is to prevent insulin resistance and lipid peroxidation to control lipid profiles where which can prevent degenerative diseases such as diabetes and dyslipidemia (Halan, Woda, & Setianingrum, 2019). The high fiber content can also reduce fasting blood glucose levels, LDL, triglycerides, and total cholesterol and increase HDL. Fiber can provide a prolonged feeling of satiety because it can absorb large amounts of water in the GI tract, limiting our food intake, especially carbohydrate and fat sources. Fiber can also slow down the absorption of food so that food glucose will be absorbed not much and will also cause a lot of fat to be burned as energy so will control that blood glucose and lipid profiles (Kurniawati & Fitriyya, 2018).

Fiber can also bind lipids in the intestines to lower cholesterol levels by up to 5% or more (Nastiti, Nurhidajah, & Yusuf, 2020). Kelor leaf powder and mengkudu have soluble fiber that can play a role in forming a gel in the small intestine. The gel can later bind lipids, cholesterol, and bile acids to be removed. Bile acids bound and discarded cause bile acids in the liver to decrease and increase the

excretion of bile acids. The impact is that the cholesterol absorbed by the body will reduce because part of the cholesterol will bind to bile acids and be removed through feces. The liver will produce bile acids again because many have been wasted because they are attached to the gel by taking cholesterol in the blood so that later it reduces cholesterol levels in the blood so that later reduces cholesterol levels in the blood (Rupiasa, Fatimah-Muis, Syaury, Tjahjono, & Anjani, 2021).

Previous research has shown the benefits of mengkudu, and kelor leaves as potential functional foods in Indonesia. One of its health benefits is that it can be used as a therapy to control blood glucose and lipid profiles. These two indicators are the originator of various types of degenerative diseases. Previous studies have shown that the administration of mengkudu extracts can reduce pre- and postprandial glucose at a dose of 750 mg/kg bb and also an amount of 125 mg/kg bb in rats Wistar DM (Anwar & Nugroho, 2015; Zega, Wowor, & Mambo, 2016). Another study using 250 ml of mengkudu cider added to manufacture plain bread lowered blood glucose levels. While kelor leaf powder at a dose of 50-100 mg/kg bb can reduce fasting blood glucose levels, the kelor leaf extract of 600 mg/kg bb can increase HDL in DM rats (Nurul Laelatunisa, Nikmatul Rizky, Rachmadanti Arum, 2019; Satrianawaty, Sumarno, & Prabowo, 2019; Villarruel-López et al., 2018). While in human studies, it has also been carried out where it gives 1000 mg/day of Moringa powder for 25 days to prediabetes subjects and is known to reduce blood glucose levels up to 20 mg/dl (Munim, Alwi, & Syam, 2019).

Analysis of Systematic Literature Review Results

Based on the results of article searches conducted on Google scholars by applying Prisma principles, four articles were obtained that are suitable for Mengkudu research and seven articles for research that discuss fiber and flavonoid levels in Moringa. The fiber in the results of the researcher's analysis was known to be 26.67 g / 100 g or 26.67%, and flavonoids 0.67 g / 100 g BW or 0.67%, while the results of the previous study showed results that the extracted mengkudu contained crude fiber by 4.49% this is different from other studies, namely mengkudu in the form of extract powder is known to have fiber by 48.55 to 49.26%, and this study shows that the extracted powder extract is two times greater than the powder that not contracted by the researcher (Nguyen et al., 2020; Saah & Adu-Poku, 2021). Mengkudu fiber-made juice or concentrate is 1.95% crude fiber (Nkeiruka M. Oly-Alawuba & Amaka A. Iwunze, 2019). Previous research that showed the results of flavonoid content in mengkudu, which was made of fresh juice and fermented, was known to contain flavonoids, namely fresh juice of 26.07 mg / 100 ml and fermented of 36.67 mg / 100ml (Z. Wang et al., 2021).

The results of the kelor powder content analysis conducted by researchers found that the fiber content was 19.86 g / 100 g or 19.86%, while the flavonoid content was 2.49 g / 100 g or 2.49%. Previous studies have been carried out which show the results of different fibers and flavonoids, showing that kelor leaves in the extract showed flavonoid content of 2.53 mg / 100 g, other studies leaves that were used as powder fiber content were 3.09% and flavonoids which were distinguished based on the type of drying were recorded with a cabin dryer (50 ° C) obtained 1.41 mg/gram while if in the oven (50 ° C) the flavonoids were 1.82 mg / g (Siskawardani, Winarsih, & Khawwee, 2021; Solichah et al., 2022). Another study of kelor leaves used as the powder was known to contain crude fiber, which was previously steam blanching 29.31%, boil water blanching at 17.74% temperature 100 °C 3 min and unblanching 23.87%. This study included the results studied by researchers using powder without blanching. While the flavonoid content is steam blanching 51.77 mg / 100grams, boil water blanching 51.73 mg / 100 g temperature 100 ° C 3 min and unblanching 56.93 mg / 100 grams (Ola-Adedoyin, Etatuvie & Olaniyan, 2021). Starchy leaves of kelor were also reported to contain crude fibers of 96.63 to 107.30 g/kg (Rane, Perunggu, & Nalle, 2021).

Kelor leaves are often also processed into dry leaves, which can be used as tea. The study conducted using dry leaves was known to contain 24.97 g / 100 g of fiber, which this study was close to the results of the researcher's analysis, namely 19.86 g / 100 g. In other studies, dry leaves contained crude fiber blanching for 3 minutes at 7.05%, without blanching at 8.03%, and flavonoid blanching for 3 minutes at 21.21% and without blanching at 19.07% (Peñalver, Martínez-zamora, Lorenzo, Ros, & Nieto, 2022; Wickramasinghe, Wickramasinghe, & Wijesekara, 2020). Kelor puree made with a small amount of water

and leaves is known to contain 6.40% crude fiber (Yumiko Murdiasa, Suparthana, & Timur Ina, 2021).

The results of previous studies showed differences in flavonoid and fiber test results in mengkudu and kelor depending on the type, geographical location, and processing method. Previous research using the kind of processing was dried and powdered was closest to the results of the researchers' analysis carried out in Indonesia. Flavonoids that are still classified as polyphenols will risk damage due to enzymatic processes. Processing such as steaming also causes the release of phenolic compounds with low molecular weight, such as flavonoids. The longer the process of withering or blanching and drying the leaves can reduce the flavonoid content significantly. Because flavonoid compounds are not heat resistant (Liana Dewi, Ari Yusasrini, & Wisaniyasa, 2021). Fiber will increase along with the increase in drying temperature, where high temperatures can reduce water content and produce an increase in carbohydrates (Kusuma, Putra, & Darmayanti, 2019). The drying and withering process can also maintain and increase foodstuff's fiber content. In this process, the water content will decrease along with the increase in carbohydrate content (Liana Dewi et al., 2021). The fiber in the previous study was mostly crude, while this used dietary fiber. Crude fiber is derived from vegetables and fruits and is known as a non-nutritional substance but has good benefits for removing feces or smoothing the gastrointestinal tract (Pandey, Pongoh, & Reo, 2021). While dietary fiber is a part of edible plants or carbohydrates that can withstand digestion and absorption in the human GI tract, based on its solubility, dietary fiber is divided into 2, namely soluble and insoluble. Several studies have proven that dietary fiber plays a role in various diseases such as cardiovascular disease (CVD), obesity, cancer, and type 2 diabetes (Veronese et al., 2018). While dietary fiber is a part of edible plants or carbohydrates that can withstand digestion and absorption in the human GI tract, based on its solubility, dietary fiber is divided into 2, namely soluble and insoluble. Several studies have proven that dietary fiber plays a role in various diseases such as cardiovascular disease (CVD), obesity, cancer, and type 2 diabetes (Dahlan, 2020).

CONCLUSION

The results of laboratory tests show that mengkudu powder and kelor leaves powder can synergistically and complement each other. Kelor leaf powder has a flavonoid content of 2.49 g / 100 grams, meaning it is 3.7 times greater than mengkudu powder, which is only 0.67 g / 100 grams. However, mengkudu powder has a twist on its fiber content of 26.61 g / 100 grams and is 1.4 times higher when compared to kelor leaves which are only 19.86 g / 100 grams. The results of the systematic review showed the highest fiber content in mengkudu, namely in extracts powder, which was 48.55 to 49.26%, while flavonoids in fermentation juice were 36.73 mg / 100 ml. The highest fiber and flavonoid content in dry kelor or powder is dietary fiber 24.97 g / 100 g DW. In contrast, flavonoids are 21.21% before drying. The leaves are blanched for 3 minutes with water at 100 ° C.

Previous research that used the type of processing was dried and powdered was closest to the results of the analysis of the research conducted now. Can make suggestions on experimental animal studies to prove the benefits of combining the two ingredients against the therapy of either simple or complex diseases such as metabolic disease.

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ANALYSIS OF NUTRITIONAL CONTENT AND ORGANOLEPTIC TEST OF SANGKURMA SNACK CUP (BANANAS, DATES, AND HONEY) AS FUNCTIONAL FOOD HIGH IN IRON AND VITAMIN C

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Abstract

Introduction: Food's functional value can be done in various ways, one of which is food modification. SangKurMa snack cup is a modified-food product made from bananas, dates, honey, and crackers. These three main ingredients (bananas, dates, and honey) contain various vitamins and minerals, including iron and vitamin C. This study aimed to determine the most preferred formulation of the SangKurMa snack cup and the amount of iron and vitamin C in each product.

Method: The analytical methods selected include the Jacobs method for iron and Spectrophotometry for vitamin C. The experimental design used in this study was a Completely Randomized Design (CRD) with four formulations. The organoleptic test uses a hedonic test on aspects of color, aroma, taste, and texture in each formula (F1, F2, F3, and F4) involving 25 semi-trained panelists using 9 Likert scales.

Results: Iron and vitamin C per 100 g include 8.48 mg and 70.25 mg in bananas, 3.39 mg and 20.45 mg in dates, 4.41 mg and 11.03 mg in honey, and 5.48 mg and 14.12 mg in crackers. The amount of iron and vitamin C per serving of SangKurMa snack cup includes 10.45 mg and 78.71 mg (F1); 11.45 mg and 86.46 mg (F2); 12.45 mg and 94.20 mg (F3) and 13.44 mg and 101.95 mg (F4). The statistical analysis of organoleptic tests showed no difference in the aspects of color, aroma, taste, and texture in all formulas (p -value>0.05).

Conclusion: SangKurMa snack cups contain high iron and vitamin C, which can fulfill 69.70-82.98% of daily iron needs, 1.05-2.02 mg of absorbed iron, and 104.96-135.94% of vitamin C requirements. In addition, the organoleptic test showed that the panelists had the same preference for color, aroma, taste, and texture in all formulas.

Keywords: Iron, SangKurMa Snack Cup, Vitamin C

INTRODUCTION

Functional food is a dish that contains energy and nutrients and has other benefits for the body, such as increasing physiological responses and reducing the risk of certain diseases (Nicoletti, 2012). This functional quality and value can be improved in several ways, such as fortification, substitution, food modification, genetic manipulation, increasing the content of certain materials, and various other efforts (Lau et al., 2013).

Snack is food or drink consumed between main meals (interludes) and has become part of people's daily consumption because it assists the implementation of dietary regulation (Chinnadurai & Sequeira, 2015; Tumuluru, 2015). These snacks can include chips, popcorn, pretzels, extruded foods, processed meats, seeds, nuts, fruits, vegetables, and other preparations (Riaz, 2015). Snack products are said to be healthy if they contain good nutrients and are low in salt and fat (Tumuluru, 2015). The SangKurMa snack cup is a modified layered snack that is packaged using a cup made of bananas, dates, honey, and crackers as additional ingredients.

Banana is a fruit whose 75% component consists of water. This fruit contains high levels of vitamin A, vitamin B, vitamin C, iron, calcium, potassium, phosphorus, sodium, magnesium, copper, fiber, and zinc (Agoes, 2011; Hasanah et al., 2017; Kumar et al., 2020). The high levels of some nutrients in Ambon bananas provide various benefits for individuals who consume them, one of which is iron. This component plays a significant role in the body, especially in synthesizing oxygen transport proteins such as hemoglobin, where about 65-74% of the iron in the body will later be used in this process (Arisman,

2017; Briawan, 2013). The availability of iron will directly affect the amount of hemoglobin formed. When hemoglobin levels are low, oxygen supply throughout the body will also decrease (Lopez et al., 2015). Low oxygen transport to all body tissues can cause various cognitive process disorders, such as reduced concentration and enthusiasm for learning and weakened individual memory skills (Chamaschella, 2015; Siva et al., 2016).

In addition to iron, bananas also contain high levels of vitamin C, which can help the process of blood formation and increase iron absorption (Mahardika & Zuraida, 2016). Consumption of 25-75 mg of vitamin C can increase the absorption of nonheme iron up to four times (Briawan, 2013). It is because vitamin C as an enhancer can reduce ferric ions (Fe^{3+}) to ferrous (Fe^{2+}) so that iron that initially enters the body in the form of ferric will be more easily absorbed by the intestines (Gropper et al., 2016).

SangKurMa snack cups also use dates as a primary ingredient. Dates are fruit with sweet characteristics, soft to hard texture, and colors ranging from yellow, green, golden brown to dark brown (Shiddiq & Greiby, 2013). Dates are also a fruit that has many benefits for the body because of their nutritional content. For example, most studies say dates have potential effects as antioxidants, anticancer, anti-hyperglycemic, and antimicrobial (Al-Shwyeh, 2019; Elhemeidy et al., 2018; Mia et al., 2020). In addition, other studies also mention that the content of iron and vitamin C in this fruit can help increase the synthesis of blood hemoglobin in anemia sufferers (Novadela & Imron, 2015; Susilowati, 2017).

Besides bananas and dates, honey is also used to manufacture this product. Honey is composed of various types of compounds which include carbohydrates in the form of sugar, water, vitamins, minerals, organic acids, bioactive compounds (antibacterial and antimicrobial), anti-inflammatory and enzymes (invertase, diastase, peroxide, and carbohydrate oxidase) (Almasaudi, 2021; Wirakusumah, 2010). Honey is also known for helping increase the body's hemoglobin levels through its iron and vitamin C content (Islamiyah, 2017; Lestari & Inti, 2019).

Until now, innovations in functional food products that are high in iron are generally in the form of cookies, snack bars, sorbets, sponge cakes, and similar cake products. Meanwhile, the SangKurMa snack cup is a new food product innovation high in iron and vitamin C, and this type of snack has never been made and researched before. This product is a modification of one kind of British dessert called Banoffee. As the name implies, Banoffee is made of 2 main ingredients, namely banana (bananas) and toffee (sugar boiled with butter until caramelized), as well as complementary ingredients in the form of cream, milk, and biscuit crumbs (Ayto, 2012). This snack is known for its sweet taste and high sugar and fat content. Therefore, to increase the functional value of the food, the researcher modified the Banoffee recipe by combining bananas with dates and honey into a SangKurMa snack cup which can potentially contain high levels of iron and vitamin C. Furthermore, the SangKurMa snack cup product is made without going through a cooking process and avoiding using ingredients that are inhibitors of iron and vitamin C work, so the risk of losing the nutritional content in the product is shallow.

Apart from the high content of nutrients in bananas, dates, and honey, these three ingredients are also relatively easy to find and obtain. Therefore, making SangKurMa snack cup products is also not difficult for all people to do. This product is expected to be able to provide a reference for new functional food preparations for the community in meeting the needs of iron and vitamin C. However, an organoleptic test is needed to determine public acceptance of the product. This test is one of the crucial stages in food development because it is directly related to product quality and consumer tastes (Ayustaningwarno, 2014). The quality of functional food will depend on the high component of certain nutrients and the results of this organoleptic test. Therefore, it is necessary to analyze iron and vitamin C in products made to determine their functional value and organoleptic tests to determine the preferred formula from several existing recipes through an assessment by a group of panelists on aspects of color, aroma, taste, and texture.

METHOD

The content of iron and vitamin C was analyzed on all ingredients. The bananas used were Ambon variety obtained from Gede Hardjonagoro Market, Surakarta City, Central Java. The dates used

were the Khalas variety, and the honey used was commercial forest honey. These two ingredients were obtained from the same shop in the Pasar Kliwon area, Pasar Kliwon District, Surakarta City, Central Java. Crackers used are commercial materials obtained from markets.

Materials needed in this analysis are HNO₃, NH₄SCN 1.5M, 1% starch (soluble starch), 0.01N iodine standard solution, and aqua dest. Meanwhile, the tools needed include porcelain crucible, muffle furnace, 100 ml and 125 ml Erlenmeyer, Genesys 20 visible spectrophotometry, waring blender, 100 ml volumetric flask, centrifuge Plc-03, pipette, burette, and stand. This analytical test was carried out at the Chem-Mix Laboratory of Pratama Bantul, Special Region of Yogyakarta.

The experimental design in this study was a completely randomized design (CRD) with four formulations consisting of:

- F1 = Bananas (100 g), dates (30 g) and honey (10 g)
- F2 = Bananas (110 g), dates (33 g) and honey (11 g)
- F3 = Bananas (120 g), dates (36 g) and honey (12 g)
- F4 = Banana (130 g), dates (39 g) and honey (13 g)

The tools used in making the SangKurma snack cup include digital scales, crackers crusher, 200 ml cup and lid, knife, spoon, plate, tissue, and hand gloves. All research activities were carried out in September-December 2021.

Analysis of Iron Content

Iron content was analyzed with NH₄SCN (ammonium thiocyanate) complexing based on research by Lestari (2013), which was modified using the Visible Spectrophotometry method. The stages of this analysis include (1) Weigh each sample (bananas, dates, honey, and crackers) as much as 5 g; (2) Ash in a muffle furnace; (3) Dissolve each ash with 50 ml of 1:3 HNO₃ solution while grinding in a porcelain crucible; (4) Filter the filtrate using filter paper into a 100 ml Erlenmeyer; (5) Take 1 ml of clear filtrate and add 2 ml of 1.5M NH₄SCN (if the sample contains iron, the solution will turn red); (5) Add aqua dest to a volume of 10 ml, then read the absorbance using a spectrophotometer with a wavelength of 510 nm; (6) Record the data obtained, then calculate using the standard iron curve.

Analysis of Vitamin C Content

This study's analysis of vitamin C content used the Jacobs method (iodine titration). The analytical procedures carried out include (1) Weigh 10-30 g of each sample (bananas, dates, honey, and crackers) that have been mashed; (2) Put each sample into a 100 ml volumetric flask and add aqua dest to the limit; (3) Separate the filtrate using a centrifuge; (4) Take 5-25 ml of the filtrate with a pipette and put it into a 125 ml Erlenmeyer; (5) Add 2 ml of 1% starch solution (add 20 ml of aqua dest if necessary); (6) Titrate with 0.01N iodine standard solution; (7) Perform the calculation of results (Ngginak et al., 2019). Calculation of vitamin C levels is carried out using the following formula:

$$\text{Vitamin C levels (mg/100 g)} = \frac{(\text{Vol I}_2 \times 0.88 \times \text{Fp})100}{\text{Ws}}$$

Information:

Vol I₂: Iodine volume (mL)

0.88: 1 mL of iodine solution (I₂) 0.01 N equivalent to 0.88 mg of ascorbic acid (vitamin C)

Fp: dilution factor

Ws: Sample weight (g)

Organoleptic Test

An organoleptic test is carried out as a preference (hedonic) test on aspects of the product's color, aroma, taste, and texture. This test involved 25 semi-trained panelists consisting of nutritionists and assistant chefs. Before conducting the organoleptic test, the researcher explained all the test procedures to be carried out and provided the opportunity for panelists who were willing to sign the consent form to become panelists. The assessment of the product is carried out using 9 Likert scales, which include (1) Dislike immensely (dislike very much); (2) Dislike very much; (3) Dislike moderately (dislike); (4) Dislike slightly; (5) Neither like nor dislike (neutral); (6) Like slightly; (7) Like moderately (like); (8)

Like very much (very much like); (9) Like extremely (very very like) (Pimentel et al., 2016). Furthermore, the results of the organoleptic test will be analyzed using the One Way ANOVA test. If there is a difference, it will be continued with Duncan's test.

RESULTS

The results of the analysis of the content of iron and vitamin C in each sample are as follows:

Table 1. The results of the study of the content of iron and vitamin C

Sample	Iron (mg/100 g)	Vitamin C (mg/100 g)
Banana of Ambon	8.48	70.25
Khalas dates	3.39	20.45
Forest honey	4.41	11.03
Crackers	5.48	14.12

Table 1 shows that the iron and vitamin C per 100 g ingredients are 8.48 mg and 70.25 mg in bananas, 3.39 mg and 20.45 mg in dates, 4.41 mg and 11.03 mg in honey, and 5.48 mg and 14.12 mg in crackers. The results of the analysis of iron and vitamin C content in each serving of the SangKurMa snack cup are presented in Table 2.

Table 2. Iron and Vitamin C Content of SangKurMa Snack Cup

Formula	Iron	Vitamin C	Absorbed Iron (10-15%)	% RDA Fulfillment*	
				Iron	Vitamin C
F1	10.45 mg	78.71 mg	1.05 – 1.57 mg	69.70%	104.96%
F2	11.45 mg	86.46 mg	1.15 – 1.71 mg	76.34%	115.29%
F3	12.45 mg	94.20 mg	1.25 – 1.87 mg	82.98%	125.61%
F4	13.44 mg	101.95 mg	1.34 – 2.02 mg	89.62%	135.94%

*Percentage of RDA based on energy needs 2100 kcal, iron 15 mg, and vitamin C 75 mg

Table 2 shows that the iron content per serving of SangKurMa snack cup in each formula includes 10.45 mg (F1), 11.45 mg (F2), 12.45 mg (F3), and 14.44 mg (F4). This amount can meet the individual daily iron needs of 69.70-82.98% and contribute 1.05 – 2.02 mg of absorbed iron. The amount of vitamin C per serving of SangKurMa snack cup includes 78.71 mg (F1), 86.46 mg (F2), 94.20 mg (F3), and 101.95 mg (F4). The following are the results of the statistical analysis of organoleptic (hedonic) tests in this study:

Table 3. Results of One Way ANOVA Analysis of SangKurMa Snack Cup Hedonic Test

Formulation	Hedonic Test								
	Colour SD	±	P value	Aroma ± SD	P value	Taste ± SD	P value	Texture SD	± P value
F1	6.76±1.05			6.76 ± 1.12		7.04±1.42		6.68±0.99	
F2	6.64±1.25			6.40±1.19		6.52±1.73		6.56±1.36	
F3	6.80±1.08		0.78	6.64±1.08	0.44	7.08±1.32	0.51	7.04±1.24	0.42
F4	6.96±0.93			6.88±0.88		6.92±1.29		6.96±1.06	

Table 3 shows that the results of organoleptic tests by 25 panelists on the color, taste, and texture of SangKurMa snack cups in all formulations were quite good, in the range of 6.5-7.49 (like moderately). In addition, the panelists' acceptance of the aroma of the SangKurMa snack cup formulations F2 was in the field 5.5-6.49 (light slightly), while F1, F3, and F4 were quite good, in the range of 6.5-7.49 (like moderately). The statistical analysis of organoleptic tests showed no difference in color, aroma, taste, and texture in each formula, indicated by p-values > 0.05. It shows that the panelists preferred the color, aroma, taste, and texture of the SangKurMa snack cup in the F1, F2, F3, and F4.

DISCUSSION

Iron and Vitamin C Content

Table 1 shows that the iron content in Ambon bananas is much higher than that of other banana

varieties, one of which is the Raja Sereh variety, which has an iron content of 0.1 mg/100 (Kemenkes RI, 2018). It indicates that Ambon has 84 times higher than Raja Sereh. Iron is an essential compound for the body that plays a role in synthesizing hemoproteins such as hemoglobin, cytochromes, and myoglobin. There are two types of iron in food, namely heme and nonheme. Heme iron is found in many animal products. About 50-60% of meat, fish, and poultry iron is heme iron, while the rest is nonheme.

In contrast to heme iron, nonheme iron is the most abundant iron found in plant products such as fruits, grains, vegetables, nuts, milk, and their derivatives (Gropper et al., 2016; Medeiros & Wildman, 2019). Between heme and nonheme, nonheme iron is the type of iron that is more difficult to absorb by the body. However, some chelators (small organic compounds that form complexes with metal ions) can bind to nonheme iron to help enhance (enhance) or inhibit its absorption (inhibit). One of these compounds that can increase (enhancer) iron absorption is ascorbic acid (vitamin C) (Gropper et al., 2016). The amount of vitamin C in Ambon bananas is also higher than in other varieties. However, research on the Raja Sereh variety also showed a lower vitamin C value of 2.9 mg/100 g (Wekti, 2018).

In line with the banana content, iron and vitamin C of dates tested in this study were also more significant than the results of research by Parvin et al. (2015) on three types of Tunisian dates (Trounja, Lagou, and Gounda). The study stated that the iron content in Trounja, Lagou, and Gounda dates was 1.9 mg, 1.79 mg, and 0.82 mg, while the vitamin C content was 0.9 mg, 0.7 mg, and 0.9 mg. mg per 100 g. Therefore, it shows that the iron and vitamin C content in Khalas dates is far superior. However, in contrast to research by Mariyam & Mary (2015), which states that the iron content in four varieties of dates (Ajwa, Safawi, Omani, and Tunisian), Tunisian dates contain the highest iron, which is 7.2 mg/100 g and Ajwa dates the lowest is 0.85 mg/100 g. In comparison, the Omani is 5.5 mg/100 g and Safawi 1.66 mg/100 g. Therefore, the difference in iron levels in this variety of Tunisian dates can be caused by the different types of dates studied. Based on these results, it can be seen that the content of Khalas dates is higher than that of Ajwa and Safawi dates but lower than that of the Omani and Tunisian varieties.

Another sample tested in this study was commercial forest honey. The analysis of the iron content in the tested honey was higher than in some varieties. For example, the results of the study by Hakim et al., (2021) on the content of two types of Kelulut honey (Trigona) showed that light-colored honey (low glucose) has an iron content of 0.77 mg/100 g and dark-colored honey (high glucose) has of 0.88 mg/100 g. In contrast, vitamin C contained in Kelulut honey was much higher, namely 110.88 mg/100 g (light-colored honey) and 93.28 mg/100 g (dark-colored honey) (Hakim et al., 2021).

Iron and vitamin C in crackers are higher than in similar food products. For example, the result of research on nutrient-rich cookies made from various flours (bananas, broccoli, tempeh, beef, chicken, and bran) showed that they contain iron of 4.07 mg/100 g and vitamin C of 0.68 mg/100 g (Lailiyana, 2012). However, the iron content of nutrient-rich cookies with tuna variant (tuna flour substitution) was superior to nutrient-rich cookies without tuna and commercial crackers, namely 8.67 mg/100 g. In contrast, the vitamin C content of tuna-rich cookies is much lower than commercial crackers and nutrient-rich cookies without tuna, which is 0.25 mg/100 g.

Iron can be oxidized into various forms, where the surrounding chemical environment strongly influences this condition. For example, Fe^{2+} (ferrous) and Fe^{3+} (ferric) are stable forms of iron in the body and food. Approximately 25% of heme iron from food can be directly absorbed by the intestine, while nonheme iron must be hydrolyzed in the gastrointestinal tract. This hydrolysis is greatly assisted by protease enzymes and hydrochloric acid found in the stomach. In addition, the absorption will increase if the source of iron consumes together with the enhancer. Conversely, if consuming the source of iron with foods containing inhibitors, the iron absorption in the body will be inhibited (Gropper et al., 2016).

Ascorbic acid (vitamin C) is one component that acts as an enhancer. Vitamin C can maintain iron in a suitable valence state for enzyme function by reducing ferric ions (Fe^{3+}) to ferrous (Fe^{2+}) so that the intestines will more readily absorb the iron that enters the body in the ferric form (Gropper et al., 2016; Mahardika & Zuraida, 2016). The Micronutrient Intervention Program (2002) in Briawan (2013) states that the consumption of 25-75 mg of vitamin C can increase the absorption of nonheme iron by four times.

Unlike the existing high-iron functional food products, the SangKurMa snack cup is a new high-iron and vitamin C product innovation. This product is a modification of one type of British dessert called Banoffee. This snack is famous for its sweet taste and is high in sugar and fat. To increase its functional value, the researchers modified the banoffee recipe by combining bananas with dates and honey into SangKurMa snack cups.

Table 2 shows the iron and vitamin C content per serving of SangKurMa snack cup in each formula. However, consuming iron is not fully absorbed by the intestines. The AKG (2019) states that the iron absorption needed in individuals is only around 1.9 mg/day or 10-15% of the daily intake. Therefore, the high vitamin C content in each serving of SangKurMa snack cup can meet daily vitamin C needs while increasing the absorption of nonheme iron in the product. As much as 65-74% of this absorbed iron will later use in the process of hemoglobin synthesis (Briawan, 2013).

The iron content in one serving of SangKurMa snack cup is relatively higher than some other high-iron products. For example, the results of research by Nopianti et al. (2019) stated that the iron content in the snack bar made from banana Kepok flour with spinach flour showed a value of 8.29 mg/kg or 0.29 mg per serving (35 g). Meanwhile, the iron content in steamed sponge cake substituted with beetroot flour and sesame powder as a functional food for anemia sufferers is 1.75 per serving (50 g) (Yuniantika, 2020). Furthermore, another study by Arviyani et al. (2022) regarding the manufacture of sorbet made from Moringa leaves and red guava also stated that each product serving contains 0.3 mg iron and 80.17 mg vitamin C.

Organoleptic Test

In addition to being superior in nutritional components, functional food quality also highly depends on consumer acceptance of the products. It can analyze through the results of organoleptic tests in the form of hedonic tests (liking).

Color

The results are in line with the research of Ruslan et al. (2015), which stated that there was no significant difference in the color between the modified brownie formulas of brown rice flour and dates because they used the same colored ingredients, namely chocolate. Color is crucial in attracting consumer interest in food (Nkuba et al., 2018). SangKurMa snack cup is a layered snack with a combination of brown and yellowish colors. Where the first layer's color is yellowish from crumbled crackers, the second layer is brown from honey, the third layer is yellowish from mashed bananas, and the fourth layer is brown from shredded dates.

The more using materials, the thickness of the layer and the proportion of colors will increase, but the variety of colors produced is still brown and yellowish. It is in line with the opinion of Selviyanti et al. (2019), which states that the more the proportion of ingredients added, the more dominant the resulting color will be. Therefore, making the SangKurMa snack cup using bananas and dates as ingredients with the highest proportion will produce a more dominant layer of yellowish and brown colors. Furthermore, it is in line with the opinion of Erawan et al. (2019), which states that bananas have various flesh colors ranging from white to orange and Ambon bananas have white to yellowish colors. At the same time, the color produced from the Khalas variety dates is brown but not as dark as other varieties. This opinion also follows the results of research by Al Harthi et al. (2015), which stated that Khalas dates have golden brown flesh and the lightest color compared to the other three varieties.

SangKurMa snack cup is only done by pulverizing and putting the ingredients into the cup sequentially without being homogenized so that the color in each formulation will be the same. Therefore, this product's color combination can give the panelists a positive impression. It is in line with the opinion of Sigarlaki et al. (2021), which states that attractive color combinations can increase consumer interest in a product.

Aroma

The result is in line with the research by Setyarini (2013), which stated that there was no difference in aroma in banana Ambon wet noodles in all treatments, which indicate by a p-value (0.82) >

0.05. Bananas are known as a fruit that has a sharp aroma. This opinion is under the explanation of Aurore et al. (2011) and Zhu et al. (2018), which state that bananas contain high volatiles, where most of the compounds consist of esters known to have a significant influence on the formation of aroma in bananas. The weak banana aroma produced in Ambon banana wet noodles causes by the presence of other more dominant ingredients, namely wheat flour and eggs (Setyarini, 2013). Meanwhile, in making the SangKurMa snack cup, although bananas dominate it, the distinctive aroma of bananas produced is very weak because it is in the 3rd layer after honey and covered by shredded dates, so the smell of the dates suppresses the scent. Therefore, dates have a weaker aroma than bananas. Mezroua et al. (2017) stated that the aromatic component in dates is relatively low. Amira et al. (2011) said that in dates found 80 volatile components that form aromas consisting of 20 esters, 19 alcohols, 13 aldehydes, 12 hydrocarbons, ten terpenes, and a small number of ketones and lactones. While in bananas, the volatile components reach 246 with 112 esters, 57 alcohols, 39 acid compounds, and small amounts of aldehydes and ketones (Aurore et al., 2011). It causes the aroma produced in the SangKurMa snack cup to be relatively weak (not typical of bananas/dates).

Aroma is an important factor for consumers in choosing food. The smell caught by the sense of smell can affect appetite and determine how big the first bite is (de Wijk et al., 2012; Morquecho-Campos et al., 2019). The difference in preference between panelists could be due to the weak characteristic aroma of bananas/dates, thus affecting the interest of some panelists in the product. It follows the opinion of Morquecho-Campos et al. (2019), who stated that the group of individuals who got flavored food had much higher interest than those with unscented food.

Taste

The test result is in line with Utami's research (2016) which states that there is no significant difference in the taste aspect in all formulations of functional biscuits substituted with sweet potato flour and dates because the main ingredients used contain high levels of sugar. Hence, the resulting sweet taste is not much different (Utami, 2016).

Snack cup SangKurMa is a snack with a dominant sweet taste from bananas, dates, and honey. Hasanah et al. (2017) stated that every 100 g of banana contains 4.64-5.22 g glucose, 4.55-5.12 g sucrose, and 4.89-5.53 g fructose. In contrast, the sweetness level of dates is much higher than bananas. The results of research support this statement by AlShwyeh & Almahasheer (2021) which states that the glucose content in Khalas dates ranges from 25.84-39.03 g/100 g. This variety has a lower level of sweetness than other varieties (AlShwyeh & Almahasheer, 2021). However, the glucose levels of Khalas dates reach 5-7 times higher than bananas. Simultaneously, using honey in making SangKurMa snack cups also affects the sweetness produced. It follows the opinion of Nasution et al. (2019), which states that forest honey contains 66.24-71.42% glucose and 3.04-4.51% sucrose. Therefore, The more ingredients used, the sweeter the resulting taste. Among the four formulations, F1 has the lowest level of sweetness, and F4 has the highest level of sweetness.

Although there is a difference in the weight of the ingredients between formulations, the levels are not much different. Therefore, it makes the sweetness levels produced in the F1, F2, F3, and F4 formulations are also not much different. This opinion is under the explanation by Utami (2016) that although there are differences in the concentration of ingredients in the making of sweet potato biscuits as a functional food, the combination of sweet dates produced is not significantly different.

Texture

The result is in line with the research results of Kusumastuti et al. (2022). They state that there was no significant difference in the texture aspect of the tomato velva with the addition of honey at various concentrations. Food texture can be felt as a result of sensory responses involving visual, audio, and tactile stimuli. This texture is vital in influencing consumer assessments of a food product (Chen and Rosenthal, 2015). The SangKurMa snack cup has a unique texture. It is because of the combination of crunchy from crumbled crackers, soft and sticky from shredded dates, and soft from mashed bananas. It follows the opinion of Al Harthi et al. (2015), which state that Khalas dates have a soft texture and are sticky. The high sugar content in this fruit can make the texture of dates sticky (Kamal-Eldin et al., 2020).

Using Ambon bananas with the highest proportion of ingredients also gives the product a softer texture. It is under the explanation of Hapsari & Lestari (2016), which state that Ambon banana flesh has a sweet taste and soft texture. Therefore, the more bananas and dates you use, the more pronounced the soft and sticky texture of the product will be.

Consumer satisfaction with the product depends on the texture of the food during the chewing process. Foods with a hard, thick, and thick texture are generally not liked by consumers (Forde & de Graaf, 2022). On the other hand, the SangKurMa snack cup has a soft, sticky, and crunchy texture that all the panelists like.

CONCLUSION

The analysis of iron content per 100 g of Ambon banana, Khalas dates, forest honey, and commercial crackers were 8.48 mg, 3.39 mg, 4.41 mg, and 5.48 mg, respectively. This value indicates that the iron content in the tested material is superior to several other varieties or similar materials. The vitamin C content in Ambon bananas, Khalas dates, forest honey, and commercial crackers, respectively, was 70.25 mg, 20.45 mg, 11.03 mg, and 14.12 mg. The content of vitamin C in bananas, dates, and crackers tends to be higher than in some varieties and similar ingredients. However, the content of commercial forest honey is still much lower than that of other varieties. Iron content per serving of SangKurMa snack cup in F1, F2, F3, and F4 ranged from 10.45-13.44 mg, and the vitamin C content was 78.71-101.96 mg. This value is superior to some existing high-iron and vitamin C food products. This figure shows that the SangKurMa snack cup can be used as an alternative snack to meet the daily iron needs of 69.70-82.98%, absorbed iron of 1.05-2.02 mg, and vitamin C needs of 104.96-135.94% (based on the RDA for energy needs of 2100 kcal, iron 15 mg and vitamin C 75 mg). The organoleptic test showed that the panelists preferred the F1, F2, F3, and F4 formulations on color, aroma, taste, and texture. Further research on the effects of consuming this product needs to be done to determine the clinical results, especially in the hemoglobin level of patients with anemia.

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STRESS LEVEL AND EMOTIONAL EATING RELATIONSHIP WITH RISK OF OBESITY ON FEMALE STUDENTS IN ISLAMIC BOARDING SCHOOL IN THE CITY OF BEKASI.

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Abstract

Introduction: the occurrence of stress in female students can come from oneself, the environment, and the mind. Female students who experience stress are prone to experiencing emotional eating or eating emotionally because of the stuffing emotion factor or choosing food as an alternative to relieve sadness, stress, loneliness, and other negative emotions. Female students who experience stress and emotional eating tend to choose sweet and high-fat foods in excess, thereby increasing the risk of obesity.

Method: this research is a quantitative study with a cross sectional design. This sample was taken by consecutive sampling technique with a total of 90 respondents. The population studied were female students who were active in Islamic boarding schools Annida Al-Islamy Bekasi is 14-18 years old.

Results: based on the results of the chi-square statistical test, obtained p-value = 0.027 (p-value<0.05) with an OR value (95%CI) = 3.439 for the relationship between stress levels and the risk of obesity, then obtained p-value = 0.021 (p-value<0.05) with OR (95%CI) = 3.938 for the relationship between emotional eating and the risk of obesity in female students.

Conclusion: there is a significant relationship between the level of stress and emotional eating with the risk of obesity in female students at the Annida Al-Islamy Islamic Boarding School, Bekasi City.

Key words : stress levels, Emotional eating, Risk of obesity.

INTRODUCTION

Women are 2.2 times more likely to experience stress than men (Kountul, et al, 2018). The density of activities carried out in Islamic boarding schools (Fadliansyah, 2013), can be associated with stressful events on female students (Fhonna, et al, 2020). Stress is the body's response to mental pressure or burden perceived life (Sari, and Defrin, 2015). The influencing factors are self, thoughts, and the environment (Musradinur, 2016).

Women are more at risk of experiencing emotional eating when they are bored, stressed and others (Putri, 2015). Emotional eating is an action taken by increasing food intake to coping with difficult feelings (Bennett et al., 2013), even when not hungry (Gavin 2014; in (Kustantri, 2020). This can happen because of the trigger factor, namely stuffing emotion and gender (Gavin 2014; inside (Kustantri, 2020).

Nutritional problems that can occur if a person experiences stress and emotional eating the risk of obesity. This statement is supported by the results of research conducted by Bennett, et al, (2013) found that about a third of the students studied experienced emotional eating to cope with stress and an average weight gain of 5 kg.

Obesity is a condition characterized by excessive fat accumulation in the in the body, so that it affects excess body weight and the health of the individual (Dewi,2015). Obesity is a state of overnutrition caused by various factors including stress and obesity emotional eating. In adolescence, obesity is a serious problem because it will continue into adulthood and can cause other health problems such as diabetes mellitus, hypertension, and other non-communicable

diseases (Telisa, Hartati and Haripamilu, 2020).

Based on the results of the Riskesdas survey, (2018), the prevalence of obesity in adolescents aged 16- 18 year by 13.5%. Then, the incidence of obesity in adolescents aged 16-18 years in the area of West Java Province by 4.51%. Bekasi City is included in the West Java Province which has a prevalence of obesity incidence for the group of adolescents aged 16-18 years of 4.11% (Riskesdas, 2018).

There are previous studies that link similar variables, such as research conducted by Demonti, (2020) found a significant relationship between the level of stress with the incidence of obesity, marked by the results of the study, which is about 50% of students with Level II obesity experiences severe stress.

Kustantri, (2020) also found an emotional connection eating with obesity (p value $0.007 < 0.05$). Based on Syarofi's research, (2019) which proves that there is a relationship between stress levels ($p=0.011$) and emotional eating ($p=0.000$) with the level of adequacy energy for regular nutrition students at Universitas Airlangga who are preparing a thesis.

Based on the above background, researchers are interested in conducting research with the title "The Relationship between Stress Levels and Emotional Eating with the Risk of Obesity in Female Students at Islamic Boarding Schools Bekasi city".

METHOD

The design of this research is correlational which aims to determine the relationship between two variables independent (independent) as a predictor with one dependent variable (dependent) as a criterion with approach using cross sectional. The location of this research is the Annida Al-Islamy Islamic Boarding School, Jalan IR. H. Juanda, Margahayu, East Bekasi, RT.001/RW.007, Bekasi Jaya, Kec. East Bekasi, Bekasi City, Java West 17113 and previously had never conducted a study with a similar title. Taking sample by consecutive sampling with a total sample of 90 active female students at the Annida Al . Islamic Boarding School Islamic Bekasi. There are inclusion criteria, namely, respondents aged 14-18 years, female students with active status in Annida Al-Islamy Islamic Boarding School, respondents are willing to participate in all research activities, physically and mentally healthy. There are exclusion criteria, namely female students who do not fill out the questionnaire completely.

The instrument used in this study was a questionnaire filled out by respondents online using google forms. Questionnaire for stress levels using the Perceived Stress Scale consisting of 10 questions with 5 answer criteria and interpreted according to the stress level score, that is, it says mild stress if the total score is 0-18 and severe stress if the total score is 19-40. Then, for emotional eating using the Dutch Eating Behavior Questionnaire (DEBQ) which consists of 13 questions with 5 answer criteria and interpreted according to the emotional eating score , that is, it says high if the total score is > 2.35 and it is said to be low if the total score is < 2.35 . Meanwhile, for the dependent variable (obesity risk) by direct measurement using a digital scale with an accuracy of 0.001 kg and microtoise related to the height and weight of female students at the Annida Al-Islamy Islamic Boarding School. Then interpreted using a score (BMI/U) which is said to be at risk of obesity if the score $1+ SD$ and said the not at risk if the score $< 1+ SD$.

The questionnaire used in this study has been tested for validity and reliability in Islamic boarding schools different, but have the same characteristics as those at the Annida Al-Islamy Islamic Boarding School in Bekasi namely the Modern Yapidh Islamic Boarding School in Bekasi. Both questionnaires have been declared valid based on at the r count value

(corrected item total correlation) $> r$ table of 0.361 with a significance level of $(\alpha) = 0.05$ and is said to be reliable based on the reliability coefficient or alpha $(\alpha) > 0.6$.

RESULTS

The Annida Al-Islamy Islamic Boarding School Bekasi is the place where this research was carried out, which is located in IR road. H. Juanda, RT.001/RW.007, Margahayu, East Bekasi, Bekasi City, West Java. Boarding school Annida Al-Islamy is a modern Islamic boarding school that was founded in 1963. There are various facilities and services available infrastructure for educational activities at the MTs, MA, and Tarbiyah Al High School of Sciences Marhalah Al Ulya with a total number of students as many as 660 students and the number of educational staff as many as 50 people. The boarding school is led by the head of the madrasa and there are teachers who competent in teaching various subjects according to the curriculum and religious knowledge.

Univariate Analysis

The univariate analysis was carried out with the aim of looking at the frequency distribution of the variable the characteristics of the respondents include age and grade level, then frequency stress levels, emotional eating, and the risk of obesity. Respondents in this study were students active daughter at the Annida Al-Islamy Islamic Boarding School in Bekasi.

Characteristics of Respondents

Grouped by age and grade level can be seen in table 3.

Table 1. Frequency Distribution of Respondents' Characteristics of Female Students at Pesantren Annida Al-Islamy Bekasi in 2022

Characteristics of respondents	N	%
Age		
15 years	20	22,2
16 years	29	32,2
17 years	25	27,8
18 years	16	17,8
Class Level		
10	29	32,2
11	31	34,4
12	30	33,3

Source: Primary Data (2022); N=90

Based on table 1 it can be seen that, most of the respondents are 16 years old, that is as many as 29 respondents (32.2%) of a total of 90 female students and most of the respondents are at the 11th grade level, as many as 31 respondents (34.4%) out of a total of 90 female students.

Stress Level

The frequency distribution of respondents' stress levels can be seen in Table 2.

Tabel 2. Frequency Distribution of Stress Levels for Female Santri in Islamic Boarding Schools Annida Al-Islamy Bekasi in 2022

Stress Level	N	%
Heavy stress	72	80
Mild stress	18	20

Source: Primary Data (2022); N=90

Based on table 2, it can be seen that, most of the respondents with high stress weight, as many as 72 respondents (80%) of a total of 90 female students.

Emotional Eating

Emotional eating frequency distribution of respondents can be seen in Table 3.

Table 3. Distribution of Emotional Eating Frequency among female students in Islamic boarding schools Annida Al-Islamy Bekasi in 2022

Emotional Eating	N	%
High	74	82,2
Low	16	17,8

Source: Primary Data (2022); N=90

Based on table 3, it can be seen that some respondents have emotional eating which is high, as many as 74 respondents (82.2%) of a total of 90 female students.

Obesity Risk

The frequency distribution of respondents' obesity risk can be seen in Table 4.

Table 4. Frequency Distribution of Obesity Risk in Female Santri at Pesantren Annida Al-Islamy Bekasi in 2022.

Obesity Risk	N	%
At Risk	46	51,1
No Risk	44	48,9

Source: Primary Data (2022); N=90

Based on table 4, it can be seen that some respondents are at risk of obesity, namely as many as 46 respondents (51.1%) from a total of 90 female students.

Bivariate Analysis

The results of the relationship between stress levels and the risk of obesity can be seen in table 5.

Table 5. Analysis of the Relationship between Stress Levels and Obesity Risk in Female Santri at the Annida Al-Islamy Islamic Boarding School Bekasi.

Stress Level	Risk of Obesity				OR (95% CI)	p-value
	At risk		No risk			
	N	%	n	%		
Heavy Stress	41	45,6	31	34,4	3, ,439 (1,109-10,666)	0,027
Mild Stress	5	5,6	13	14,4		

Source: Primary Data (2022); N=90

Based on table 5 it is known that, the results of the analysis of the relationship between stress levels and The risk of obesity can be said as many as 41 respondents (45.6%) with severe stress levels have a risk of obesity. While respondents with mild stress levels were 5 respondents (5.6%) are at risk for obesity. Based on the results of the Chi square statistical test , p-value = 0.027 (p <0.05). That matter shows that H_0 is rejected, so there is a significant relationship between stress levels with the risk of obesity in female students at the Annida Al-Islamy Islamic Boarding School Bekasi. From the results of the analysis also obtained the value of OR = 3.439. So that it can be said that respondents with severe stress levels 3,439 times the risk of obesity compared to mild stress levels. The results of the relationship between emotional eating and the risk of obesity can be seen in table 6.

Table 6. Analysis of the Relationship between Emotional Eating and the Risk of Obesity in female students at the Annida Al-Islamy Islamic Boarding School Bekasi.

Emotional Eating	Risk of Obesity				OR (95% CI)	p-value
	At risk		No risk			
	N	%	n	%		
High	42	46,7	32	35,6	3,038	

Low	4	4,4	12	13,3	(1,161- 13,357)	0,021

Source: Primary Data (2022); N=90

Based on table 6, it is known that, the results of the analysis of the relationship between emotional eating with the risk of obesity, it can be said as many as 42 respondents (46.7%) with emotional eating have a high risk of obesity. Meanwhile, respondents with low emotional eating were 4 respondents (4.4%) have a risk of obesity. Based on the results of the Chi square statistical test, $p\text{-value} = 0.021$ ($p < 0.05$). That matter shows H_0 is rejected, so there is a significant relationship between emotional eating and the risk of obesity in female students at the Annida Al-Islamy Islamic Boarding School Bekasi. From the results of the analysis too obtained the value of $OR = 3.938$. So it can be said that respondents with high emotional eating 3,938 times more likely to have obesity risk compared to low emotional eating.

DISCUSSION

Characteristics of Respondents

Respondents in this study were female students at the Annida Al-Islamy Islamic Boarding School Bekasi, as many as 90 active female students who are willing to be research respondents. Characteristic data Respondents taken in this study were age and grade level.

For characteristics based on age, the results obtained are the most female students who become respondents in this study were 16 years old. Then, for characteristics based on the class level, the results showed that the most female students were in class 11 who became respondents in this research.

The results of this study are in line with the results of research from Rahmawati, (2016) which states: found that, demographic characteristics by age who experienced severe stress Most were aged 16-18 (60.3%) out of a total of 237 respondents at SMA Negeri 23 West Jakarta.

The age of 16 is the age of transition from adolescence to adulthood. In phase there are many new challenges to be experienced. So if female students don't face these challenges well, you will easily experience stress. In this phase, female students will experience increased feelings of loneliness, anxiety and depression. This increases the risk of female students experiencing emotional eating as an alternative to calm down. This opinion is in accordance with Romadhoni, (2019) which states that ages 14-19 years, including the age of transition from adolescence to adulthood. Then reinforced with opinion of Kaligis, (2021), which states that in the transition phase, the individual will have new experiences and challenges and experience increased feelings of loneliness, stress, anxiety, and depression in dealing with the problems that occur.

Stress level

In this study, the stress level was measured on 90 active female students in Islamic boarding schools Annida Al-Islamy Bekasi uses a Perceived Scale Stress (PSS) questionnaire with a total of 10. The questions consist of 6 negative questions and 4 positive questions that have been validated and reliable. The results obtained are most of the female students at the Annida Al-Islamy Islamic Boarding School with severe stress level.

The results of this study are in line with the results of Saputri's research, (2017) which found that From a total sample of 245 students at the Darul Ihsan Islamic Boarding School, Aceh City, there were 122 students experiencing severe stress. Another researcher, Dyah, (2020) also

found that out of a total of 100 students, girls at the Raudlotul Huda Islamic Boarding School, Temanggung Regency, there are 5 female students experiencing stress heavy.

Stress is a condition that results from several factors or what is called a stressor. so that individuals who experience stress will feel depressed. This is in accordance with the opinion from Barseli, Ifdil and Nikmarijal, (2017) which states that stress is defined as a depressed condition experienced by a person because of a mismatch between demands and abilities possessed Thus, resulting in mental stress or the burden of life perceived in the individual (Sari, Nurdin and Defrin, 2015).

Stressors can come from the environment, oneself, or from the mind of the individual. These three factors play an important role as a trigger for stress. Stress caused by environment could be due to demands from parents, teachers and themselves who requires to be positive in the environment and obedient in meeting all demands. Thus, the burden on the individual's mind increases and triggers stress. This is in line with the opinion of Musradinur, (2016) which states that there are environmental demands, self, and thoughts can trigger stress. Other researchers namely Lazarus and Folkman (2018); in Budianto, et al (2021) also stated that stress Internal factors result from physical, environmental, and social demands.

The level of severe stress that occurs in female students at Annida Al-Islamy can be caused by learning activities that are too dense, regulations that are too strict in pesantren, academic and non-academic activities, coupled with religious activities, as well as the obligation of female students to make a memorizing deposit of the Al-Quran letter to the teacher. Then, the female students also feel sad because they are far from their parents and family as well sometimes there are differences between friends because of differences of opinion. those factors that This makes female students complain a lot and are prone to experiencing severe stress.

This opinion is in line with the opinion of Fauziah, (2021) which states that and learning applied in Islamic boarding schools such as being required to memorize the Koran and hadith as well as other regulations that must be obeyed and implemented, so that students are vulnerable to stress. Reinforced by the opinion of (Laily, 2019) who found that the factors causing stress on female students at Al-Falah Islamic Boarding School, Kediri City, which comes from internal and external such as lack of parental love and differences between students.

Emotional eating

In this study, measurements of emotional eating were carried out on 90 active female students in Pesantren Annida Al-Islamy Bekasi using the Dutch Eating Behavior Questionnaire (DEBQ) with a total of 13 questions that have been validated and reliable. Obtained the results of most female students at the Annida Al-Islamy Islamic Boarding School with high emotional eating. The results in this study are in line with the results of research from Ramadhani and Mahmudiono, (2021) who found that from a total sample of 133 students aged 15-17 years in SMA6 Surabaya, there are 68 students (51.1%) experiencing emotional eating. Then, some students think that eating can improve mood and minimize discomfort. Emotional eating or interpreted as eating emotionally is an individual act in consuming food as an alternative to calm oneself from negative emotions such as stress, anxiety, and depression, even when not hungry.

High emotional eating in female students at the Annida Al-Islamy Islamic Boarding School can this is because they are susceptible to negative emotions such as stress, anxiety, and depression. These negative emotions can be triggered from various factors, such as the demands of parents who requires female students to get satisfactory grades, the demands of teachers who

requires female students to memorize the Al-Quran and Hadith, then there is a test that will take place had to be faced by female students resulted in increased feelings of anxiety, stress, and depression. Thus, they choose to eat mainly sweet and fried foods as a temporary way to calm down even if you're not hungry.

In line with the opinion stated by Bennett, et al, (2013) that Emotional eating is a negative action due to an increase in food intake to overcome feelings the hard one. Reinforced by the opinion of (Al-Musharaf, 2020) which states that emotional eating is an individual's tendency to overeat as a coping mechanisms in regulating stress, anxiety, and depression.

Risk of Obesity

In this study, the risk of obesity was measured in 90 active female students in Islamic boarding schools Annida Al-Islamy Bekasi uses anthropometric measurements consisting of body weight and height, then interpreted using the category z-score calculation (BMI/U) for determine whether the respondent is at risk of obesity or not. Thus, we get a partial result Most female students at the Annida Al-Islamy Islamic Boarding School are at risk of obesity.

The results of this study are in line with the results of research from Puspitasari, (2018) which found that women are 1.7 times more likely to be obese ($p=0.001$) than men. Results from Nugroho and Sudirman, (2020) also found the risk of obesity in 416 adolescents and young adults aged 13-24 years in Samarinda City.

The risk of obesity that occurs in female students at the Annida Al-Islamy Islamic Boarding School can be caused by several factors, such as stress that causes excessive hunger and there are factors that cause obesity. Supporting factors are physical activity factors and age factors. The female students admitted that they liked it consuming sweet foods, snacks, fried foods, and fast food. Thing This can be a trigger for the risk of obesity because the students liketo consume sweet foods and high in fat in the long term to relieve yourself from stress.

According to the Indonesian Ministry of Health, (2019) there are risky foods such as food and drinks sweet, salty foods, instant foods, fried and fatty foods. According to Ramadhani, (2021) if consuming these risky foods can increase the risk of obesity. Sigh, et al, (2020) also added that by consuming fast food, or fast food and snacks such as chips can increase the risk of obesity.

Then from the physical activity factor, female students admitted that they rarely do sports, they only doing sedentary physical activity in accordance with daily conditions such as cleaning room, study, and recite. This opinion is supported by Shadrina, (2017) who found that light physical activity dominates around 105 students (43.9%) of the 239 students at Pesantren X Bogor city. Ainun et al, (2021) also added that the findings showed that the activity of sedentary is the most practiced by young women at the Darul Aman Gombara Islamic Boarding School, Makassar namely reciting, studying, and dhikr.

Then from the age factor, female students aged 14-18 years are included in the mass group vulnerable adolescents are at risk of obesity. Because according to Juniarta and Darmayanti, (2020) during adolescence, there is an increased need for nutritional intake for development her body. However, if the intake of nutrients is excessive and uncontrolled, it can increase obesity risk. Sugiatmi and Handayani, (2018) added that during adolescence the number of fat in women increases twice as much as to prepare for pregnancy, so women are more likely to be obese. In adolescence, obesity is a problem serious because it will continue into adulthood (Telisa, Hartati and Haripamilu, 2020).

The Relationship Between Stress Levels and The Risk of Obesity in Female Students in

Islamic Boarding Schools Annida Al-Islamy Bekasi

Based on the results of the analysis of the relationship between stress levels and the risk of obesity in female students at Pesantren Annida Al-Islamy Bekasi which can be seen in table 5 is known that, p-value of 0.027 ($p < 0.05$), so it can be said that there is a relationship between stress levels and risk obesity in female students at the Annida Al-Islamy Islamic Boarding School Bekasi. From the results of the analysis, it can be seen that the value of OR = 3.439 which means that female students with severe stress levels 3,439 times the chance to have obesity risk compared to female students with heavy stress.

The results of this study are in line with the results of research from Masdar et al., (2016) who found a significant relationship between stress and obesity ($p = 0.028$) (OR = 0.443) in 132 students from SMA Negeri in Pekanbaru City. Then, reinforced by the results of research from Mohammad and Dasuki, (2021) who found that there was a relationship between stress levels and obesity ($p=0.016$) in 60 young women aged 15-18 years in Sukoharjo City.

The results of this study and the results of previous studies prove that there is relationship between stress levels with the risk of obesity in the group of adolescent girls. This statement is corroborated by Nurrahmawati and Fatmaningrum, (2018) which states that, the higher the level of the stress experienced, the higher the risk of obesity in the individual.

There is a difference between the environment in the pesantren and the environment outside the pesantren. This is the reason why young women are vulnerable to stress. Before entering the boarding school, young women have unlimited activities, activities that are not too crowded and feel the facilities adequate house. Meanwhile, after they enter the pesantren environment, young women are required to be able to become independent students, able to participate in all activities and activities dense, and must be able to accept all the facilities provided by the pesantren.

The statement above is in accordance with the opinion of Suharsono and Anwar, (2020) which states that, every female student has differences in dealing with environmental conditions such as a new place to live, new friends and a new culture. According to Rahmawati and Hadiansyah, (2021) states that, if the individual is not able to adjust well the individual is susceptible to stress.

The stress experienced by female students can also come from mental pressure such as there is pressure from parents of female students who demand their children to get grades good academic, there is pressure in facing academic exams and religious exams such as depositing the memorization of the Qur'an and hadith, then there is a difference between friends because competition, and sometimes feelings of sadness arise because they are far from both parents and family.

This statement is in accordance with the opinion of Musradinur, (2016) which states, that the demands of the environment, self, and thoughts can trigger stress. Basar, Indriasari and Battung, (2020) also added that stressors can come from internal such as health and mental conditions, emotional control, etc. as well as external such as environmental conditions, family problems, academic environment, friends, the learning process, etc (Basar, Indriasari and Battung, 2020).

Female students who experience stress will easily feel hungry. Because in times of stress, the body will release the hormone cortisol which has an impact on increasing hunger. Hormone Cortisol is a hormone that functions in metabolism to digest sugar and fat be a source of energy. According to Gavin (2014) in Kustantri (2020) states that when the body is in a state of stress, the body needs a lot of energy.

The role of the hormone cortisol in these situations is to trigger the desire to consume foods that contain sugar and fat to be used as a source of energy resulting in excessive hunger resulting in excessive eating patterns, especially high desire to consume sweet foods and high-fat foods. According to Gavin (2014) in Kustantri (2020), with the increase in the hormone cortisol, this results in high activity of lipoprotein lipase in adipose tissue. So that it can increase fat stores in the body.

Based on this theory, female students with severe stress will experience an increase in energy caused by the hormone cortisol so female students tend to choose food sweet and high in fat. Then, if the food is not controlled both in terms of quantity and quality, then the role of lipoprotein lipase hormone is more active to store fat in the body, so female students are at high risk of obesity.

This statement is also proven by the results of research by Ramadhani and Mahmudiono (2021) which states that there is a relationship between stress and increased food consumption fast food, dairy products and sweet foods in adolescents. This statement is reinforced by Lubis, (2021), which states that if a person cannot control food either from in terms of the amount and type of food such as fatty and sweet foods, then a person it will be easy to become obese.

The relationship between emotional eating and the risk of obesity in female students in Islamic boarding schools Annida Al-Islamy Bekasi

Based on the results of the analysis of the relationship between emotional eating and the risk of obesity in female students at the Annida Al-Islamy Islamic Boarding School in Bekasi, which can be seen in table 6, it is known that, the p-value of 0.021 ($p < 0.05$), so it can be said that there is a relationship between emotional eating and the risk of obesity in female students at the Annida Al-Islamy Islamic Boarding School Bekasi. From the results of the analysis too it can be seen that the OR value is OR = which means that female students with emotional eating high has a 3,938 times chance of having a risk of obesity compared to female students with low emotional eating.

The results of this study are in line with the results of research by Kustantri, et al, (2020) who found there is a relationship between emotional eating and the incidence of obesity in regional health center officers District Manyar Gresik Regency that is equal to 48.2%. Supported by research results from Shriver et al, (2021) who found a positive relationship between emotional eating and incidence of obesity in adolescents.

The incident of emotional eating in female students at the Annida Al-Islamy Islamic boarding school in Bekasi was triggered by the perceived negative emotions and the stuffing emotion factor. Stuffing emotions is the action of female students who tend to choose food as a temporary alternative to relieve yourself of negative emotions. This is evidenced from the results of the answers to the questionnaire that some female students answered that when they were upset, anxious, bored, depressed, anxious, lonely and disappointed, they tend to prefer eating as a way to calm yourself, especially sweet foods even though you are not hungry.

In accordance with the opinion of Gavin (2014) in Kustantri (2020) which states that stuffing emotion is someone who thinks that with food is the way temporarily to relieve stress, discomfort, anger, fear, sadness, anxiety, hatred, lonely and shy. Someone who has such an assumption will focus on consuming food even if you are not hungry, so it is very risky for obesity. This statement is supported by Wijayanti et al, (2019) who stated that emotional eating is an example of unhealthy eating behavior that can affect weight gain body.

There is another factor in emotional eating, namely gender. Women are more at risk experience emotional eating when bored, stressed and others (Putri, 2015). So that it can be said that women have a stronger urge to eat when they experience negative emotions such as stress, depression, boredom and others than men. This opinion is supported by the opinion of Hamilton and Fagot in Gryzela and Ariana, (2021) which states that women are more dominant in using emotion focused coping than men who tend to use problem focused coping.

According to Morin, (2021) *problem focused coping* is when a person directly face the source of the problem and solve it and eliminate everything that makes stress, while *emotion focused coping* is when a person only focuses on maintaining feelings and emotions without any desire to change the situation that is causing the problem and stress. Thus, women tend to choose food as an alternative to maintain emotional feeling.

These two factors are the basis for female students at the Annida Al-Islamy Islamic Boarding school Bekasi has *emotional eating*, which puts female students at risk of obesity. If there is no will to change the situation, then the risk of obesity will be higher. This thing is supported by Frayn et al, (2018) which states that poor coping skills effective for dealing with negative emotions such as eating more emotionally consistently associated with obesity.

CONCLUSION

There are several conclusions in this study entitled "Stress Levels And Emotional Eating Relationship With Risk Of Obesity On Female Students In Islamic Boarding School In The City Of Bekasi" as following:

1. Characteristics of respondents in this study were obtained from a total of 90 female students active female students at the Annida Al-Islamy Islamic Boarding School Bekasi are dominated by female students who is 16 years old and is in 11th grade on average.
2. Respondents in this study experienced a level of severe stress as many as 72 respondents of the total active respondents as many as 90 female students caused by the environment, self themselves, and thoughts.
3. Respondents in this study on average experienced high *emotional eating* as much as 74 respondents from the total active respondents as many as 90 female students caused by santri daughters are susceptible to negative emotions and the presence of a stuffing emotion factor.
4. Respondents in this study had an average risk of obesity as many as 46 respondents Of the total active respondents, 90 female students were caused by female students experience stress that leads to overeating (*emotional eating*) and Supporting factors are physical activity factors and age factors.
5. There is a significant relationship between stress levels and the risk of obesity in female students at Pesantren Annida Al-Islamy Bekasi with *p-value* = 0.027 ($p < 0.05$) and OR = 3.439.
6. There is a significant relationship between *emotional eating* and the risk of obesity in students girls at the Annida Al-Islamy Islamic Boarding School Bekasi with *p-value* = 0.021 ($p < 0.05$) and OR = 3,938.

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PRODUCT DEVELOPMENT, NUTRITIONAL CONTENT, AND DIETARY FIBER KIDNEY BEANS TEMPEH (*Phaseolus vulgaris* L.) AS FUNCTIONAL FOOD

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Abstract

Introduction: The trend of vegetarian food consumption is currently increasingly in demand by the public for various reasons such as belief, the need for a healthy life, to allergies to certain animal foods. A vegetarian diet has several health benefits such as lowering the risk of cardiovascular disease, obesity, and high blood pressure. This is because the vegan group has a food consumption pattern that does not eat animal meat at all so it tends to be low in fat, low in cholesterol and high in fiber. Processing kidney beans by fermentation into tempeh as one of the foods has several benefits, such as increasing dissolved protein levels and nutritional quality. The aims of this research is to study the process of making kidney beans tempeh (*Phaseolus vulgaris* L.) and analyzing the chemical properties (water content, ash, protein, fat, carbohydrates and dietary fiber) of kidney bean tempeh (*Phaseolus vulgaris* L.).

Method: The study used an experimental design that was carried out for 7 months, namely August 2017 to September 2017 and December 2017 to April 2018.

Results: Kidney beans tempeh (*Phaseolus vulgaris* L.) contains 61.08 (% w/w wb) of moisture and 0.55, 0.69, 12.62, 25.06, and 1.85 (% w/w wb) of ash, fat, protein, carbohydrate, and dietary fiber respectively.

Conclusion: Kidney beans tempeh (*Phaseolus vulgaris* L.) has potential as a functional food and a type of food for a vegetarian diet

Key words : degenerative disease, fermentation, functional food, kidney beans tempeh (*Phaseolus vulgaris* L.)

INTRODUCTION

The trend of vegetarian food consumption is currently increasingly in demand by the public for various reasons such as belief, the need for a healthy life, to allergies to certain animal foods. According to Key et al. (2006), a vegetarian is someone who does not eat meat, poultry, or fish. A vegetarian diet has several health benefits such as lowering the risk of cardiovascular disease, obesity, and high blood pressure. This is because the vegan group has a food consumption pattern that does not consume animal meat at all so that it tends to be low in fat, low in cholesterol and high in fiber (Craig 2009). Some of the foodstuffs commonly consumed by the vegetarian group are vegetables, fruits, nuts, seeds, and grains.

One of the beans that is widely developed in the manufacture of vegetarian patties is kidney beans. Kidney beans are food ingredients that have a hypocholesterolemic effect because they contain dietary fiber and flavonoids, namely proanthocyanidins and isoflavones (Nakamura *et al.* 2010). Processing kidney beans by fermentation into tempeh as one of the raw materials for vegetarian patties has several benefits such as increasing dissolved protein levels and nutritional quality. Dissolved protein shows the level of water-soluble protein so that the protein is easily digested (Purwoko and Handajani 2007). Fermentation produces proteolytic enzymes by molds that break down proteins into amino acids so that the dissolved nitrogen increases and decreases antinutrient compounds and oligosaccharides (Susi 2012).

Therefore, the development of kidney bean tempeh (*Phaseolus vulgaris* L.) needs to be done as an effort to develop products that are useful in terms of nutritional content, have potential as functional food products and food for vegetarians.

METHOD

Place and Time of Research

The research was conducted for 7 months, namely August 2017 to September 2017 and December 2017 to April 2018. Trial and error of product manufacture was carried out at the Food Experimental Laboratory, Department of Community Nutrition, FEMA IPB. The organoleptic test of the product was carried out at the Organoleptic Laboratory, Department of Community Nutrition, FEMA IPB. Proximate and dietary fiber analyzes were carried out in the Laboratory of Biochemistry and Micronutrient Analysis, Department of Community Nutrition, FEMA IPB. Mineral and amino acid analysis was carried out at the Integrated Chemistry Laboratory, Baranangsiang Campus of IPB.

Materials and Tools

The main ingredient in this study was kidney bean (*Phaseolus vulgaris* L.) which was obtained from Pasar Anyar, Bogor Regency. Inoculants for kidney bean fermentation were obtained from Tegal Regency, Central Java. In addition, the materials used for chemical analysis include oil paper, concentrated H₂SO₄, selenium mix, H₃BO₃ 3%, Methyl Red Indicator: Methyl Blue, 30% NaOH, red litmus paper, 0.1 M HCl, cotton, technical hexane, Na₂HPO₄ · 2H₂O, NaHPO₄ · 2H₂O, 1 N NaOH, tarmamyl enzyme, 1 M HCl, pepsin enzyme, pancreatin enzyme, 95% ethanol, acetone, HNO₃, deionized water, distilled water, orthohtaldehide, brij-30 30% solution 2-mercaptoethanol, aqueous solution standard amino acid 0.5 mol/mL, Na-EDTA, methanol, tetrahydrofuran (ENT), Na-acetate, and high pure aquadest.

The Making of Kidney Beans Tempeh (*Phaseolus vulgaris* L.)

The kidney beans used were kidney beans obtained from Pasar Anyar, Bogor Regency. The basic principles in making tempeh consist of cleaning, washing, boiling, soaking, washing, adding inoculants, packaging, and fermentation. The procedure for making kidney bean tempeh in this study refers to the procedure for making soybean tempeh in Utari's research (2011) with modifications.

Analysis of the Nutritional Content Kidney Beans Tempeh (*Phaseolus vulgaris* L.)

The nutritional content analysis carried out on kidney bean tempeh was in the form of water content analysis using the gravimetric method (AOAC 2005), ash content using the gravimetric method (AOAC 2005), fat content using the Soxhlet method (AOAC 2005), protein content using the micro kjeldahl method (AOAC 2005), and protein content using the micro-kjeldahl method (AOAC 2005). carbohydrate by difference method (AOAC 2005). In addition, another analysis carried out on kidney bean tempeh was the enzymatic method of food fiber analysis (AOAC 2005).

RESULTS

The Making of Kidney Beans Tempeh (*Phaseolus vulgaris* L.)

Kidney beans are food ingredients that have a hypocholesterolemic effect because they contain dietary fiber and flavonoids, namely proanthocyanidins and isoflavones (Nakamura *et al.* 2010). Proanthocyanidins have a function in lowering cholesterol levels by inhibiting lipase activity (Boateng *et al.* 2008). According to Santoso (2011), dietary fiber provides health benefits such as controlling weight or controlling diabetes, preventing gastrointestinal disorders, colon cancer, and reducing blood cholesterol levels and cardiovascular disease.

Tempe is one of the food ingredients from soybeans that have gone through a fermentation process. Processing kidney beans by fermentation into tempeh as one of the raw materials for vegetarian patties has several benefits such as increasing dissolved protein levels and nutritional quality. Dissolved protein shows the level of water-soluble protein so that the protein is easily digested (Purwoko and Handajani 2007).

Fermentation produces proteolytic enzymes by molds that break down proteins into amino acids so that the dissolved nitrogen increases and decreases antinutrient compounds and oligosaccharides (Susi

2012). The procedure for making kidney bean tempeh in this study refers to the procedure for making soybean tempeh in Utari's research (2011) with modifications. The stages of making kidney bean tempeh consist of cleaning, washing, boiling, soaking, washing, adding inoculants (yeast), packaging, and the fermentation process. The stages of the process of making kidney bean tempeh can be briefly seen in Figure 1.

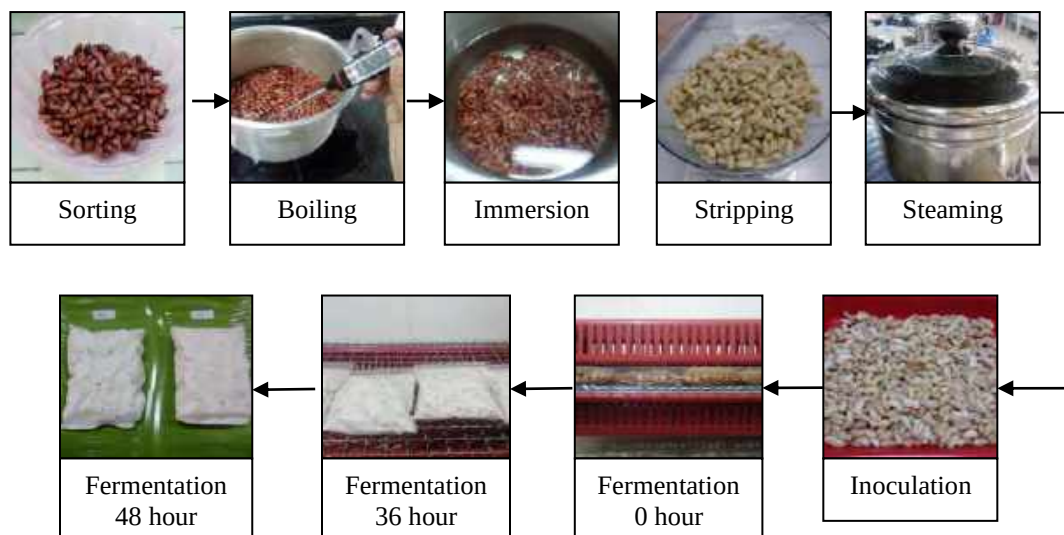


Figure 1. The Making of Kidney Beans Tempeh (*Phaseolus vulgaris* L.)

Kidney beans tempeh was then analyzed for its nutritional content, including water content, ash, fat, protein, carbohydrates, and dietary fiber. The results of the analysis were then compared with the SNI for soybean tempe as a food ingredient (SNI 01-3144-2009). The following is Table 1 which shows the results of the chemical analysis of kidney bean tempeh per 100 grams.

Table 2 Nutritional content and dietary fiber of kidney bean tempeh		
Nutritional content	Kidney beans tempeh	SNI of soybeans tempeh
Moisture(%wb)	61.08±0.11	Maks 65
Ash (%wb)	0.55±0.50	Maks 1.5
Fat (%wb)	0.69±0.01	Min 10
Protein (%wb)	12.62±0.60	Min 16
Carbohydratet (%wb)	25.06±0.54	-
Dietary fiber (%wb)	1.85±0.14	-

DISCUSSION

The making of kidney beans tempeh begins with sorting and washing to obtain good quality kidney beans. The next stage is boiling when the water temperature has reached 90-95°C with a ratio between kidney beans and water of 1:3 for 10 minutes. This boiling is intended so that the kidney beans can absorb as much water as possible so that the texture becomes softer and kills contaminant microorganisms found in kidney beans. Next, kidney beans were soaked in water that had been added with vinegar (2% glacial acetic acid and 98% water) as much as 10% of the total volume of water used during soaking to produce a pH value of 3.94-5.01 for 7 hours. The pH value of the soaking water was in accordance with the required pH value for the growth of tempeh molds, namely 3.5-5.2 (Sapuan & Noer 2001). The addition of vinegar is useful for shortening the immersion time and helping the growth of bacteria for the synthesis of vitamin B2, vitamin B6, vitamin B12, niacin, biotin, folic acid and

pantothenic acid (Herman & Karmini 1999).

After soaking for 7 hours, the kidney bean skin was peeled to facilitate the growth of mycelium during the fermentation process. The kidney beans that have been removed from the skin are then washed thoroughly to remove the mucus on the kidney beans, then steamed for 15 minutes after the steaming water boils at a temperature of 90-95°C so that the texture becomes softer and cleaner. The steamed kidney beans were then air-dried at room temperature for 30 minutes before the fermentation process. The next stage is sowing yeast (inoculants) as much as 0.2% of the weight of kidney beans after steaming. The addition of 0.2% yeast refers to the addition of soy yeast made by soybean tempeh craftsmen in Bogor Regency as much as 1 tablespoon (equivalent to $\pm$ 10 grams) for every 5 kg of soybeans after steaming and cooling. So that after being converted it produces a conversion factor of adding yeast of 0.2% by weight of kidney beans after steaming and cooling.

After the addition of yeast, kidney beans are packaged using food-grade polyethylene plastic because it is safe, inert to food, permeable to oxygen so that it can support mold growth (Dirim *et al.* 2004). Packaging using this plastic also aims to monitor the growth of mycelium in kidney beans during the fermentation process. The successful process of making kidney bean tempeh is characterized by a good appearance, a compact texture, and a white mycelium that covers the entire surface of the tempeh. The growth of mold mycelium is influenced by the type of mold used, soluble viability, temperature, and pH (de Reu *et al.* 1993).

This study used 200 grams of kidney beans to produce as much as (339 $\pm$ 1.41) grams of kidney bean tempeh. The yield was obtained from the ratio of the weight of kidney bean tempeh produced to the total dry kidney beans before fermentation. The yield of kidney bean tempeh was (169.5 $\pm$ 0.71) %. According to Shurtleff & Aoyagi (1979), during the fermentation process, mycelium growth occurs which is indicated by the penetration of the mold mycelium into the bean and covers the beans, causing an increase in final weight.

The moisture content in foodstuffs determines the acceptability, freshness and durability of these foodstuffs (Winarno 2008). The average water content of kidney bean tempeh is 61.08%. According to the Ministry of Health (1992) the water content of whole kidney beans is 12.00%. The water content of kidney beans increased when the fermentation process was carried out. During the fermentation process, the release of water vapor by molds occurs as a result of the decomposition of complex compounds that are blocked by plastic so that the water content of tempeh will increase (Susi 2012). According to BSN (2009), the maximum water content in soybean tempeh is 65%. This shows that the water content of kidney bean tempeh is in accordance with the BSN requirements for the water content of soybean tempeh.

The ash content of kidney bean tempeh is 0.55%. When compared with the ash content of soybean tempeh, which is a maximum of 1.50% (BSN 2009), then the ash content of kidney bean tempeh is in accordance with the BSN requirements for the ash content of soybean tempeh. The ash content in food shows its mineral content. The low ash content in kidney bean tempeh indicates low mineral content in kidney bean tempeh.

The fat content of kidney bean tempeh is 0.69%. When compared with the fat content in soybean tempeh, which is at least 10% (BSN 2009), then the fat content in kidney bean tempeh is below the BSN requirements for the fat content of soybean tempeh. Kidney beans have a fat content of 1.5% (Astawan 2009) to 1.7% (Ministry of Health 1992).

Kidney beans have a lower protein content when compared to soybeans, which is 23% (Ministry of Health 1992). Protein content in kidney beans that have been fermented into tempeh has increased to 12.62% (32.35% db). According to Susi (2012), during the fermentation process, molds produce proteolytic enzymes that break down proteins into amino acids so that the dissolved nitrogen increases. When compared with the protein content in soybean tempeh, which is at least 16% (BSN 2009), then the protein content in kidney bean tempeh is below the BSN requirement for soy tempeh protein content.

The carbohydrate content of kidney bean tempeh was calculated using the by difference method. Calculation of carbohydrate content by difference is done by subtracting the number one hundred with

the moisture content, ash content, fat content, and protein content. The carbohydrate content of kidney bean tempeh in this study was 25.06%.

Total dietary fiber (total dietary fiber, TDF) consists of components of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) (Muchtadi 1989). According to Qi *et al.* (2011), water soluble dietary fiber is fiber that can dissolve in water such as pectin and carrageenan. Meanwhile, water insoluble dietary fiber consists of cellulose, hemicellulose, lignin, and chitosan. The dietary fiber content in kidney bean tempeh is 4.74%.

CONCLUSION

Kidney beans tempeh (*Phaseolus vulgaris* L.) contains 61.08 (% w/w wb) of moisture and 0.55, 0.69, 12.62, 25.06, and 1.85 (% w/w wb) of ash, fat, protein, carbohydrate, and dietary fiber respectively. Kidney beans tempeh (*Phaseolus vulgaris* L.) has potential as a functional food and a type of food for a vegetarian diet. Research related to consumer acceptance and product shelf life needs to be done in order to provide a more optimal picture when consumed by the wider community.

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ANTIBACTERIAL EFFECT OF AMLAKI (*PHYLLANTHUS EMBLICA*) EXTRACT AGAINST *PSEUDOMONAS AERUGINOSA*

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Abstract

Introduction: Natural herbal remedies have shown promising antimicrobial properties and fewer side effects compared to synthetic. In this regard, medicinal plants, *Phyllanthus emblica* (Amlaki) was investigated for potential antibacterial effect against the most common nosocomial pathogen (*Pseudomonas aeruginosa*). The experimental study was to determine the antibacterial effect of aqueous and ethanolic extract of *P. emblica* against standard strains of *P. aeruginosa*.

Method: The study was conducted during the period of July 2018 to June 2019 in collaboration with the Department of Pharmacology and Therapeutics and Microbiology, Mymensingh Medical College, Mymensingh, Bangladesh. Six separate experiments were done e.g. (**Expt-I**) Determination of the antibacterial activity of aqueous extract of Amlaki (AAE) and (**Expt-II**) ethanolic extract (EAE) against *P. aeruginosa* by Kirby-Bauer disc diffusion method, (**Expt-III**) Determination of minimum inhibitory concentration (MIC) of aqueous extract, (**Expt-IV**) ethanolic extract, (**Expt-V**) a standard antibiotic ciprofloxacin against test organism by broth dilution technique and making a comparison with MIC of AAE and EAE and (**Expt-VI**) Subculture studies of materials from effective AAE, EAE and ciprofloxacin preparations for confirmation of respective results of Experiments III, IV and V.

Results: Aqueous and ethanolic extracts were used in six different concentrations 100 µg/ml, 200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml and 1000µg/ml. For aqueous extract and ethanolic extracts dose dependent inhibitory effect was observed against the test organism. Showing greater inhibitory effect at the same concentrations[AAE vs EAE: 6vs6, 6vs8, 7.5vs17, 15.5vs23.5, 24vs25, 27.5vs35]. The MIC of ciprofloxacin was lowest in comparison to MICs of AAE and EAE for the test organism. The subculture studies also confirmed the results of the previous experiments.

Conclusion: This study demonstrates that there is definite antibacterial effect of both aqueous and ethanolic extract of *P. emblica* against *P. aeruginosa*.

Key words : *Phyllanthus emblica* (Amlaki), *Pseudomonas aeruginosa*, aqueous and ethanolic extract.

INTRODUCTION

Infectious diseases are the leading cause of untimely death world-wide and it has become a global concern. The clinical efficacy of many existing antibiotics is being threatened by rapid emergence of multidrug-resistant pathogens. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Kumar et al. (2012) there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. Yebpella et al. (2011) different species of medicinal plants have been analyzed for biologically active ingredients known to have pharmacological properties and many of the studied plants have shown antimicrobial property. Plant extracts highlight a continuous effort aimed at finding new compounds against microorganisms. Malik et al. (2017) the use of plant extracts, with known antimicrobial properties, can be of great significance in the treatment of various microbial infection. Mukherjee et al. (2006), contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects. Therefore, much attention has been paid to the discovery and development of new antimicrobial agents of plant origin that might act against these resistant organisms and Amlaki could be an interesting candidate.

Phyllanthus emblica (Gaertn) (syn. *Emblia officinalis*) belongs to the Family Euphorbiaceae. *Phyllanthus emblica* is very large genus consisting of approximately 550 to 750 species and is subdivided into 10 or 11 subgenera. *Phyllanthus emblica* L. (syn. *Emblia officinalis*) has been extensively used, both as fruit and as tonic for its therapeutic potentials. Anbuselvi et al. (2015), it is highly nutritious and is rich natural source of vitamin C, carotene, thiamine, riboflavin, niacin, amino acids, and minerals such as

calcium, phosphorus, iron. It also contains tannins and colloidal substances, phyllembic acid, lipids, gallic acid, ellagic acid, trigalloylglucose, terchebin, corilagin and emblicol. Phyllembin and mucic acid have been isolated from the fruit pulp. Studies have shown that the active compounds contained by *P. emblica* have significant medicinal value. It has its beneficial role in treatment of diabetes, cough, asthma, bronchitis, dyspepsia, colitis, flatulence, hyper acidity, peptic ulcer, skin diseases, leprosy, inflammations, anaemia, hepatopathy, jaundice, diarrhoea, dysentery, haemorrhage, leucorrhoea, cardiac disorders, intermittent fevers and greying of hair and is given to cancer. Nair et al. (2007), it has anti-viral, anti-bacterial, anticancer, anti-allergic, and anti-mutagenic properties. Amlaki is proven to rejuvenate all the organ systems of the body, provide strength and wellness.

To overcome antibiotic resistance, essential oils and medicinal plant extracts present alternative solutions that are safer, more efficacious and multifunctional. Amlaki consist of polyphenols, flavonoids, gallic acid, ellagic acid and other bioactive compound that possess antioxidant, antibacterial, antiseptic, antiviral and antifungal properties. Amlaki has been reported to inhibit the growth of several antibiotic resistant strains of bacteria. The present study is aimed to investigate the antibacterial activity of Amlaki against *Pseudomonas aeruginosa*. So that Amlaki can be used against these microorganisms in antibiotic resistance.

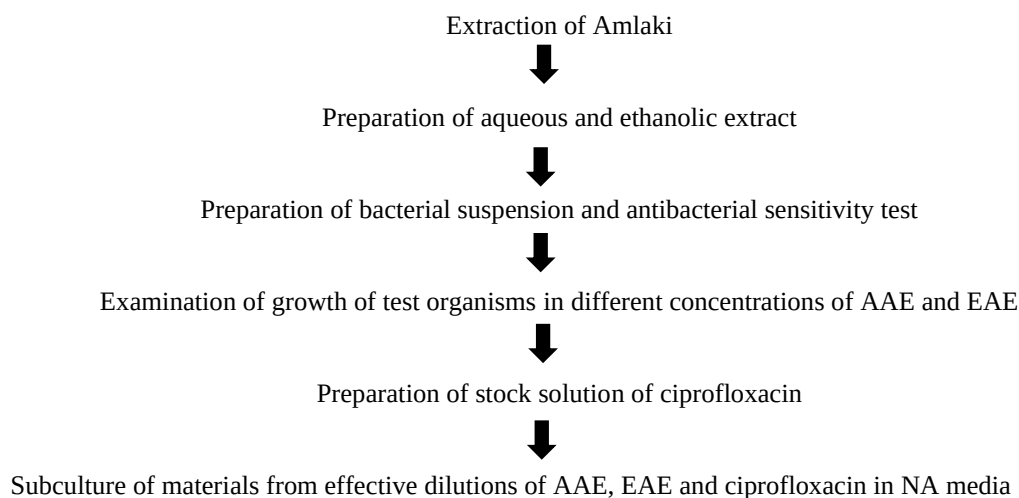
METHOD

This experimental study was conducted in the Department of Pharmacology & Therapeutics in collaboration with the Department of Microbiology, Mymensingh Medical College, Mymensingh, between July 2018 to June 2019. The test organism *Pseudomonas aeruginosa*, ATCC 27853 and all the test organisms (Reference strains) were collected from Department of Microbiology of same medical College. Amlaki were bought from local market of Mymensingh. A Quinolones antibiotic (ciprofloxacin) which was bought in injectable form (200 mg/100 ml) manufactured by Opsonin Pharma Limited, Bangladesh. The whole study was fragmented into six separate experiments [Table-1]. In this study antimicrobial activity of Amlaki was analysed in terms of the Zone of inhibition (ZOI) and Minimum Inhibitory Concentration (MIC) for the test organism, Ghani et al (2003).

Table 1: Experiment carried out

Experiment No.	Targets
Expt-I	Antibacterial sensitivity testing of aqueous extract of <i>Phyllanthus emblica</i> (Amlaki) against <i>Pseudomonas aeruginosa</i> by using disc diffusion method.
Expt-II	Antibacterial sensitivity testing of ethanolic extract of Amlaki against test organisms by disc diffusion method.
Expt-III	Determination of Minimum Inhibitory Concentration (MIC) of aqueous extract of Amlaki against test organisms by broth dilution technique.
Expt-IV	Determination of MIC of ethanolic extract of Amlaki against test organisms by broth dilution technique.
Expt-V	Determination of MIC of ciprofloxacin against test organisms by broth dilution technique.
Expt-VI	Subculture studies of materials from effective aqueous extract, ethanolic extract and ciprofloxacin preparations for confirmations of respective results of Experiments III, IV and V.

Steps followed for the above experiment:



RESULTS

For AAE, *P. aeruginosa* started showing definite activity from 600 µg/ml where was 15.5 mm (Fig:1). Negative control (disc containing only sterile D/W) showed no zone against any bacteria.

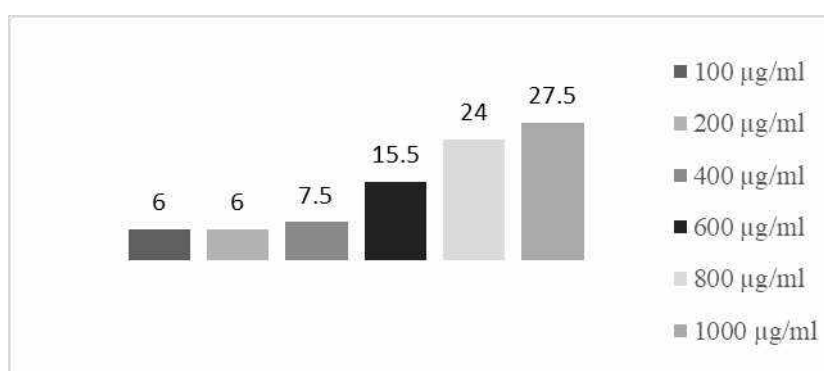


Figure 1: Zone of inhibition at different concentrations of AAE

For EAE, *P. aeruginosa* started showing definite activity from 400 µg/ml with ZOI of 17 mm compared to Negative control. Maximum ZOI was 35 mm at 1000 µg/ml against aforesaid bacteria. [Fig-2]. The MICs of AAE was 500 µg/ml whereas for ethanolic extract of Amlaki the MIC was 300 µg/ml. In case of ciprofloxacin the MIC was 1 µg/ml. The result of expt III-IV was confirmed by the subculture studies.

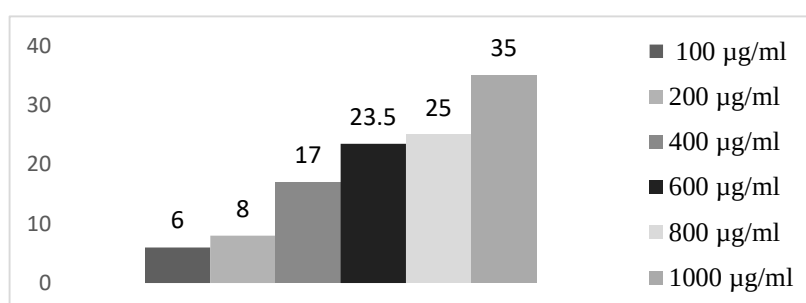


Figure 2: Zone of inhibition at different concentration of EAE

DISCUSSION

In **Experiment-I**, Zone of Inhibition of AAE for *P. aeruginosa*, were 6 mm, 6 mm, 7.5 mm, 15.5 mm, 24 mm and 27.5 mm at different concentrations respectively (100, 200, 300, 400, 600, 800, 1000 µg/ml) Dharajiya et al. (2015), analyzed the antimicrobial activities of *E. officinalis* fruits against different pathogenic bacteria, *E. coli*, *Serratia marcescens*, *P. aeruginosa* and *Bacillus cereus* by agar well diffusion method using different solvents such as aqueous, ethanol, methanol and hexane. In aqueous extract, ZOI for *P. aeruginosa* was 12±1.0 mm at 100 and 50 mg/ml which is observed closer to ZOI (15.5 mm) at 600 µg/ml in our study. Another study was carried out by Selvamohan et al. (2012) to assess the antibacterial activities of *P. emblica* against pathogens including *Klebsiella pneumonia*, *S. aureus*, *P. aeruginosa* and *E. coli*. Three different solvents such as aqueous, ethanol, and methanol were used in this case at various concentrations (20, 30, 40, 50, 60 µl). In aqueous extract of *P. emblica* has high impact on the human pathogenic bacteria such as 22mm, 21 mm, 21 mm and 18 mm against *Klebsiella* sp., *Staphylococcus* sp., *Pseudomonas* sp. and *E. coli* respectively. In present study for *P. aeruginosa* 24 mm ZOI was seen at 800 µg/ml which somewhat coincide with the aforesaid research.

In **Expt-II** for *P. aeruginosa*, ZOI were 6 mm, 8 mm, 17 mm, 23.5 mm, 25 mm and 35 mm at same concentrations respectively. As per Dharajiya et al. (2015) in ethanolic extract, for *P. aeruginosa* ZOI was 13±1.0 mm. But in present study it was 17 mm at 400 µg/ml for the same test organism. However, Selvamohan et al. (2012), found better impact (19-23 mm) against all pathogens in case of ethanol extract. However highest antimicrobial activity (ZOI:22 mm) was observed against *E. coli* and *Pseudomonas* sp. respectively, for the methanolic extracts of *P. emblica*. In the present study, *Pseudomonas* sp. 35 mm which show dissimilarity with aforementioned study.

In **Expt -III**, the Minimum Inhibitory concentration (MIC) of aqueous extract of Amlaki (AAE) was determined by broth dilution technique against the test organisms. In present study the MICs of aqueous *P. emblica* extract for *P. aeruginosa* 500 µg/ml. Dharajiya et al. (2015), determined the MIC of aqueous extract of *P. emblica* by the dilution method against pathogenic organisms including *P. aeruginosa*, *E. coli* and in that study the MIC for, *P. aeruginosa* it was 25 mg/ml (or 25000 µg/ml) which is way apart from that of the current study. This dissimilarity might be due to different technical procedure.

In **Expt -IV**, the MIC of ethanolic extract of Amlaki (EAE) was determined by broth dilution technique against the test organisms. Nahor et al. (2012), determined the MIC of ethanolic extract of Amlaki (EAE) by the dilution method against pathogenic organisms including *S. aureus*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae*. In that study the MIC, for *P. aeruginosa* was 25.0 µg/ml which is again observed to be inconsistent with our findings which can be explained by the different extract and different technical procedure.

CONCLUSION

Our study suggests that both aqueous and ethanolic extracts of Amlaki have antibacterial effect against *P. aeruginosa*. Effect of ethanolic extract was more antimicrobial effect against *P. aeruginosa*. However, the MIC for EAE is almost half than that for AAE. Further study is required to isolate the active compounds responsible for the antibacterial activity of Amlaki and also to know their efficacy, mechanism of action, toxicity profile and safety margin for its therapeutic use. Hopefully, that would lead to the discovery of new and more potent antimicrobial agents originated from Amlaki.

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SCREENING OF SECONDARY METABOLIT COMPOUNDS IN CITRONELLA (*Cymbopogon nardus*) USING REFLUX AND SOXHLETATION METHODS

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Abstract

Introduction: Citronella (*Cymbopogon Nardus*) belongs to the Poaceae family. Citronella (*Cymbopogon Nardus*) has antibacterial, anti-inflammatory, diuretic, antioxidant, anticancer, and antipyretic properties. Therefore, it is necessary to conduct research on the phytochemical screening of n-hexane extract of citronella stems by reflux and soxhletation methods. The purpose of this study was to determine the content of secondary metabolites of Citronella (*Cymbopogon Nardus*) using reflux and soxhletation methods.

Method: This research method is experimental with a variety of methods, namely the reflux method and the soxhlet method, the sample used is Citronella Wangi stems obtained from the Wonosari farm, Kendal. Data analysis was done descriptively.

Results: The results of this study the yield obtained by the reflux method and the soxhletation method did not meet the requirements of the Indonesian herbal pharmacopoeia, namely 7.2% because for reflux the results were 2.4% and the soxhletation method 3.8%.

Conclusion: The conclusion of this study is that the yield obtained does not meet the requirements of Indonesian herbal pharmacopoeias and that Lemongrass contains alkaloids, flavonoids, and tannins.

Key Words: Fragrant Lemongrass (*Cymbopogon Nardus*), secondary metabolites, N-Heksana, reflux, Soxhlet, Yield.

INTRODUCTION

Nurcholis *et al* (2019) states that the diversity of biological natural resources in Indonesia consists of a variety of plant species. Indonesia's geographical location in the tropics is one of the biological centers in the world in the form of various types of plants spread throughout Indonesia. Many plants have been used as traditional medicine for generations. People prefer to use traditional medicines because they are cheap, can be formulated by themselves and have little side effects compared to drugs from pharmaceutical products. The way to develop traditional medicine is by conducting phytochemical screening to determine the active chemical components contained in medicinal plants.

According to Purbowati *et al.* (2021) Phytochemical screening is a method used to determine the components of active compounds contained in samples, namely their chemical structure, biosynthesis, natural distribution and biological function, isolation, comparison of chemical compositions of various types of plants. Susanty and Bachmid (2016) explained that phytochemical compounds contained in plants can be obtained by extraction. Extraction is the process of separating a substance from a mixture using a solvent. Extraction can be carried out by various methods, one of which is reflux and soxhletation, both methods are included in the hot extraction method. Reflux is an extraction method that is carried out at the boiling point of the solvent, for a certain time and a certain amount of solvent using the presence of a cooler (condenser). Soxhletation is extraction using a solvent that is always new, generally carried out using a special tool so that constant extraction occurs using a recooling (condenser). In the extraction process using solvents, the selection of solvents in the extraction process usually uses the principle of like dissolves like, where a non-polar compound will dissolve in a non-polar compound and a polar compound will dissolve in a polar compound.

One of the medicinal plants that have potential as medicinal ingredients is Lemongrass (*Cymbopogon nardus*). In the research of Anwar and Siringoringo (2020) Lemongrass is a plant that is widely cultivated in tropical and subtropical areas. In addition, lemongrass is commonly used by the community. Several studies have reported that lemongrass has antibacterial, anti-inflammatory, diuretic, antioxidant, anticancer, and antipyretic properties. In addition, lemongrass can relieve coughs, treatment of typhus, seizures, and rheumatism. In a previous study conducted by Anwar and Siringoringo (2020) it has been explained that the Citronella plant has several groups of compounds such as D-limonene, citronellal, citronellol, geraniol by means of citronella essential oil identified by Gas-Mass Spectrometry (GC-MS)

chromatherapy)

Previous research conducted by Desmiaty *et al.* (2019) using a different sample, namely *R. fraxinifolius*, it was found that the reflux method gave more yield, but the Soxhlet method was able to extract more polyphenolic compounds and antioxidant compounds. In this study, we want to use the method of reflux and soxhletation. Reflux is used because it is simpler, cheaper, and has a shorter time, while soxhletation is used because it can produce more extract and use less solvent and can be used repeatedly. Research conducted by Hilmarni *et al.* (2021) the sample used was citronella leaves taken in Bukittinggi, 1 kg in wet sorting, then finely chopped and extracted by percolation with n-hexane as solvent. In the research of Hilmarni *et al.* (2021) it is proven that lemongrass contains alkaloids, flavonoids, and saponins.

Based on the above background, it is necessary to conduct research on the phytochemical screening of citronella by reflux and soxhletation methods. The results of this study are expected to provide information regarding the content of secondary metabolites in citronella

METHOD

Research Design

The research design used in this study was experimental.

Location and Research Time

This research was conducted at the Chemical Laboratory of Stikes Mitra Keluarga Bekasi Timur, the research was carried out for approximately 3 months from March to May 2022.

Population and Research Sample

The population as well as the sample in this study were 15 kg of fragrant lemongrass (*Cymbopogon nardus*) stems obtained from Wonosari, Kec. Kendal, Kendal Regency, Central Java.

Research Variabel

In this study, the independent variable is a single variable (Sugiyono, 2017). The independent variables in this study were the percentage yield of the thick extract and the phytochemical screening of the lemongrass stem extract using reflux and soxhletation methods.

Research Tools and Materials

Tools

The tools used in this study were knife, cutting board, dropper, analytical balance, test tube (Iwaki Pyrex®), measuring cup (Iwaki Pyrex®), blender (Philips), beaker glass (Iwaki Pyrex®), reflux, soxletation, rotary evaporator, filter paper, mattress thread, steam dish (Iwaki Pyrex®) stir bar, thermometer, horn spoon, spatula.

Research Materials

The materials used in this study were Citronella, magnesium powder, concentrated HCL, 2 N HCL (Hydrochloric acid, distilled water), N-Hexane, Mayer (chloride, aquades, potassium iodide), ethyl acetate, concentrated sulfuric acid, Dragendorff (Nitrate), concentrated nitric acid, potassium iodide, distilled water), Wagner (Aquades, iodine, potassium iodide), 1% FeCl₃ (FeCl₃, aquades).

Method Reflux

In the reflux method, the mashed Lemongrass sample was then weighed as much as 50 g, then put in a round bottom flask and 500 ml of N-Hexane solvent was added. After that, a reflux device was assembled, until it was extracted at 50°C for 2 hours. The solution that has been extracted is filtered through filter paper and then put in an Erlenmeyer.

Method Soxletation

In the soxhletation method, the first method is to weigh the *Simplicia Citronella Wangi* (*Cymbopogon nardus*) as much as 50 g then wrapped in filter paper and then tied with mattress thread. Then assemble the

soxhlet apparatus. Next, filter paper containing Citronella (*Cymbopogon Nardus*) was added, then put it into the soxlet apparatus plus 500 ml of N-Hexane solvent, then heated at 50°C with a hot plate until the cycle drops were colorless.

Extract Evaporation

After obtaining the results of the reflux and soxhletation methods, the next step is to separate the solvent from the Citronella extract using a rotary evaporator and then evaporate it at a temperature of 50°C with a speed of 100 rpm. After obtaining the results, the yield is calculated using the following formula:

$$\text{Thick extract yield \%} = \frac{\text{Extract weight}}{\text{Citronella weight}} \times 100\%$$

(Susanty and Bachmid, 2016)

RESULTS

The results of the yield on the thick extract of Citronella stem stems using N-hexane as solvent can be seen in table 5.3 below.

Table 5.3 Yield Results of Concentrated Lemongrass Stem Extract

Extraction Method	Extracted Powder Weight (grams)	Weight of Extracted Extract (grams)	Yield Value (%)
Reflux	50	1,2	2,4
Soxletation	50	1,9	3,8

From table 5.3 it can be seen that the yield of the thick extract of the Citronella stem extract showed the higher yield value was the soxhletation method of 3.8%.

Phytochemical Screening

When a thick extract is obtained, phytochemical screening is carried out including alkaloids, flavonoids, saponins, tannins, steroids/terpenoids. The results of phytochemical screening can be seen in table 5.4. **Table 5.1 Phytochemical Screening**

Phytochemical Screening	Reagent	Method Reflux	Method Soxletation	Description
Alkaloid	Mayer	+	+	Sediment white
	Dragendroff	+	+	Sediment orange
	Wagner	+	+	Sediment brown
Flavonoid	Mg + HCl pekat	+	+	yellow
Saponin	Aquadest	-	-	No foam
Tanin	FeCl ₃ 1%	+	+	Brownish green
Steroid/terpenoid	Etil + asam asetat anhidrat + asam sulfat pekat	-	-	Unstable green color

Description: + = Positive result

- = Negative result

The results of phytochemical screening of citronella stem extract using the reflux method and the soxhletmethod showed the presence of alkaloids, flavonoids and tannins

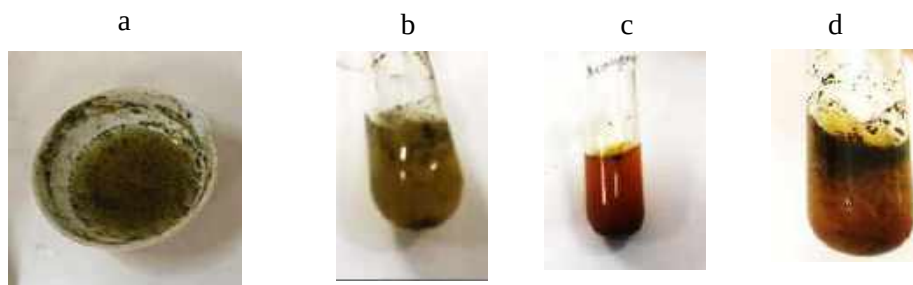


Figure 5.5. The results of the alkaloid test using the reflux method and the soxhlet method before and after the reagent was given

Description:

a = Before

b, c, d = After added reagents (b. mayer, c. dragendrof, d. wagner)

Figure 5.5 shows the results of the alkaloid test with three reagents, namely Mayer, Wagner and Dragendroff reagents. In Mayer's reagent positive for alkaloids was shown a white precipitate, Wagner's reagent was positive for alkaloids indicated the presence of brown deposits, and positive Dragendroff's reagent indicated the presence of red precipitates.



Figure 5.6. The results of the flavanoid test using the reflux method and the soxhlet method before and after the reagent was given

Description:

a = Before

b = After

In Figure 5.6, the positive flavonoid test results with mg and HCL powder reagents show a yellow color. The data obtained in table 5.4 is complemented by Figure 5.7 which can be seen in the stem samples of Citronella (*Cymbopogon nardus*) during phytochemical screening.



Figure 5.7. The results of the steroid/terpenoid test using the reflux method and the soxhlet method before and after the reagent was given

Description:

a = Before

b = After

Figure 5.7 shows the negative steroid/terpenoid test results with ethyl reagent, anhydrous acetic acid, and concentrated sulfuric acid. Because it does not form a green color if positive for steroids and red if positive for terpenoids. The data obtained in table 5.4 is complemented by Figure 5.8 which can be seen in the stem samples of Citronella (*Cymbopogon nardus*) during phytochemical screening.

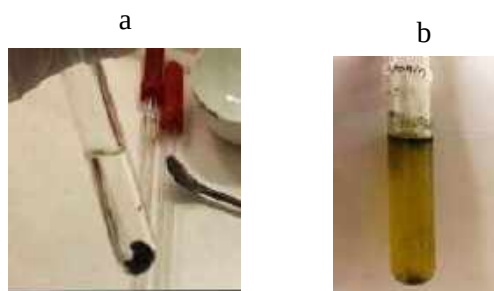


Figure 5.8. The results of the saponin test using the reflux method and the soxhlet method before and after the reagent was given

Description:

a = Before

b = After

In Figure 5.8, the results of the saponin test are negative because it does not produce foam after being shaken for ± 1 minute. The data obtained in table 5.4 is complemented by Figure 5.9 which can be seen in the stem samples of Citronella Wangi (*Cymbopogon nardus*) during phytochemical screening.

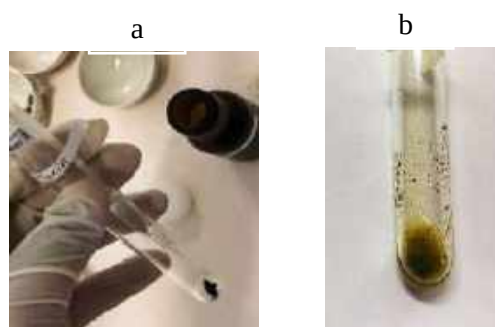


Figure 5.8. The results of the tannin test using the reflux method and the soxhlet method before and after the reagent was given

Description:

a = Before

b = After

In Figure 5.9 the results of the positive tannin test with FeCl_3 reagent produce a brownish green color.

DISCUSSION

Based on the results of the viscous extract research, the yield calculation was carried out, the results obtained were 2.4% for the reflux method and 3.8% for the soxhlet method. The yield percentage results in this study did not meet the requirements of the Indonesian Herbal Pharmacopoeia because it was less than 7.2% (DEPKES, 2017).

According to Sari *et al.* (2021) the longer the simplicia is extracted, the lower the yield will be. This is because the temperature and pressure increase so that the yield of the viscous extract decreases. In addition to the extracting factors, the moisture content of the sample also affects the yield of the thick extract, so that prior to extraction, drying is carried out to reduce the moisture content of the material. The yield of thick extract was influenced by the way of handling before and after extraction.

In this study, positive results were obtained on phytochemical screening including alkaloids, flavonoids and tannins. The alkaloids were tested using extracts and reacted with Mayer, Dragendorff, and Wagner reagents. The Mayer reagent produces a white precipitate because nitrogen in the alkaloids will react with metal ions K^+ from $K_2[HgI_4]$ (potassium tetraiodomercurate (II)) to form a precipitated potassium-alkaloid complex. Dragendorff's test obtained positive results indicated by orange color, because nitrogen is used to form coordinate covalent bonds with K^+ which is a metal ion. The Wagner test got a positive result to get a brown precipitate, because the metal ion K^+ will form a coordinate covalent bond with nitrogen in the alkaloid. The flavonoid test got positive results with yellow results, due to the addition of magnesium powder and the addition of HCL, to reduce the benzopyron core bonds contained in the flavonoid structure that form flavylum salts. The tannin test got a positive result because it showed a brownish green color and it can be concluded that the citronella has a phenol group (Harahap, 2021).

CONCLUSION

Based on the research that has been done, it can be concluded that the results of the yield of the thick extract of Lemongrass Wangi showed that none of the results met the requirements contained in the Indonesian herbal pharmacopoeia as much as 7.2%. Citronella thick extract using two methods, reflux and soxhletation, was proven to contain secondary metabolites in the form of alkaloids, flavonoids, and tannins.

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ANALYSIS OF RHODAMIN B IN BLUSH ON SOLD ON MARKETPLACE X USING TLC METHOD (THIN LAYER CHROMATOGRAPHY)

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Abstract

Introduction: Blush on is a cosmetic to color the cheeks with various colors such as red, pink, to peach. The use of blush on is often misused by adding harmful dyes such as Rhodamine B. The impact of Rhodamine B can cause skin irritation and respiratory tract, and it is a carcinogen if continuously exposed and accumulates in the body; therefore, it is necessary to research the detection of Rhodamine B content in blush on cosmetic sold on Marketplace X. **Method:** Identification of Rhodamine B by optimizing the mobile phase using the Thin Layer Chromatography method. The design of this research is experimental with a purposive sampling technique and samples are taken according to the criteria: Red and Pink color, imported products, solid powder with a price range of Rp. 5000–25,000, does not have a notification number from National Agency of Drug and Food Control. Data analysis using R_f and R_s values obtained.

Results: 12 samples of blush on using TLC method with optimal mobile phase mixture of n- Butanol: Ethanol 96%: Aquadest (20:12:15), Samples that were positive for Rhodamine B produced round/elliptical and tailless stains. Visually it shows a bright pink color, and under UV light, 254 nm and 366 nm fluoresces yellow or orange. Sample A3 has an average R_f value 0.79 and R_s 3.02. Sample A4 average R_f value 0.77 and R_s 2.35. Sample A6 has an average R_f value of 0.68 and an R_s of 2.02. Sample A7 has an average R_f value of 0.76 and an R_s of 2.19. Sample A8 average R_f value of 0.69 with an R_s value of 2.69. Sample A11 has an average R_f value of 0.68 and an R_s of 4.23, and sample A12 has an average R_f value of 0.69 with an average R_s value of 1.73. **Conclusion:** 7 of 12 samples blush on contain Rhodamine B

Key words : Mobile phase, Cosmetic, Rhodamine B, Blush on, Thin Layer Chromatography

INTRODUCTION

Cosmetic products in Indonesia are currently increasing rapidly, due to the many cosmetic products sold in the market and Marketplace at affordable prices ranging from well-known brands to illegal cosmetic products so that many consumers are interested in buying cosmetic products without knowing the ingredients contained in the cosmetics. One of the cosmetics that is often used by women is blush on. Blush on is a cosmetic to color the cheeks in the form of cream, liquid, powder or gel with various colors such as red, pink, to peach. Blush contains high concentrations of dyes and pigments, zinc oxide and titanium dioxide (Andayani et al., 2019). The use of blush is often misused by adding harmful dyes such as Rhodamine B. Rhodamine B is commonly used as a textile dye, dye for cotton, bark, paper, wool, nylon and varnish, in the form of a greenish crystalline powder, in dissolved form at high concentrations. purplish red and at low concentrations bright red (Putri et al., 2017). The side effects of rhodamine B can cause skin irritation, and are carcinogenic if continuously exposed and accumulated in the body (Nanda and Darayani, 2018). According to BPOM Regulation Number 23 of 2019 concerning Technical Requirements for Cosmetic Ingredients, it is stated that Rhodamine B is prohibited from being used for cosmetic additives, so it is necessary to conduct research on the content of Rhodamine B in blush on cosmetic sold in marketplace X using the Thin Layer chromatography method.

METHOD

Research Tools and Materials

The tools used in this study were GF254 silica gel plate, Analytical balance (Ohaus), 100 mL measuring cup (Herma), 100 mL volumetric flask (Pyrex), 25 mL volumetric flask (Pyrex), 10 mL volumetric flask (Pyrex). 10 mL Beaker glass (Iwaki), Oven (Ika), UV Lamp (CAMAG), Chamber (CAMAG), Stirring Rod, Funnel (Pyrex), Volume pipette (Iwaki), Dropper pipette (Pyrex), Micropipette Socorex 2-20 μ l and filter paper, while the materials used included samples of blush sold in Marketplace X, Rhodamin B Standard (Sigma), Methanol p.a (Merck), Aquadest, Ethylacetate p.a (Merck), Ammonia p.a (Merck), n- Butanol p.a (Merck), Hydrochloric acid p.a.

Analysis Qualitativ:

Preparation of standard solution

Dissolve 10 mg of Rhodamine B standard into a 100 mL volumetric flask using methanol p.a, stir homogeneously, then add methanol p.a to the mark of the volumetric flask and stir until homogeneous (100 ppm) (Elfasyari et al., 2020).

Preparation of standard solution

A total 500 mg of the blush sample and put it in a beaker glass then add 4 drops of 4 M HCl and enter 2 mL of methanol p.a then stir homogeneously and then enter it into a 25 mL volumetric flask and homogenize (Elfasyari et al., 2020). Add methanol p.a ad 25 mL, then stir homogeneously and filter the solution using filter paper (Elfasyari et al., 2020).

Mobile Phase Optimization

Optimization is done by spotting a test solution containing Rhodamine B and a comparison solution, namely Rhodamine B standard solution. Optimization is carried out with various types of solution mixture compositions and with various concentrations that have been carried out by previous researchers as follows:

1. Ethyl acetate: Methanol: Ammonia (25:6:1) (Ratnaningsih, 2013)
2. n-Butanol: Ethanol 96%: Aquadest (20:12:15) (Fatimah et al., 2016)
3. Ethyl acetate: Methanol: Ammonia (15:6:3) (Riyanti et al., 2018)
4. Ethyl acetate: Methanol: Ammonia + Aquadest (15:3:3) (Justina and Nandu, 2016)
5. Ethyl acetate: Methanol: Ammonia (15:3:3) (Afriyeni and Utari, 2016)
6. n-Butanol: Ethyl acetate: Ammonia (55:20:25) (Yuniarto and Rosalina, 2019)

Qualitative Analysis of Thin Layer Chromatography Method

1. Activate the TLC plate measuring 4x10 cm by heating it for 30 minutes in the oven at 100°C.
2. The Standard solution and the test solution were spotted as much as 3 l using a micropipette on the plate with a distance of 1 cm from the bottom of the plate and 1 cm for the distance between the stains.
3. Allow the plate containing the sample to dry for a while then insert the plate into a chamber that has been saturated with the selected mobile phase.
4. Remove the plate from the fully eluted vessel.
5. Observations were made on the color of the stain on the plate visually (pink) and under UV lamps of 254 nm and 366 (yellow or orange fluorescence), this indicated the presence of Rhodamine B dye. The R_f value was calculated.
6. The klt plate was sharpened with HCl spray reagent (producing a pink color) (Tjuana et al., 2021).

RESULTS

In this study, apart from looking at the shape and color of the stain on the TLC plate, it also calculated the R_f value, R_f difference, R_s value and homogeneity of the mobile phase solvent mixture. The results of the R_f and R_s values in this study are shown in table 1.

Table 1. Mobile Phase Optimization

(MPI) Mobile Phase Etil asetat : Methanol : Ammoniak (25:6:1) (PI:4,71)				
Sample	R _f value	R _f value difference	Rs (Resolution)	Description
Rhodamine B	0,34	TD	TD	Homogeneous mobile phase
I	0,34	0	3,05	
II	0,34	0	2,05	
III	0,34	0	2,05	
(MPII) Mobile Phase n-Butanol : Ethanol 96 % : Aquadest (20:12:15) (PI:6,22)				
Sample	R _f value	R _f value difference	Rs (Resolution)	Description
Rhodamine B	0,68	TD	TD	Homogeneous mobile phase
I	0,68	0	3,35	
II	0,68	0	5,03	
III	0,69	0,01	3,03	
(MPIII) Mobile Phase Etil asetat : Methanol : Ammoniak (15:6:3) (PI:5,3)				
Sample	R _f value	R _f value difference	Rs (Resolution)	Description
Rhodamine B	0,88	TD	TD	Homogeneous mobile phase
I	0,88	0	3,53	
II	0,88	0	4,03	
III	0,88	0	2,03	
(MPV) Mobile Phase Etil asetat : Methanol : Ammoniak + Aquadest (15:6:3) (PI:5,3)				
Sample	R _f value	R _f value difference	Rs (Resolution)	Description
Rhodamine B				The mobile
I	ND	ND	ND	phase is not homogeneous to form 2 layers
II				
III				
(MPV) Mobile Phase Etil asetat : Methanol : Ammoniak (15:6:3) (PI:5,32)				
Sample	R _f value	R _f value difference	Rs (Resolution)	Description
Rhodamine B				The mobile
I	ND	ND	ND	phase is not homogeneous to form 2 layers
II				
III				
(MPVI) Mobile Phase n-Butanol : Etil asetat : Ammoniak (55:20:25) (PI:5,55)				
Sample	R _f value	R _f value difference	Rs (Resolution)	Description
Rhodamine B				The mobile
I	ND	ND	ND	phase is not homogeneous to form 2 layers
II				
III				
Information				
MP	: Mobile phase	I	: Replication 1	
ND	: Not detected	II	: Replication 2	
PI	: Polarity index	III	: Replication 3	

Table 1 shows the results of the optimization of the mobile phase, the mixture (MPII) of n-Butanol: ethanol 96%: aquadest (20:12:15) best met the criteria, namely a clear and homogeneous solution with an R_f value range of 0.2-0.8 the difference between the R_f values of the sample with R_f Rhodamin B 0.2 and the value of Rs 1.5 compared to other mobile phase mixtures, the FGII mixture has a good R_f value, a good value of Rs or separation and produces a clear and homogeneous solution mixture.

The results of the optimization of the mobile phase in this study showed the shape and color of the stain on the blush sample. The results of the stains seen visually on the TLC plate can be shown in Figure 1.

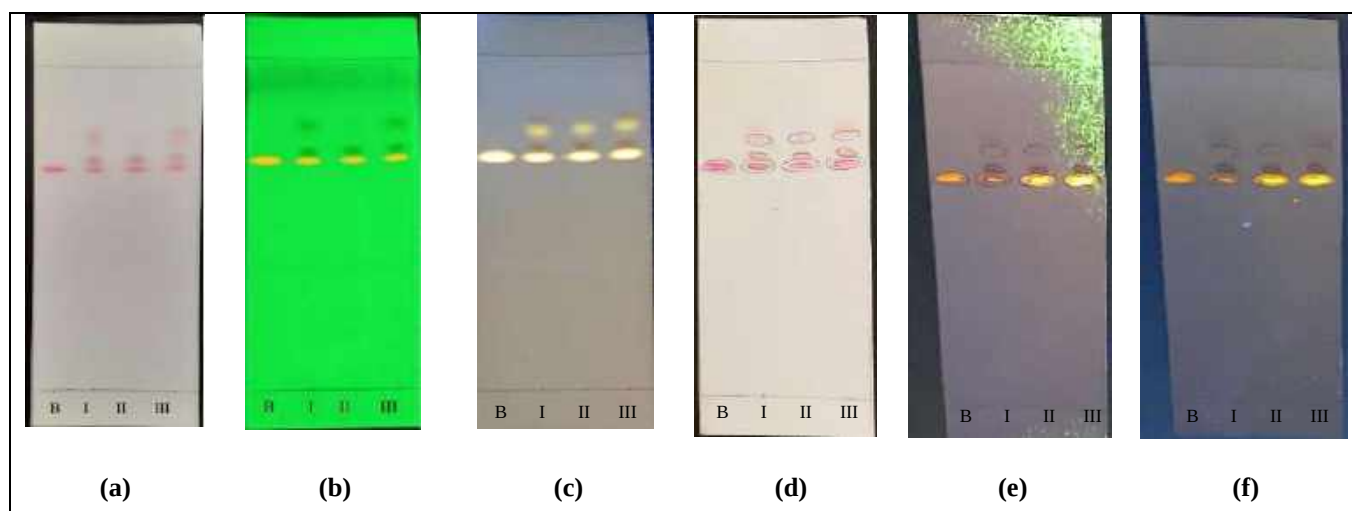


Figure 1. Spots of mobile phase n-Butanol : Ethanol : Aquadest (20:12:15)

Information : (a) Visual
 (b) Under UV light 245 nm
 (c) Under UV light 366 nm
 (d) Visual after sparying HCl
 (e) Under UV light 254 nm after spraying HCl
 (f) Under UV light 366 nm after spraying HCl

B: Standard
 I : Replication 1
 II : Replication 2
 III : Replication 3

Figure 1 shows stain under 366 nm UV light after being sprayed with 4 M HCl based on the shape and color of the stain, a positive result of a blush sample containing Rhodamine B is indicated by a yellow or orange fluorescence stain and a round/elliptical shape of the stain, while the spots are stained with yellow or orange fluorescence. stains on samples that do not contain Rhodamine B are indicated by stains that do not fluoresce yellow or orange.

Table 2. Results of Cosmetic Blush Samples using mobile phase n-butanol : Ethanol 96 % : Aquadest (20:12:15)

Rep.	Sample	Visual color	UV 254 nm	UV 366 nm	Rf value	Rf value difference	Average difference Rf	Rs value	Average Rs value	Result
A1	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.70	ND	ND	ND	ND	
	I	Faded pink	non-fluorescent	non-fluorescent	0.73	0.03		4.01		
	II	Faded pink	non-fluorescent	non-fluorescent	0.73	0.03	0.03	3.23	3.75	(-)
	III	Faded pink	non-fluorescent	non-fluorescent	0.73	0.03		4.03		
A2	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.74	ND	ND	ND	ND	
	I	Faded pink	non-fluorescent	non-fluorescent	0.75	0.01		9.03		
	II	Faded pink	non-fluorescent	non-fluorescent	0.75	0.01	0.01	9.03	9.03	(-)
	III	Faded pink	non-fluorescent	non-fluorescent	0.75	0.01		9.03		
A3	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.75	ND	ND	ND	ND	(+)

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	I	Pink	Orange fluorescence	Orange fluorescence	0.79	0.04		3.02	
	II	Pink	Orange fluorescence	Orange fluorescence	0.79	0.04	0.04	3.02	3.02
	III	Pink	Orange fluorescence	Orange fluorescence	0.79	0.04		3.02	
A4	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.75	ND	ND	ND	ND
	I	Pink	Orange fluorescence	Orange fluorescence	0.77	0.02		2.01	
	II	Pink	Orange fluorescence	Orange fluorescence	0.77	0.02	0.02	2.01	(+)
	III	Pink	Orange fluorescence	Orange fluorescence	0.78	0.03		3.03	
A5	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.71	ND	ND	ND	ND
	I	Brick red	non- fluorescent	non- fluorescent	0.76	0.04		4.38	
	II	Brick red	non- fluorescent	non- fluorescent	0.78	0.06	0.06	4.04	(-)
	III	Brick red	non- fluorescent	non- fluorescent	0.79	0.08		4.84	
A6	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.66	ND	ND	ND	ND
	I	Pink	Orange fluorescence	Orange fluorescence	0.68	0.02		2.02	
	II	Pink	Orange fluorescence	Orange fluorescence	0.68	0.02	0.02	2.02	(+)
	III	Pink	Orange fluorescence	Orange fluorescence	0.68	0.02		2.02	
2A7	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.75	ND	ND	ND	ND
	I	Pink	Orange fluorescence	Orange fluorescence	0.77	0.02		1.51	
	II	Pink	Orange fluorescence	Orange fluorescence	0.76	0.01	0.01	2.03	(+)
	III	Pink	Orange fluorescence	Orange fluorescence	0.76	0.01		3.03	
A8	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.68	ND	ND	ND	ND
	I	Pink	Orange fluorescence	Orange fluorescence	0.69	0.01		2.02	
	II	Pink	Orange fluorescence	Orange fluorescence	0.69	0.01	0.01	3.02	(+)
	III	Pink	Orange fluorescence	Orange fluorescence	0.69	0.01		3.02	
A9	Rhodamine B	Pink	non- fluorescent	non- fluorescent	0.66	ND	ND	ND	ND
	I	Faded brick red	non- fluorescent	non- fluorescent	0.68	0.02		11.08	
	II	Faded brick red	non- fluorescent	non- fluorescent	0.68	0.02	0.02	10.57	(-)
	III	Faded brick red	non- fluorescent	non- fluorescent	0.69	0.03		9.54	

A10	Rhodamine B	Pink	non-fluorescent	non-fluorescent	0.68	ND	ND	ND	ND	
	I	Faded brick red	non-fluorescent	non-fluorescent	0.70	0.02		4.54		
	II	Faded brick red	non-fluorescent	non-fluorescent	0.71	0.03	0.03	4.04	4.54	(-)
	III	Faded brick red	non-fluorescent	non-fluorescent	0.71	0.03		5.05		
A11	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.69	ND	ND	ND	ND	
	I	Pink	Orange fluorescence	Orange fluorescence	0.68	0.01		5.01		
	II	Pink	Orange fluorescence	Orange fluorescence	0.68	0.01	0.01	2.68	4.23	(+)
	III		Orange fluorescence	Orange fluorescence	0.68	0.01		5.01		
A12	Rhodamine B	Pink	Orange fluorescence	Orange fluorescence	0.69	ND	ND	ND	ND	
	I	Pink	Orange fluorescence	Orange fluorescence	0.69	0		1.51		
	II	Pink	Orange fluorescence	Orange fluorescence	0.69	0	0	2.03	1.73	(+)
	III	Pink	Orange fluorescence	Orange fluorescence	0.69	0		1.68		

Table 2. Shows the results of cosmetic blush samples using a mixture of mobile phase n-Butanol : 95% Ethanol : Aquadest (20:12:15) resulting from 12 samples there are 7 positive samples shown in the sample with the code A3; A4; A6; A7; A8; A11 and A12 with stain criteria visually bright pink and fluoresces yellow or orange under UV light at 254 nm and 366 nm, respectively. The R_f value is in the price range of 0.2-0.8 with the difference in the R_f value between the Rhodamine B standard and the sample of 0.2 and the value of R_s 1.5.

The results of the sample using the mobile phase n-Butanol: Ethanol 96%: Aquadest (20:12:15) in this study showed the shape and color of the stain on the blush sample. The results of the stains seen visually on the TLC plate can be shown in Figure 2.

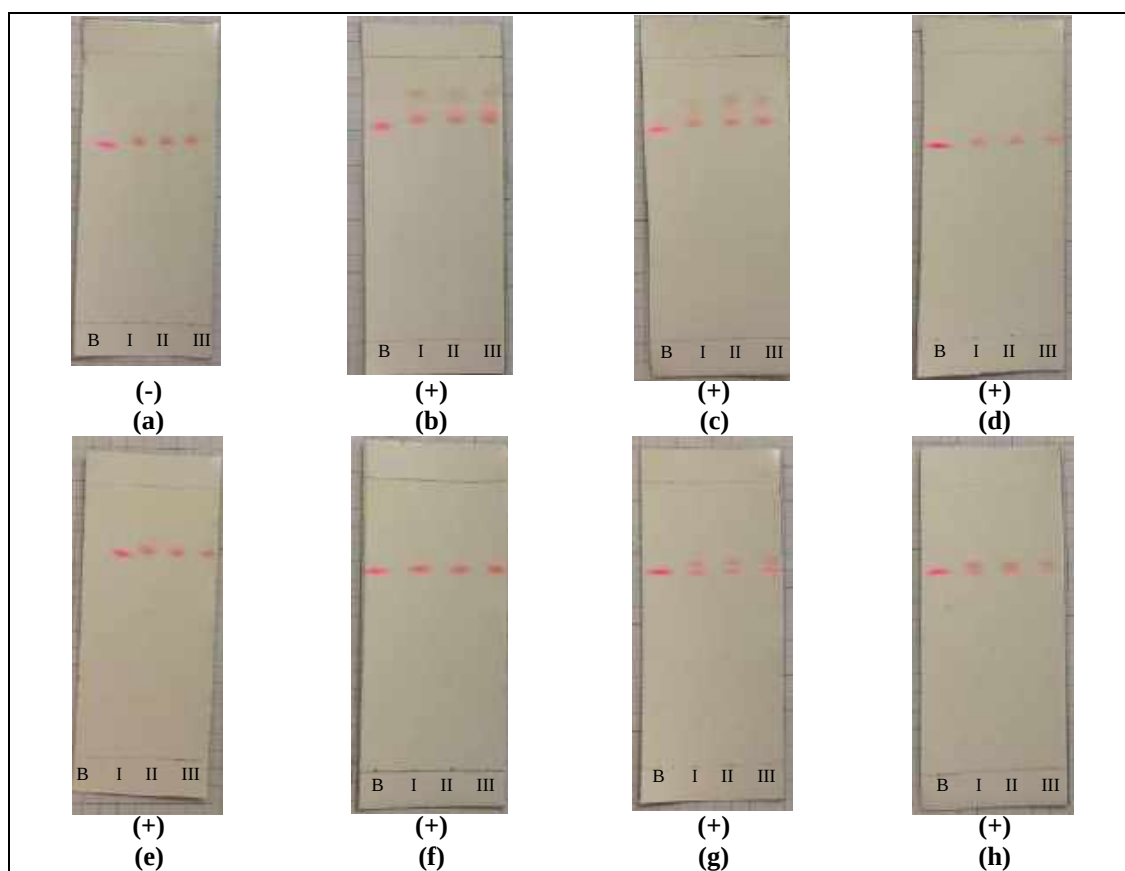


Figure 2. Visually spot sample

Information	:	(a) Sample negative	(e) Sample A7
		(b) Sample A3	(f) Sample A8
		(c) Sample A4	(g) Sample A11
		(d) Sample A6	(h) Sample A12
		B : Standard	II : Replikasi 2
		I : Replikasi 1	III : Replikasi 3

Figure 2. shows the sample stain visually based on the shape and color, a positive result of a sample containing Rhodamine B is indicated by a bright pink stain and a round/elliptical stain in Figure (b); (c); (d); (e); (f); (g) and (h).

The positive and negative results on samples containing Rhodamine B at 245 nm UV light can be seen in Figure 3.

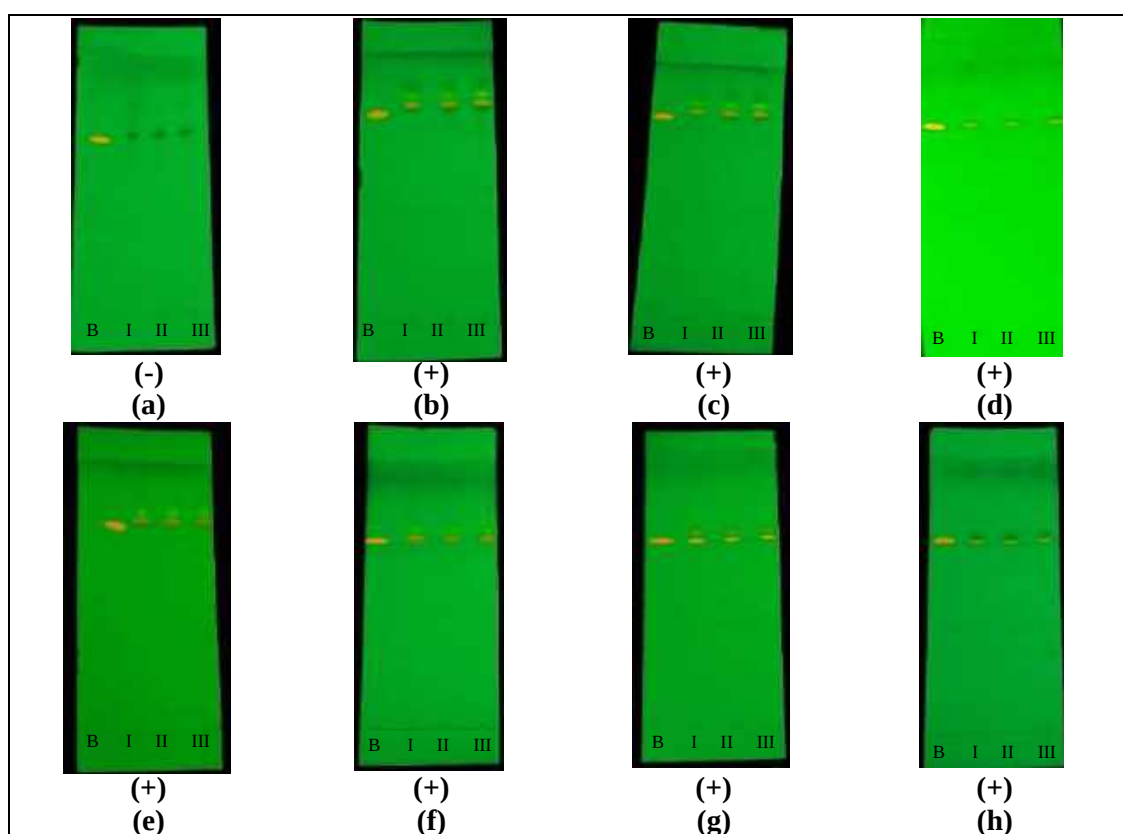


Figure 3. Sample spot under 254 nm UV light

Information	: (a) Sample negative	(e) Sample A7
	(b) Sample A3	(f) Sample A8
	(c) Sample A4	(g) Sample A11
	(d) Sample A6	(h) Sample A12
	B : Standard	II : Replikasi 2
	I : Replikasi 1	III : Replikasi 3

Figure 3. shows sample stain under 254 nm UV light based on the shape and color of the stain, a positive result of a blush sample containing Rhodamine B is indicated by a yellow or orange fluorescent stain and a round/elliptical shape of the stain (b); (c); (d); (e); (f); (g) and (h), while the stains on the blush sample that did not contain Rhodamine B were shown by stains that did not fluoresce yellow or orange in Figure (a).

The positive and negative results on samples containing Rhodamine B at 366 nm UV light can be seen in Figure 4.

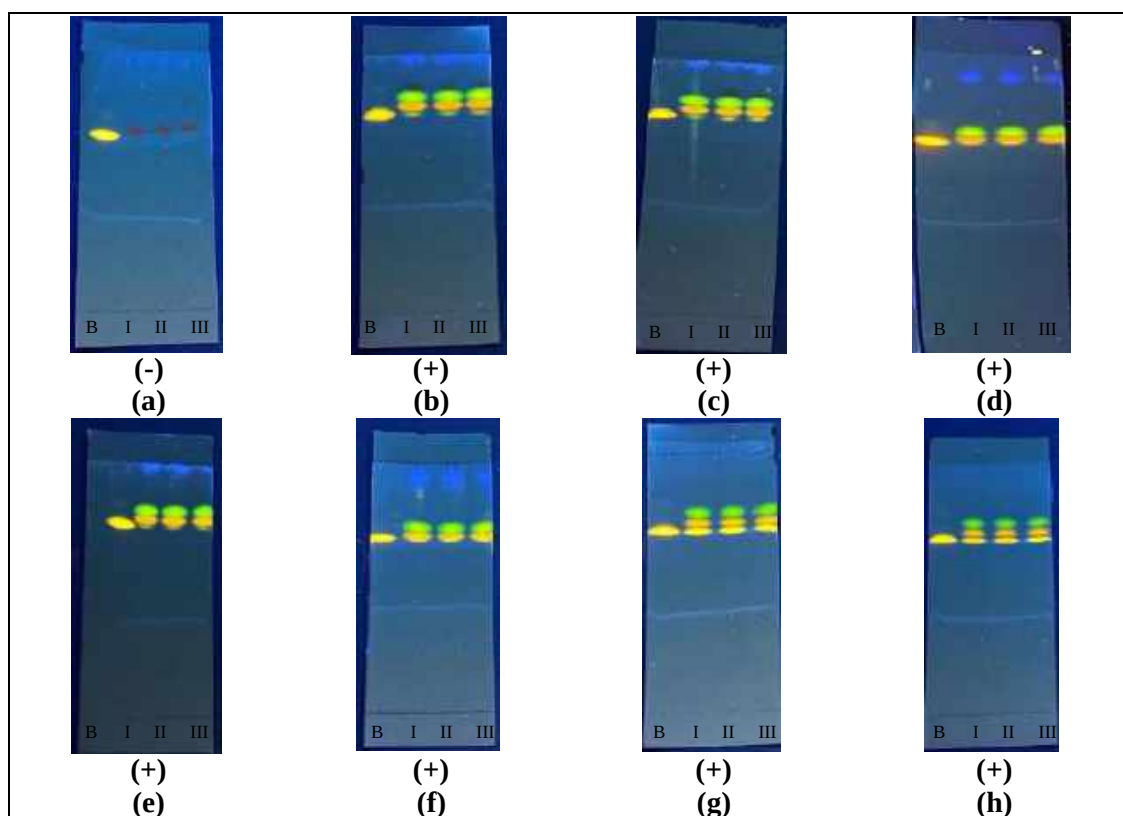


Figure 4. Sample spot under 366 nm UV light

Information : (a) Sample negative (e) Sample A7
 (b) Sample A3 (f) Sample A8
 (c) Sample A4 (g) Sample A11
 (d) Sample A6 (h) Sample A12
 B : Standard II : Replikasi 2
 I : Replikasi 1 III : Replikasi 3

Figure 4. shows stain under 366 nm UV light based on the shape and color of the stain, positive results of samples containing Rhodamine B are indicated by yellow or orange fluorescent stains and round/elliptical stains in Figure (b); (c); (d); (e); (f); (g) and (h).

DISCUSSION

Identification of Rhodamine B dye in cosmetic blush on sold in Marketplace X can be done using the TLC (Thin Layer Chromatography) test method, the TLC method is a method that can detect Rhodamine B compounds because Rhodamine B has chromophore and auxochrome groups so that Rhodamine B can fluoresce if observed under UV 254 nm and UV 366 nm. The standard solution is made by dissolving Rhodamine B powder in methanol as a position control and a solution is made by dripping 4 M HCl as much as 4 drops which serves to destroy the compound in the blush on sample and the Rhodamine B compound in the blush on sample so that it does not ionize into a neutral form (Yuniarto et al., 2019) which was then dissolved by methanol and filtered to separate the insoluble particles. Rhodamine B has the property of being very soluble in organic solvents such as methanol so that methanol is used to dissolve Rhodamine B because methanol is polar so it can dissolve organic substances as well as polar (Sari et al., 2022).

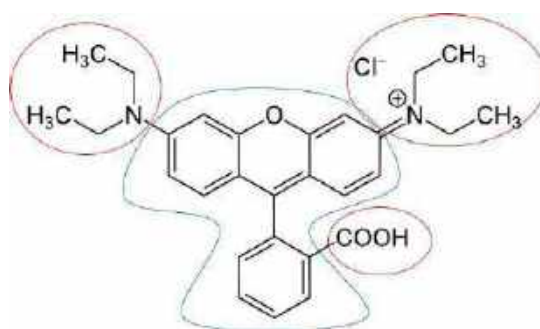


Figure 5. Chromophore and Auxochrome groups of Rhodamine B Compounds

— = Chromophore group
— = Auxochrome group

Rhodamine B has auxochrome and chromophore groups which can be seen in Figure 5. Rhodamine B has chromophore groups that are able to absorb UV and visible light in organic compounds, namely carboxyl groups and aromatic compounds. Rhodamine B also has an auxochrome group, which is a group that has a free electron pair such as NR₂ (Fauziah et al., 2020). In this study, silica gel TLC plate type Gf254 was used, which means that the silica gel can fluoresce at UV 254 nm (Indrayani et al., 2017). silica gel contains silanol (Si-OH) and siloxane (Si-O-Si) groups on its surface where the silanol groups can interact with the analyte because it forms hydrogen interactions. The silanol group can interact with water so that the TLC plate (silica gel) can be deactivated, so it is necessary to heat it in the oven to dry the water molecules on the plate so that the TLC plate becomes active (Rosamih, 2019). On the surface of the silica gel can be reactivated and the silanol group can interact with the analyte (Ediningtyas, 2012)

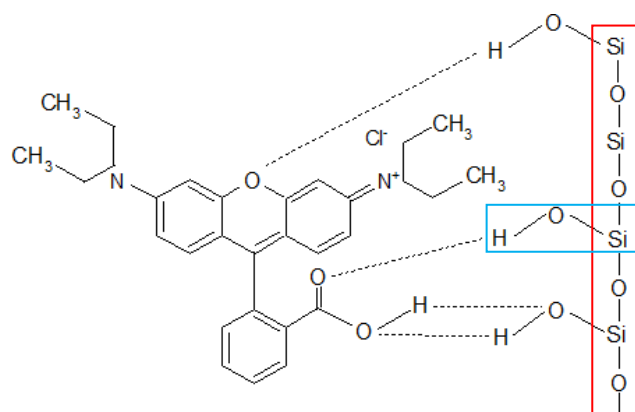


Figure 6. Rhodamine B . Hydrogen Bond Interaction

..... = Hydrpgen interaction
— = Siloxane group
— = Silanol group

The interaction between silica gel and Rhodamine B compounds in Figure 6. will form hydrogen bonds in which Rhodamine B is polar and the silica gel is also polar which will cause strong adsorption so that the Rhodamine B analyte compound will be retained on the silica gel and the analyte on the silica gel will propagate longer (Ediningtyas, 2012). Rhodamine B is polar because its molecular structure has a carboxyl group with a lone pair of electrons so that the choice of eluent needs to be adjusted. Rhodamine B has a carboxyl and amine group that will form intermolecular hydrogen bonds with polar solvents so that Rhodamine B compounds are easily soluble and can produce good separation between Rhodamine B compounds and other compounds (Nafiq et al., 2020). In this study, the chamber that has been filled with the mobile phase needs to be saturated. The saturation function is to ensure that the particles from the mobile phase will be evenly distributed throughout the chamber so that the spots in the stationary phase will move optimally by the mobile phase, which means that the saturation is to optimize the rise of the eluent (Samosir et al., 2018). Optimization of the mobile phase was carried out to find a suitable mobile phase for the separation of Rhodamine B compounds contained in blush on cosmetic so as to obtain optimal separation, namely to produce round/elliptical and tailless spots (Elfasyari et al., 2020). The

sample is declared positive if it enters the sample stain parameter criteria that are parallel to the Rhodamine B standard which is visually bright pink if the distance between the Rf value of the stain between the standard and the sample is the same or close to each other with a price difference of 0.2 (Fauziah et al., 2020), fluoresces orange under UV light of 254 nm and 366 nm (Hiola et al., 2021) the Rf value enters the range of 0.2-0.8 (Elfasyari et al., 2020) and there is a good separation between the 2 stains if the value of Rs 1.5 (Harum, 2021).

CONCLUSION

Based on the results of the research on Rhodamine B content analysis by optimizing the Mobile Phase Using the Thin Layer Chromatography Method in Blush On Cosmetics sold on Marketplace X, it can be concluded that: Spots with positive results are visually round/elliptical and light pink in color according to the color of the standard Rhodamine B stain. Observations of spot under UV light of 254 nm and 366 nm fluoresce in yellow or orange. The optimal separation of Rhodamine compounds is the mobile phase mixture of n-Butanol: Ethanol 96%: Aquadest (20:12:15). 12 of blush samples studied, there were 7 samples containing Rhodamine B with an Rf value of 0.79 for sample A3; sample A4 0.77; sample A6 0.68; sample A7 0.76; sample A8 0.69; sample A11 0.68 and sample A12 Rf value of 0.69.

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PHYTOCHEMICAL SCREENING OF JAPANESE PAPAYA LEAF EXTRACTS (*Cnidoscolus Aconitifolius* Mill.) WITH THE MACERATION METHOD USING DIFFERENT TYPES OF SOLVENTS

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Abstract

Introduction: Japanese Papaya Leaf is a wild bush plant that grows up to 6 meters, and this leaf is easy to grow in tropical countries like Indonesia. The flavonoid compounds contained can lower cholesterol levels in the blood. **Method:** In this study, population and samples of Japanese Papaya Leaf (*Cnidoscolus aconitifolius* Mill.) were used that grow in Bekasi Timur Regency, Bekasi. This research is an experimental qualitative descriptive study that aims to determine the results of the macroscopic examination, and phytochemical screening using a test tube on Japanese papaya leaves extracted by maceration using 70% ethanol, n-hexane, and aqua distillate as solvents. **Results:** The results of the research uses an macroscopic examination, which included leaf morphology test showed dark green color with a slightly shiny slippery surface and round and finger shape leaves. The results of the extract yield from various solvents, obtained aqua distillate by 27%, ethanol 70% by 10.2%, and n-hexane by 4.5%. In this case the amount of yield has a different polarity. Phytochemical screening test of Japanese Papaya leaf extract from 70% ethanol and aqua distillate show positive for alkaloids, flavonoids, saponins, triterpenoids/steroids, tannins, while for n-hexane solvent showed positive for flavonoids and steroids. **Conclusion:** The conclusion of this study is that extracts from Japanese Papaya leaves contain secondary metabolites, namely alkaloids, flavonoids, saponins, tannins, triterpenoids and steroids. **Key words :** Extract Yield, Japanese Papaya, Macroscopic, Phytochemical Screening

INTRODUCTION

Japanese Papaya comes from Yucatan, Mexico, and Central America. They were first discovered in an open forest area by I.M. Johnst. Local people commonly use this plant as vegetables and medicine. The benefits of Japanese Papaya leaves cannot be separated from the content of substances in them, such as Protein, Calcium, Iron, Phosphorus, Vitamin A, Niacin, Riboflavin, and Vitamin C. In contrast, the carposide content in Japanese Papaya leaves is efficacious as an anthelmintic. According to Loarca-Pina (2010) in Fatimah, (2016) stated that in addition to the above content, Japanese papaya is also beneficial in biological activities such as antimutagenic, antioxidant, hypoglycemic, and anti-inflammatory, antiprotozoal, and antibacterial. According to Purwati *et al.* (2017) in Hasibuan *et al.*, (2021) explained that phytochemical screening is the first step to determining the active ingredients which are secondary metabolites in plants. The flavonoid compounds contained in Japanese papaya leaves are known to be suitable for lowering cholesterol levels in the blood (Rahmawati, 2018). Extraction is the first step taken to get the compound to be extracted. The choice of extraction method is also adjusted to the presence of compounds contained in it. In this case, maceration with the appropriate solvent is used, which meets the specified criteria (Agustina *et al.*, 2018). An important factor in the extraction process is the selection of an appropriate solvent. The solvent used must be able to extract most of the secondary metabolites in the simplicia. The effectiveness of the solvent for extracting is very dependent on the solubility of the compound in the solvent, has the principle of like dissolves like, where a polar solvent will dissolve polar compounds and non-polar solvents will dissolve non-polar compounds. The use of the type of solvent can influence the yield of the resulting compound (Kemit *et al.*, 2017). The results of research from Obichi *et al.*, (2015) on Japanese papaya leaves show the content of tannins, saponins, alkaloids, and flavonoids. The difference between the previous research and the one studied is in the solvent and the sampling site used so researchers are interested in conducting research with phytochemical screening to identify secondary

metabolites of Japanese Papaya leaves in the maceration method with three different types of solvents.

METHOD

A. Research Design

The research design is a descriptive qualitative experimental study that was conducted to determine these secondary metabolites contained in Japanese Papaya (*Cnidioscolus aconitifolius* Mill.) leaves by maceration extraction using three solvents, namely 70% ethanol, n-hexane, and aqua distillate.

B. Research Sites

This research was conducted at the Laboratory of the Mitra Keluarga College of Health, East Bekasi.

C. Research Population

The population is the object of research in the form of animals, humans, objects, and also plants that have certain characteristics research conducted. In this study, the population of Japanese Papaya (*Cnidioscolus aconitifolius* Mill.) leaves growing in Bekasi Timur Regency, Bekasi.

D. Research Variable

The independent variables in this study were the content of secondary metabolites in the phytochemical screening test of Japanese Papaya leaf extract (*Cnidioscolus aconitifolius* Mill.) and the percentage yield of Japanese Papaya leaf extract (*Cnidioscolus aconitifolius* Mill.).

E. Japanese Papaya Leaf Sampling

Sampling began in February 2022 in the Bekasi Timur Regency area, Bekasi. The plant part used is the leaf part of the Japanese Papaya plant. Japanese Papaya leaves have been collected and then weighed to determine the weight obtained.

F. Extract Making

The Japanese Papaya leaves used were fresh Japanese Papaya leaves which were dark green as much as 100 grams each then macerated with 70% ethanol, n-hexane, and 500 mL aqua distillate as a solvent. Each Japanese Papaya leaf powder was covered with aluminum foil to protect it from light and left for 3 x 24 hours and homogenized by stirring occasionally. Then the macerate in the form of a liquid extract was filtered using filter paper until a filtrate was produced (accommodated). After that, the residue sample was continued by maceration 1 x 24 hours with aqua distilled solvent, 70% ethanol, and 250 mL of new n-hexane. Furthermore, the maceration sample was filtered and the filtrate obtained was collected into one with the macerate filtrate. The solvent filtrate of 70% ethanol, n-hexane, and aqua distillate will be concentrated or thickened using a rotary evaporator at 400 C to obtain a thick extract (Erlina *et al.*, 2018).

RESULTS

A. Japanese Papaya Leaf Morphology Examination

Based on the data in Table 3.1 shows the morphological results of Japanese Papaya leaves, namely with a length of 16-17.5 cm, a width of 14.4-30 cm, dark green, leaf shape round and fingered, leaf tip (apex) tapered, leaf base (base), grooved / incised, leaf margins (margin) are jagged, leaf surface texture is slippery, slightly shiny, leaf veins spread, and the leaf arrangement is single.

Table 3.1 Japanese Papaya Leaf Morphology Examination

Parameter	Observation result	References (Harahap, 2017)
Length	16-17,5 cm	10-30 cm
Width	14,4-30 cm	10-30 cm
Color	Dark green	Dark green
Leaf shape	Round and Finger	Round and Finger
Leaf tip (apex)	Tapered	Tapered
Leaf base	Notched/cut	Notched/cut

Leaf edge (margin)	Jagged	Jagged
Surface texture	Slick a little shiny	Slick a little shiny
Leaf bone	Spread	Spread
Leaf arrangement	Single	Single

B. Powder Organoleptic Test

Based on Table 3.2 states that the results of the organoleptic test on Japanese Papaya leaf powder are powder form/texture, dark green/green leaf color, strong distinctive odor/aroma, and bitter taste.

Table 3.2 Powder Organoleptic Test Results

Organoleptic Test	Observation Result
Shape/texture	Powder
Color	Dark green/green leaf
Smell/scent	Strong characteristic
Flavor	Bitter

C. Extract Organoleptic Test

Based on Table 3.3 the organoleptic test of Japanese Papaya leaf extract stated that the results of the three types of solvents were aqua distillate and 70% ethanol showed the results of the form/texture of the extract being thick, brown-black in color, and characteristic odor/smell, and n-hexane showing the results. green-black shape/texture, green-black color, and distinctive smell/aroma.

Table 3.3 Extract Organoleptic Test Results

Solvent variation	Observation Result		
	Shape/texture	Color	Smell/scent
Aqua Distillate	Thick extract	Dark brown	Strong characteristic
Ethanol 70%	Thick extract	Dark brown	Strong characteristic
N-Hexane	Thick extract	Green-black	Strong characteristic

D. Extract Yield Calculation

Based on Table 3.4, the results of the calculation of the extract yield show that the 3 types of solvents are aqua distillate solvent as much as 27%, ethanol 70% as much as 10.2%, and n-hexane as much as 4.5%.

Table 3.4 Extract Yield Calculation Results

Solvent variation	sample weight (gram)	extract weight (gram)	Yield (%)	Yield Requirements (Depkes RI, 2008)
Aqua distillate	100	27	27	No less than 7,2%
Ethanol 70%	100	10,2	10,2	
N-Hexane	100	4,5	4,5	

E. Phytochemical Screening

Based on Table 3.5, the results show that the phytochemical screening test on aqua distillate was positive for alkaloids, flavonoids, saponins, triterpenoids, tannins, and 70% ethanol solvent was positive for secondary metabolites of alkaloids, flavonoids, saponins, triterpenoids, and tannins, while for the solvent n-hexane was positive. contains flavonoids and steroids.

Table 3.5 Phytochemical Screening Results

Phytochemical Test	Observation Results		
	Ethanol 70%	N-Hexane	Aqua distillate
Alkaloids	Dragendorff's: (+)	Dragendorff's: (-)	Dragendorff's: (+)
	Mayer: (+)	Mayer: (-)	Mayer: (+)
	Wagner: (+)	Wagner: (-)	Wagner: (+)
Flavonoids	(+)	(+)	(+)

Saponins	(+)	(-)	(+)
Steroids	(-)	(+)	(-)
Triterpenoids	(+)	(-)	(+)
Tannins	(+)	(-)	(+)
Glycoside	(-)	(-)	(-)

DISCUSSION

This study aims to determine the morphology, organoleptic and secondary metabolites present in Japanese papaya leaves with different types of solvents, namely aqua distillate, 70% ethanol, and n-hexane. Tests based on physical parameters include organoleptic examination of powders and extracts and leaf morphology. The organoleptic test aims to observe the shape, color, and smell of Japanese papaya leaves. Based on organoleptic observations of Japanese papaya leaf powder, the results showed that the leaves were dark green, had a powder shape or texture, a distinctive odor or aroma, and a bitter taste. In the research that has been done on organoleptic extracts, the results obtained are that 70% ethanol and aqua distillata have a thick extract form/texture, brown-black in color, with a characteristic odor. As for the solvent n-hexane, the extract organoleptic results were the form/texture of the extract was thick, green- black in color, had a distinctive smell. The purpose of the yield calculation is to determine the percentage of extract obtained so that it will be known the number of simplicia needed to make a certain number of extracts (Lusiyaningrum, 2021). The results obtained from this study indicate that the yield of aqua distillate has the largest yield of 27% and 70% ethanol has a yield of 10.2%, from the results obtained it is included in the extract yield requirements in the Indonesian Herbal Pharmacopoeia (2008) which is more than 7, 2%, while in the solvent n-hexane has the smallest yield of 4.5% so it is not included in the yield requirements. From the yield data, it has something to do with secondary metabolite compounds contained in a sample so that if the number of yield samples increases, the number of secondary metabolites contained in the sample will also increase. The amount of yield depends on each solvent that has a different polarity (Wijaya *et al.*, 2022).

The results of a qualitative test through phytochemical screening on samples of Japanese Papaya (*Cnidoscopus aconitifolius* Mill.) leaves growing in the Bekasi Timur Regency area, Bekasi using a variety of solvents, namely 70% ethanol, n-hexane, and also aqua distillate. while on the results of the qualitative test through phytochemical screening conducted by (Nyam *et al.*, 2020) on samples of Japanese papaya leaves (*Cnidoscopus aconitifolius* Mill.) from the Department of Plant Science & Biotechnology, University of Jos, Nigeria using 70% ethanol as solvent. showed positive secondary metabolite compounds, namely alkaloids, tannins, flavonoids, terpenoids. Thus, in these two studies, there were similarities in secondary metabolites, the only difference being terpenoids and glycosides. In this study (Nyam *et al.*, 2020) glycoside compounds were detected (positive), while in this study no glycoside compounds were detected (negative).

The results of this study showed positive secondary metabolites with aqua distillate solvent, namely alkaloids, flavonoids, saponins, triterpenoids, and tannins, while the qualitative test results through phytochemical screening conducted by (Ogbu and Igboanusi, 2019) on samples of Japanese papaya leaves (*Cnidoscopus aconitifolius* Mill.) from Ogbatai, Woji in Obio Akpor, South-south region, Nigeria using aqua solvent, namely alkaloids, tannins, saponins, and steroids. So in the two studies, there are similarities in secondary metabolite compounds, only the difference is in terpenoids and glycosides. In this study (Ogbu & Igboanusi, 2019) phytochemical screening for terpenoids and glycosides was not carried out, while in this study, phytochemical screening results obtained were terpenoids (positive) and glycosides (negative).

The results of this study showed a positive secondary metabolite compound with n-hexane solvent, namely flavonoids and steroids, while the results of the qualitative test through phytochemical screening conducted by (Abdulmumeen *et al.*, 2016) on samples of Japanese papaya leaves from Mr. Bolu Ajayi of the Department of Plant Biology, University of Ilorin, Nigeria, used n-hexane as a solvent, with positive results on glycosides, steroids, flavonoids, and terpenoids. So, in these two studies, there were similar

secondary metabolites, only that they differed in glycosides and terpenoids. In this study (Abdumumeen et al., 2016) glycosides (+) and terpenoids (+) were detected, while in this study, glycosides (-) and terpenoids (-) were not found. The negative results produced on secondary metabolites can occur because secondary metabolites can be influenced by environmental factors such as soil fertility, land height, climate, temperature, and pollution (Rudianti, 2018). Other factors include the nature of chemical compounds, solvents used, and available tools (Azizah et al., 2018).

The relationship between the results of phytochemical screening and the yield contained in a sample is that if the number of yield samples increases, the number of secondary metabolites contained in the sample also increases. In this study, the most screening results were obtained in 70% ethanol and aqua distillate solvents which contain secondary metabolites of alkaloids, flavonoids, saponins, triterpenoids, and tannins. The secondary metabolites detected in each extract are compounds that can provide antioxidant, antibacterial, and antidiabetic activity (Hasan et al., 2021)

CONCLUSION

Based on what has been described, it can be concluded that:

1. Macroscopic appearance on the leaves of Japanese Papaya (*Cnidoscolus aconitifolius* Mill.) on leaf morphology is 16-17.5 cm long, 14.4-30 cm wide, green, round leaf shape, leaf tip (apex) is tapered, Leaf base is notched/cut, leaf margin is jagged, leaf surface texture is (slightly shiny, smooth, spreading leaf veins and single leaf arrangement and from powder organoleptic test, powder shape/texture, green, characteristic odor, and bitter taste), whereas in the organoleptic test the extracts, namely 70% ethanol and aqua distillate, had a thick extract from, blackish-brown color, and a characteristic odor, and in n-hexane, it had a thick extract from, blackish green color, and a distinctive smell.
2. The content of secondary metabolites of Japanese Papaya leaves from a variety of solvents, namely in aqua distillate solvent positive containing alkaloids, flavonoids, saponins, triterpenoids, and tannins, 70% ethanol solvent positive containing alkaloids, flavonoids, saponins, triterpenoids, and tannins, and while in positive n-hexane solvent contains flavonoids and steroids.

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EVALUATION OF THE USE OF FLUOROQUINOLONE ANTIBIOTICS IN PATIENTS WITH SPECIFIC DIARRHEA IN THE INPATIENT INSTALLATION OF A PRIVATE HOSPITAL IN THE WEST BEKASI REGION FOR THE 2020 PERIOD

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Abstract

Introduction: The rational use of antibiotics is the selection of antibiotics that are selective against infecting microorganisms and effectively destroy infecting microorganisms. The consequences of giving antibiotics that are not appropriate can lead to several negative impacts, one of which is bacterial resistance to antibiotics. Specific diarrhea with certain infectious causes is included in the top 10 diseases of patients undergoing hospitalization. Diarrhea is very common in developing countries, one of which is Indonesia. This study was conducted with the aim of evaluating the rationale of the use of fluoroquinolone antibiotics in inpatients at a private hospital in the West Bekasi region in the period 2020.

Methods: The design of this study was a retrospective non-experimental study using patient medical records. Evaluation of antibiotics carried out is the accuracy of prescribing fluoroquinolone antibiotics. The sample of this study was 83 diarrhea patients who were treated in an inpatient installation of a private hospital in the West Bekasi area for the period 2020.

Results: Evaluation of the use of antibiotics in this study included the right indication, the right patient, the right dose, the right drug, and the right duration of drug administration. The results showed that the right indication was 100%, the patient was 100% right, the dose was 100% right, the drug was 87.9%, and the duration of the drug was 81.93%.

Conclusion: It can be concluded that based on the results of this study, it was found that the use of antibiotics in patients with specific diarrheal diseases affected the rationality of antibiotics, namely the right indication, the right patient, the right dose, the right drug, and the right time of administration.

Keywords: Specific Diarrhea, Antibiotics, Fluoroquinolones, Retrospective

INTRODUCTION

Diarrhea is a disease condition with symptoms of bowel movements that have a liquid or soft consistency, and occur with a frequency of 3 or more times a day. The causes of diarrhea are divided into 6 groups, namely bacterial infections, viral infections, malabsorption, poisoning, allergies, immune disorders and other causes of disease. Diarrhea has become a health problem with very high morbidity and mortality in several developing countries such as Indonesia (Firmansyah, 2020). Diarrhea is a very endemic disease in West Java Province. According to the 2015 Diarrhea Rapid Survey data, cases of diarrhea of all ages nationally were 270 cases/1,000 population. The target that is the reach of services for diarrhea sufferers of all ages at health facilities is 10% of the number of diarrhea sufferers of all ages. The results of the presentation of service coverage for diarrhea sufferers in 2019 in districts/cities of West Java Province were 75.8% (Jabar Health Office, 2019).

The management of specific diarrhea is the administration of antibiotic therapy. Antibiotic therapy is used to provide therapy for diarrhea that occurs due to bacterial infection or bloody diarrhea and there are symptoms of systemic infection experienced by the patient (Dewi, 2016). The administration of antibiotic therapy is very dangerous if it is not consumed according to the dose because it will cause bacterial resistance to these antibiotics. Thus, antibiotic management requires attention to achieve rational administration. In the administration of antibiotics that are not appropriate will cause the risk of resistance. Several types of antibiotics in Indonesia are resistant to several bacteria that cause diarrhea, resulting in a decrease in the ability of antibiotic therapy when treating infections experienced by humans which will make treatment much more difficult (Frida, 2018).

Based on this, considering the high incidence of diarrhea and the high use of fluoroquinolone antibiotics. In addition, the occurrence of irrationality in the use of antibiotics, it is necessary to conduct research on the rationale of using fluoroquinolone antibiotic therapy as the first line in diarrhea patients. This study was conducted to evaluate the right indication, the right patient, the right dose, the right drug, and the right time of administration for the use of fluoroquinolone antibiotics.

METHOD

This study uses a non-experimental descriptive observational method. The study was conducted through retrospective medical record data from diarrhea patients who received antibiotics at the inpatient installation of the West Bekasi Region Private Hospital for the period 2020. The observational study design was where the researcher did not treat the variables and only observed the situation that had occurred. The study was conducted at a private hospital in West Bekasi from February to March 2022. The study population was all patients with a diagnosis of diarrhea at an inpatient installation at a private hospital in the West Bekasi region for the period 2020. The number of samples in this study were 83 patients. is the population that has met the inclusion criteria and exclusion criteria.

Slovin's formula:

$$n = \frac{N}{1 + N (e)^2}$$

Information:

n = Sample Unit (Number of Respondents Required)

N = Population (sum of population size of specific diarrheal patients in one of the West Bekasi Private Hospitals in 2020)

e = Error Value Used

The researcher uses an error value of 5%, the number of samples used is based on the formula, namely:

$$\begin{aligned} n &= \frac{92}{1 + (92 (0,5^2))} \\ &= \frac{92}{3,5} \\ &= 74,5 \sim 75 \end{aligned}$$

With the addition of a drop out of 10%, the sample size of this study was 83 patients.

RESULTS

A. Characteristic Data

Patient data if it has been obtained then grouped by gender and age to determine the distribution of specific diarrhea patients who are hospitalized in a private hospital in the West Bekasi area.

1. By Gender

In table 1, it can be seen that patients with a diagnosis of specific diarrhea are mostly male with the amount of data obtained is 45 with a percentage of 54.2%, while for female sex the data obtained is 38, the percentage is 45.8%. The results showed that the percentage of patients with specific diarrhea by gender who underwent hospitalization was higher than that of women.

Table 1. Characteristics by Gender

Gender	Medical Record	Percentage
Laki-laki	45	54,2%
Perempuan	38	45,8%
Total	83	100%

2. By Age

The age data in this study was used to determine the number of patients with specific diarrhea suffered by adolescents, adults to geriatrics. The results obtained in adolescent to geriatric patients suffered the most in the age range of 20-44 years, namely 47 patients (56.6%), and the 45-54 year age group as many as 21 patients (25.3%). Then the lowest age range is the age group 60-69 (2.4%).

Table 2. Characteristics by Age

Age group	Medical Record	Percentage
10-14 year	-	-
15-19 year	4	4,8%
20-44 year	47	56,6%
45-54 year	21	25,3%
55-59 year	6	7,2%
60-69 year	2	2,4%
>70 year	3	3,6%

Total	83	100%

3. Route of Antibiotic Administration

On the route of giving antibiotics to specific diarrheal patients inpatients at private hospitals in the West Bekasi area for the 2020 period, the most were orally with a large percentage of 96.4%, intravenously 1.2% and drip 2.4%.

Table 3. Distribution of Patients by Route of Antibiotics

Drug Route	Medical Record	Percentage
Oral	80	96,4%
Intravena	1	1,2%
Drip	2	2,4%
Total	83	100%

4. Duration of Antibiotics

There is a long period of giving antibiotics to inpatient diarrhea patients at a private hospital in the West Bekasi area for the 2020 period, which can be seen from 1-7 days as many as 82 patients (98.8%) and 8-14 days as many as 1 patient (1.2%).

Table 4. Distribution of Patients with Duration of Antibiotics

Giving Time	Medical Record	Percentage
1-7 day	82	98,8%
8-14 day	1	1,2%
Total	83	100%

A. Pattern of Antibiotic Use

The pattern of single use of antibiotics can be shown in table 5.

Table 5. Patterns of Antibiotic Use of Diarrhea Patients in Private Hospitals in the West Bekasi Region for the 2020 Period

Pattern of Drug	Nama Obat	Medical Record	Percentage
Single	Ciprofloxacin oral	76 Oral	91,6%
Single	Ciprofloxacin Drip	1 Drip	1,2%
Single	Levofloxacin Oral	4 Oral	4,8%
Single	Levofloxacin IV	1 IV	1,2%
Single	Levofloxacin Drip	1 Drip	1,2%
Total		83	100%

The results of the evaluation of the accuracy of the administration of fluroquinolone antibiotic therapy in this study were based on the guidelines of the Minister of Health in 2011 and according to the NEJM (The New England Journal of Medicine) Acute Infectious Diarrhea. This evaluation includes several forms of criteria including the right patient, the right indication, the right dose, the right drug, and the right time of administration.

B. Evaluation of Antibiotic Use

The results of the evaluation of the accuracy of the administration of fluroquinolone antibiotic therapy in this study were based on the guidelines of the Minister of Health in 2011 and according to the NEJM (The New England Journal of Medicine) Acute Infectious Diarrhea. This evaluation includes several forms of criteria including the right patient, the right indication, the right dose, the right drug, and the right time of administration.

Table 6. Evaluation of Antibiotic Use in Patients with Specific Diarrhea in Private Hospitals in the West Bekasi Region for the 2020 Period

Parameter	(%) Accuracy	(%) Inaccuracy
Right Patient	100%	-
Right Indication	100%	-
Right Dose	100%	-
Right Drug	87,95%	12,05%
Right Time	81,93%	-
(Not right / Too Long)	-	6,02%
(Not right / Too Short)	-	12,05%

DISCUSSION

Characteristic Data

Based on the results of the study in table 1. regarding the distribution of patients according to gender characteristics above, it can be seen that patients with a diagnosis of diarrhea suffered mostly by men with the amount of data obtained by 45 patients with a large percentage of 54.2%, while for the female gender the data obtained as much as 38 percentages of 45.8%. The form of lifestyle, level of sanitation, and physical activity are factors that influence the occurrence of diarrhea. Male patients are very susceptible to diarrhea due to their lifestyle and poor sanitation levels compared to women (Riskesdas, 2013).

Based on table 2. the age data of the patients in this study, patients with specific diarrhea patients included adolescents, adults to geriatrics. The number of patients aged 20-44 years were 47 patients (56.6%), and the group aged 45-54 years were 21 patients (25.3%). This result cannot be said to be in accordance with the results of previous studies regarding the prevalence of diarrhea, stating that diarrhea is very common in children. Diarrhea that occurs in children is defined as the second leading cause of death in the world after pneumonia. The results of this study can be used as a basis, because adults are also more susceptible to diarrhea. The results of this study are in line with research conducted by Dewi (2016) which showed that there were more diarrhea patients in the adult age group (59%) compared to children.

Based on the results of a study regarding the route of antibiotic administration in hospitalized specific diarrheal patients at a private hospital in the West Bekasi area for the 2020 period the most orally with a total of 80 patients (96.4%), intravenously as many as 1 patient (1.2%) and drip in 2 patients (2.4%). Basically the route of administration of oral antibiotics should be the main choice for infection therapy, but if the patient's infection is severe, then parenteral antibiotics can be reconsidered (Kemenkes RI, 2011a). Administration of routes such as intravenous and drip are usually given for cases of patients who are weak, have nausea, severe vomiting, or are unconscious when they come to the hospital to get a quick drug effect, but if the

patient's condition is outside of these conditions, parenteral antibiotics should be replaced immediately. with oral antibiotics (Kemenkes RI, 2011a).

Based on the results of the study regarding the duration of administration in table 4 antibiotics in specific diarrhea patients hospitalized in a private hospital in the West Bekasi area for the 2020 period 1-7 days as many as 82 patients (98.8%) and 8-14 days as many as 1 patient (1.2%). It can be seen that the duration of drug administration until the patient is declared allowed to go home from the hospital is about 5-7 days for the category of diseases caused by infection (Kemenkes, 2011).

Antibiotic Usage Profile

One of the pharmacological therapies for patients with diarrhea is antibiotics. Based on table 5.4, the profile of the use of antibiotics in the research sample is ciprofloxacin and levofloxacin. This antibiotic is a fluoroquinolone class of antibiotics with a broad antibacterial spectrum. The mechanism of this antibiotic is to play a role in inhibiting DNA replication so that it has bactericidal properties. Fluoroquinolone class of antibiotics has excellent activity against gram-negative and gram-positive bacteria. In the results of table 5.4, it can be seen that the most widely used fluoroquinolone class of antibiotics is ciprofloxacin. Ciprofloxacin is a fluoroquinolone antibiotic that is used as first-line treatment for patients with infections due to invasive bacterial pathogens or in empiric treatment. Empirical treatment is therapy that uses antibiotics before the type of bacteria that infects the patient is known. Antibiotics that have a broad spectrum are very effective as empirical therapy and therefore provide optimal outcomes. The fluoroquinolone group is a class of antibiotics that according to previous researchers was considered the first choice therapy for diarrhea patients, especially in Traveler's diarrhea.

Evaluation of Antibiotic Use

The evaluation carried out in this study was based on data on the use of specific diarrheal patients using guidelines, such as the Regulation of the Minister of Health of the Republic of Indonesia No. 2406 of 2011, Dipiro 2008 : Pharmacotherapy: A Pathophysiologic Approach. Sevebth and NEJM (The New England Journal of Medicine) Acute Infectious Diarrhea. Evaluations carried out on the use of antibiotics include the right patient, the right indication, the right dose, the right drug, and the right time of administration. Patients can be said to be rational when using drugs if they receive therapy according to clinical needs including a dose, timeliness and affordable price. The use of appropriate drugs is recommended in improving the quality of health and patient therapy (Eldesi, 2015).

Right Patient

Evaluation of the accuracy of patients in the study sample is shown in table 5.6. Right patient is a form of therapy that is used by considering the patient's condition first which is generally included in the medical record that there is a patient history such as a hypersensitivity reaction (allergy). Antibiotic monotherapy given to patients in this study was ciprofloxacin in 77 cases (92.8%) while 4 cases (7.2%) were given levofloxacin. In this study, there were no patients who experienced hypersensitivity (allergy) to the fluoroquinolone antibiotics given such as ciprofloxacin and levofloxacin. Based on medical record data, the patient's exact criteria have met the criteria of 100%.

Research conducted by Winarti (2021) stated that all patients did not have complaints of hypersensitivity (allergic) reactions to the antibiotics given, so that the use of antibiotics in patients in this study could be said to be 100% correct. However, if the patient is allergic to certain

antibiotics but the antibiotics are still prescribed, then the antibiotic is declared inappropriate for the patient.

Precise Indication

Evaluation of the accuracy of the indications in the study sample is shown in table 5.6. Precise indication is the accuracy of drug administration given to patients diagnosed with diarrhea by doctors who have a specific therapeutic spectrum so that the administration is adjusted to the patient's diagnosis. Diarrhea diagnosis is determined by the doctor based on the symptoms and the results of the patient's stool test. Based on the exact criteria, the indications can be stated to have met the criteria of 100% because the administration of antibiotics is in accordance with the diagnosis determined by the doctor, namely Specific Diarrhea.

Research conducted by Okpri (2020), based on the accuracy of the indications for the use of antibiotics for diarrhea patients at the Friendship Hospital based on the Friendship Hospital Guidelines with 90 samples, said to be 100% accurate. Indications by providing therapy to patients based on physical examination, laboratory results and by only listening to the complaints experienced by patients to provide the best therapeutic action (Okpri, 2020).

Right Dosage

Evaluation of dose accuracy in the study sample is shown in table 5.6. Appropriate dosing is a drug use that must be adjusted to the therapeutic range of the drug. Based on table 5.4, it can be stated that all patients with specific diarrhea in this study sample had received antibiotics (ciprofloxacin and levofloxacin) which met the dose accuracy of 100%. Evaluation of dose accuracy was carried out based on the NEJM (The New England Journal of Medicine) Acute Infectious Diarrhea Guidelines for empirical and definitive antibiotic therapy in diarrhea patients. The recommended dose of ciprofloxacin is 500 mg orally twice daily and levofloxacin 500 mg orally once daily.

The accuracy of the dose is very necessary in the success of therapy (Baxter, K., 2008). If the use of antibiotics is given at a dose that exceeds the normal dose it can cause resistance, while the use of antibiotics with a lower dose can result in the expected therapeutic effect not being achieved because the antibiotic does not reach the MIC (Athifah, 2019). The use of appropriate antibiotics depends on the value of clinical parameters and the type of infecting bacteria as well as selecting by optimizing the pharmacodynamic dose of antibiotic use (Athifah, 2019). Monitoring of antibiotics needs to be done to see if the antibiotics given have reached therapeutic levels during treatment (Athifah, 2019).

Research conducted by Vidya (2017) showed that there were 10 cases of inappropriate antibiotic doses given to patients with diarrhea under five. Dosage inaccuracy is divided into two categories, namely underdose in 7 cases (13.46%) and overdose category in 3 cases (7.69%). Overdose is the dose or frequency given that exceeds the standard dose according to the Pediatric Dosage Handbook guidelines, while underdose is the dose or frequency given less than the standard dose according to the Pediatric Dosage Handbook guidelines. According to the analysis of the study, 41 cases (78.85%) of antibiotic doses were correct (Vidya, 2017). Dosage inaccuracy is divided into two categories, namely underdose in 7 cases (13.46%) and overdose category in 3 cases (7.69%). Overdose is the dose or frequency given that exceeds the standard dose according to the Pediatric Dosage Handbook guidelines, while underdose is the dose or frequency given less than the standard dose according to the Pediatric Dosage Handbook guidelines. According to the

analysis of the study, the correct dose of antibiotics was obtained in 41 cases (78.85%) at the right dose (Vidya, 2017).

Right Medicine

Evaluation of drug accuracy in the study sample is shown in table 5.6. The right drug is a form of conformity in the selection of antibiotics by taking into account the effectiveness of the antibiotic therapy. The choice of antibiotics used in this study both empirically and definitively was in accordance with the NEJM (The New England Journal of Medicine) Acute Infectious Diarrhea Guidelines on the use of fluoroquinolone antibiotics such as ciprofloxacin and levofloxacin for patients with specific diarrhea. Based on the criteria for the right drug, it can be stated that all patients in this study had met the criteria for the right drug by 73 patients (87.95%) and 10 patients (12.05%). The accuracy of fluoroquinolone antibiotics in this study is based on the fact that fluoroquinolones are the first of choice for the treatment of specific diarrhea (Guerrant, 2004). The inaccuracy of antibiotics is due to the use of fluoroquinolone antibiotics in patients whose stool test results show fungal infection. Fluoroquinolone antibiotics are broad-spectrum antibiotics that can inhibit or kill bacteria from both gram-positive and gram-negative groups. The mechanism of action of the fluoroquinolone class of antibiotics is to inhibit nucleic acid synthesis, by inhibiting the action of DNA gyrase (topoisomerase II) and topoisomerase IV. The half-life of ciprofloxacin is 3-5 hours and the half-life of levofloxacin is 5-7 hours (Katzung, 2018).

Research conducted by Andriani et al., (2016) obtained the results of 38 patients who received therapy according to the 2005 WHO standards, which were 100% right indication, 76.31% right drug, 76.31% right patient and 71.05% right dose (Andriani et al., 2016). If based on research conducted by Subur et al., (2020) the evaluation of drug use on the right indication, right patient and right dose indicators has reached 100%, but on the right drug indicator it is 37% and the wrong drug is 63%, the drug inaccuracy occurs in This study was due to the use of antibiotics without laboratory results showing acute diarrhea due to infection (Subur et al., 2020).

Right Time of Giving

Evaluation of drug accuracy in the study sample is shown in table 5.6. The duration of administration of antibiotic therapy depends on the severity and type of infecting bacteria. The inaccuracy of the duration of administration is if the duration of administration of the prescribed antibiotic is less than that specified by the NEJM (The New England Journal of Medicine) Acute Infectious Diarrhea Guidelines regarding the use of fluoroquinolone antibiotics. Short duration of antibiotic administration resulted in the expected clinical outcome could not be achieved, whereas if the administration of antibiotics was too long it could increase the risk of resistance, which would slow down healing. Stopping the use of antibiotics can also lead to resistance. Resistance is the resistance of microbes to certain antimicrobial substances. Resistance can occur through several mechanisms, namely, microorganisms produce enzymes that damage the active substance, microorganisms change membrane permeability to drugs, changes in the target structure of drugs, changes in metabolic pathways, and microorganisms change enzymes that function for their metabolism to become less active against drugs (Burke.A. Chunhe, 2015). Based on the proper evaluation of the duration of administration to specific diarrhea patients in the inpatient installation of a private hospital in the West Bekasi area, there is an inaccuracy in the length of administration, namely too short and too long. The accuracy of antibiotics used by specific diarrhea patients was 68 patients (81.92%) while the inaccuracy of antibiotics occurred in 15 patients, namely 5 patients

(6.02%) incorrectly with too long duration and 10 patients (12.05%) incorrectly with duration is too short. Assessment of the suitability of the duration of antibiotic administration in patients with specific diarrhea based on the suitability of the duration of antibiotic administration with the NEJM (The New England Journal of Medicine) Acute Infectious DiarrheaI guidelines. Based on the results of the study there were 10 patients (12.05%) with definitive therapy and 73 patients (87.95%) with empiric therapy. Empirical treatment is the most widely used treatment with antibiotics given before the results of the culture test or the type of bacteria that infect the patient are known, therapy can be seen from the patient's specific symptoms by first-line administration of the patient according to the guidelines. Antibiotics that have a broad spectrum are very effective as empirical therapy and therefore provide optimal outcomes. Definitive treatment is a form of therapy given, when the patient has been tested for faecal culture and the type of infected bacteria is known. According to the NEJM (The New England Journal of Medicine) Acute Infectious Diarrhea guidelines, the duration of empiric antibiotic administration in diarrheal patients is at least 3 days, while definitive therapy is at least 5 days (Guerrant, 2004).

Research conducted by Okpri (2020) related to the evaluation of the accuracy of the duration of antibiotic administration in inpatient diarrhea patients at the Friendship Hospital, it was still found that antibiotics were given with the wrong duration of administration with a duration of administration that was too short or too long. The accuracy of giving antibiotics in this study was 49 patients (46.2%), and the inappropriateness of giving antibiotics with too long a duration was 27.3%, and the administration was too short and too short was 26.40% (Okpri, 2020).

CONCLUSION

Based on the results of this study which was carried out at a private hospital in the West Bekasi area for the 2020 period, it can be concluded that based on the characteristics, the gender of the patient that dominates is male 54.2% compared to female 45.8% and based on the age of the patient with specific diarrhea. includes adolescents, adults and geriatrics. The number of patients aged 20-44 years were 47 patients (56.6%), and 45-54 years were 21 patients (25.3%).

The profile of the use of antibiotics in the study sample was treatment that used antibiotics alone, namely the fluoroquinolone group such as ciprofloxacin and levofloxacin. The results of the rationale evaluation of therapy showed that the right patient was 100%, the indication was 100%, the dose was 100%, the drug was 87.9%, and the duration of drug administration was 81.93%.

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ANALYSIS OF DRUG CHEMICALS (BKO) DICLOFENAC SODIUM IN RHEUMATIC HERBAL PRODUCTS CIRCULATED IN BEKASI CITY USING UV-VISIBLE SPECTROPHOTOMETRY METHOD

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Abstract

Introduction: Rheumatic herbal products are an alternative treatment to relieve rheumatic pain. It is not uncommon to find rheumatic herbal products containing drug chemicals (BKO) to provide a better therapeutic effect. Based on PERMENKES No. 7 of 2012, states that herbal medicine is prohibited from containing BKO. This study aims to analyze the content of BKO diclofenac sodium in five rheumatic herbal products circulated in Bekasi City.

Method: The type of study is non-experimental descriptive with non-probability purposive sampling method. The study was carried out qualitatively with organoleptic and color tests with KMnO_4 and quantitatively with UV-Visible spectrophotometry.

Results: The results of the qualitative analysis showed that five samples of herbal medicine were identified as positive because they reacted with KMnO_4 . The maximum wavelength of diclofenac sodium was found at 292 nm. The linear equation is $y = 0.0981x - 0.6501$ with a value of $r^2 = 0.9986$. The analysis results of BKO diclofenac sodium in rheumatic herbal products were found to be 152,176 mg; 144,183 mg; 128,582 mg; 170,963 mg; and 146,656 mg.

Conclusion: Based on these results, it is concluded that the content of BKO in rheumatic herbal products is contrary to PERMENKES No. 7 of 2012, which states that herbal medicine is prohibited from containing BKO.

Keywords: BKO, diclofenac sodium, drug chemical, rheumatic herbal products, UV-Visible Spectrophotometry

INTRODUCTION

Indonesia has long been able to create treatment methods based on natural ingredients that have been known empirically. This treatment method is called traditional medicine which is still trusted and used by the community (Pertiwi and Suariyani 2020). The use of traditional medicine in society is inseparable from the role of parents in preserving the nation's culture (Andriati and Wahjudi 2016). People tend to prefer traditional medicine because apprehensive of synthetic drugs' side effects and think traditional medicine is better (Ege 2021). The use of traditional medicine is considered by the community to have a quick effect on disease at affordable prices, although this can happen the other way around (Sidoretno and Oktaviani 2018).

The level of public awareness to make more use of natural ingredients as treatment has made the traditional medicine industry in Indonesia grow rapidly. The development of traditional medicine needs to be supported by various scientific studies as standardization that can be adapted to the applicable health care system (Hanum et al. 2017). On the other hand, this triggers the production of traditional medicines that do not meet CPOTB (Indonesia's Drug GMP) regulations (Pertiwi and Suariyani 2020). This has also led to the distribution of illegal traditional medicines by adding drug chemicals (BKO) to the products. The distribution of illegal traditional medicines containing BKO is certainly disturbing to the public because it can have harmful effects on health, such as side effects, contraindications, overdose, and even death (Hanum et al. 2017).

In October 2021, the Food and Drug Supervisory Agency of the Republic of Indonesia (BPOM) issued a public warning No. HM. 01.1.2.10.21.18 in the form of findings the traditional medicinal products and health supplements containing drug chemicals including diclofenac sodium, ephedrine HCl, sildenafil citrate, paracetamol, dexamethasone, and phenylbutazone. The product findings then will be followed up administratively, in the form of revocation of distribution permits, product withdrawals from the market, and product destruction. The discovery of many herbal products containing drug chemicals on the market has encouraged researchers to identify drug chemicals in traditional medicinal products in various regions of Indonesia.

A study on the analysis of drug chemicals in traditional medicinal products conducted by Tahir et al. (2018) in Makassar City, found that 3 out of 7 brands of herbal medicine contained diclofenac sodium. A subsequent study in Mataram City by Rosyada et al. (2019), also found that 3 out of 10 herbal medicine brands contained diclofenac sodium with the highest amount of 135,1982 mg. Similarly, a study on the

variant of rheumatic herbal medicine conducted by Dewi et al. (2019) in Subang City, found that 3 out of 10 herbal medicine brands contained diclofenac sodium with the highest amount of 16,11 mg.

With BPOM regulation and supervision of traditional medicinal products added with drug chemicals that do not cover the entire territory of Indonesia and there was research evidence that traditional medicinal products added with drug chemicals are still found, the researchers wanted to analyze the medicinal chemical diclofenac sodium in rheumatic herbal medicine products circulated in Bekasi City, Indonesia. This study was conducted using the UV-Visible spectrophotometric method for analysis of BKO diclofenac sodium.

METHOD

Design of Study

The design of this study is descriptive non-experimental.

Location and Time of Study

This study was conducted at STIKes Mitra Keluarga Chemical Laboratory, Bekasi City, West Java, Indonesia from February to March 2022.

Population and Sample

Samples were taken by non-probability purposive sampling technique. The study population consisted of all traditional drug stores selling rheumatic herbal products in the Bekasi City area. The sample is based on the calculation of the SLOVIN formula and applies the following criteria: products with indications of rheumatic herbal products, products in powder form, with no expiration date, and products that are of interest to the public. The number of samples determined was 5 (five) brands of rheumatic herbal products labeled with brands A, B, C, D, and E.

Instruments and materials

The instruments used were glassware (*Iwaki, Pyrex*), Analytical Balance (*Ohaus*), UV-VIS Spectrophotometer (*Thermo Scientific Genesys 10S UV-Vis v4.006*), and Micropipette (*Socorex*). The materials used were 5 (five) brands of rheumatic herbal products, diclofenac sodium standard (*Aarti*), Methanol p.a. (*Merck*), and KMnO_4 (*Merck*).

Qualitative Analysis

Organoleptic Test

The organoleptic test was carried out by observing samples of rheumatic herbal products in terms of shape, color, smell, and taste with the human senses without the help of any instruments (Ningrum, Wirasti, and Sugeng 2018).

Preparation of 1% KMnO_4 reagent

100 mg of KMnO_4 dissolved in 100 mL of distilled water, stir until dissolved. Heat at 70°C for 15 minutes. The solution was allowed to stand and then filtered. Store the solution in a tightly closed bottle and lined with aluminum foil (Maulida, Hakim, and Mohtar 2020).

Color Test with 1% KMnO_4

50 mg of samples were dissolved in methanol p.a. 1 mL, stir until dissolved and filtered. Prepare a positive control solution and a negative control solution. Positive control solution was 50 mg of diclofenac sodium standard dissolved in methanol p.a. 1 mL. The negative control solution was methanol p.a. 1 mL. The entire solution was reacted with $\pm$ 2-3 drops of 1% KMnO_4 reagent until it turned brown. The color change indicates that the solution has a double-bond compound (Dewi, Hendrayanti, and Nurhayati 2019).

Analysis Method Verification

Preparation of Diclofenac Sodium Standard Solution

50 mg of diclofenac sodium standard was dissolved in 50 mL methanol p.a., stir until dissolved (main standard solution 1000 ppm). The main solution was then diluted to 100 ppm by taking 1 mL of the solution and diluted with methanol p.a. 10 mL (standard solution 100 ppm) (Rosyada, Muliasari, and Yuanita 2019).

Maximum Wavelength Measurement of Diclofenac Sodium

Standard solution of 100 ppm was taken as much as 10 ppm and 20 ppm, then measured with UV-Vis spectrophotometry in the range of 200 – 400 nm. The result of the wavelength spectrum with the highest absorbance is defined as the maximum wavelength (Rosyada et al. 2019).

Linearity

Standard solutions of 100 ppm were made into series solutions in concentrations of 10, 11, 12, 13, 14, and 15 ppm. The blank solution (methanol p.a.) was prepared. Measure the absorbance of the entire solution with

UV-Vis spectrophotometry at a predetermined maximum wavelength. The absorbance obtained then plotted on the linear equation of concentration to absorbance. The test was replicated three times (Rosyada et al. 2019).

Accuracy

The accuracy test was carried out by the addition method. The accuracy test was carried out in the range of 80-120% with a sample and standard ratio of 70: 30. Measure the absorbance of the solution with a UV-Vis spectrophotometer and replicated it three times. Accuracy is expressed in the percentage of recovery (% recovery). The accuracy value is acceptable in the range of 80-110% (Riyanto 2016).

Precision

The standard solution of 100 ppm was taken at 10 ppm, 11 ppm, and 12 ppm, then the concentration of the solution was measured using a UV-Vis spectrophotometer and replicated three times. Precision is expressed in %RSD value. The precision value is acceptable if the %RSD value is $\leq 2\%$ (Riyanto 2016).

Quantitative Analysis

Preparation of Rheumatic Herbal Products Sample Solution

Samples of brands A, B, C, D, and E were 50 mg each and dissolved in methanol p.a. as much as 50 mL. Stir until dissolved and filtered (main sample solution 1000 ppm). Then the solution was diluted to 100 ppm (sample solution 100 ppm) (Rosyada et al. 2019).

Determination of the Amount of Diclofenac Sodium in the Sample

The sample solution of 100 ppm was measured for absorbance at the maximum wavelength that had been determined by UV-Visible spectrophotometry. The test was replicated three times. The amount of diclofenac sodium was determined by the following formula:

$$\text{Amount in the packaging (mg)} = \text{Amount in sample (mg)} \times \frac{\text{Weight of packaging (mg)}}{\text{Weight of sample (mg)}}$$

Data analysis

Data analysis was carried out based on the test results obtained in the form of maximum wavelength and the ratio of concentration to absorbance. From the graph, the statistical data will be processed descriptively.

RESULTS

Preliminary Analysis of Sample Packaging

Samples of rheumatic herbal products that have been labeled will be observed on the product tag on the packaging. The results of the analysis are shown in Table 1.

Table 1. Result of Preliminary Analysis of Sample Packaging

Product Tag	Sample

	A	B	C	D	E
Product name	+	+	+	+	+
Name and address of the manufacturer	+	+	+	+	+
Batch Number	-	-	-	-	-
Registration Number	+	+	-	+	+
Expired Date	-	-	-	-	-
Indication	+	+	+	+	+
Herbal product logo	+	+	-	+	+
Herbal product label	-	+	-	+	+

Qualitative Analysis

Organoleptic Test

An organoleptic test was carried out on each sample by observing the sample in terms of shape, color, smell, and taste. The organoleptic test results are listed in Table 2. and Figure 1.

Table 2. Result of Organoleptic Test

Sample Code	Organoleptic Test			
	Form	Odor	Flavor	Color
A	Powder	Typical herb odor	Bitter	Yellowish dark green
B	Powder	Typical herb odor	Bitter	Dark yellow
C	Powder	Typical herb odor	Bitter	White, with other colors like black, brown, and orange
D	Powder	Typical herb odor	Bitter	Light yellow
E	Powder	Typical herb odor	Bitter	White, with other colors like black, brown, and orange



Figure 1. Result of Organoleptic Test

Color test with KMnO_4

The color test was carried out to determine the presence of a redox reaction in the diclofenac sodium double bond in each sample by reacting the sample solution of rheumatic herbal medicine with KMnO_4 . A positive result is indicated by a change in the color of the solution to a brown color. The color test results are listed in Table 3. and Figure 2.

Table 3. Result of Color Test

Sample Code	Result of Color Test		Conclusion
	Before Test	After Test	
A	Dark yellow	Light brown	+
B	Light yellow	Light brown	+
C	Clear	Light brown	+
D	Light yellow	Light brown	+
E	Clear	Light brown	+
Control (+)	Clear	Dark brown	+
Control (-)	Clear	Purple	-

Conclusion :

+ : Contain double-bond groups

- : Does not contain double-bond groups

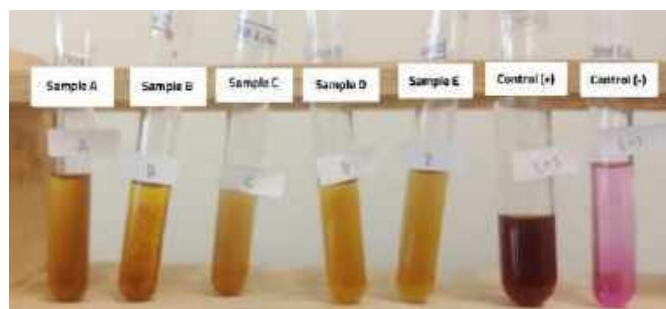


Figure 2. Result of Color Test

Analysis Method Verification

Determination of the Maximum Wavelength of Diclofenac Sodium

Determination of diclofenac sodium levels using the stand of diclofenac sodium with concentrations of 10 ppm and 20 ppm. The maximum wavelength was found at 292 nm. The determination of the maximum wavelength is shown in Figure 2.

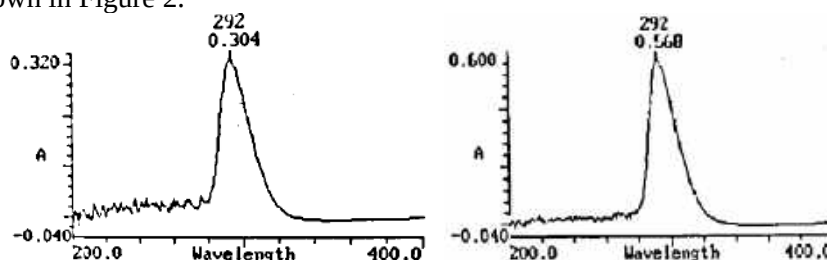


Figure 2. Maximum wavelength diclofenac sodium at 10 dan 20 ppm

Linearity

The linearity test was carried out by measuring the absorbance of diclofenac sodium standard solution series with concentrations of 10, 11, 12, 13, 14, and 15 ppm at a maximum wavelength of 292 nm. The absorbance value against the concentration was plotted on a linear regression equation which then obtained the following results: $y = 0.0981x - 0.6501$ with r^2 value = 0.9986. The standard curve of diclofenac sodium is shown in Figure 3.

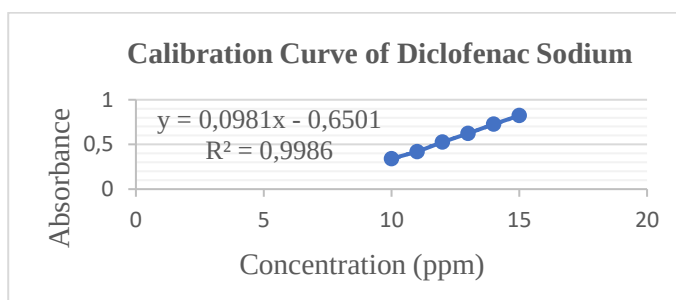


Figure 3. Calibration Curve of Diclofenac Sodium

Precision

The precision test was carried out by measuring the absorbance of the standard solution of diclofenac sodium at concentrations of 10, 11, and 12 ppm with three replications. The results of the precision test showed that the %RSD at each concentration was 0.24%; 1.02%; and 0.70%. The test results have met the precision requirements, namely %RSD 2%. The precision test results are shown in Table 4.

Table 4. Precision Test

Conc. (ppm)	Abs	Measured Conc. (ppm)	Avg. Conc. (ppm)	SD	RSD (%)
10	0,402	10,725	10,749	0,026	0,24%
	0,404	10,745			
	0,407	10,776			

11	0,453	11,245	11,306	0,115	1,02%
	0,452	11,234			
	0,472	11,438			
12	0,590	12,641	12,563	0,088	0,70%
	0,573	12,468			
	0,584	12,580			

Accuracy

The accuracy test was carried out by measuring the absorbance of the standard solution to which the sample solution had been added with a ratio of 70: 30. The accuracy test was carried out in the range of 80%, 100%, and 120% with three replications. The results of the test show that each range has met the accuracy requirements, 80 – 110%. The results of the accuracy test are shown in Table 5.

Table 5. Accuracy Test

Range	Abs	Sample Conc. (ppm)	Standard Conc. (ppm)	% Recovery	Avg. Recovery (%)
80%	0,297	6,72	2,88	101,89%	101,89%
	0,288	6,72	2,88	98,70%	
	0,306	6,72	2,88	105,08%	
100%	0,537	8,40	3,60	102,80%	101,10%
	0,525	8,40	3,60	99,41%	
	0,531	8,40	3,60	101,10%	
120%	0,773	10,08	4,32	102,47%	101,92%
	0,764	10,08	4,32	100,34%	
	0,775	10,08	4,32	102,94%	
Entire Avg conc. (%)					101,64%

Quantitative Analysis

Determination of the Amount of Diclofenac Sodium in Sample

Determination of the amount of diclofenac sodium in the sample was carried out by measuring the absorbance of the 100 ppm sample solution. The absorbance was plotted on a linear regression equation so that the diclofenac sodium content in the sample was obtained. The results of diclofenac sodium levels (ppm) were converted into the form of amounts (mg). The results of the precision test are shown in Table 6.

Table 6. Determination of the Amount of Diclofenac Sodium in Sample

Sample Code	Abs	Amount in Sample (mg)	Amount in the packaging (mg)	Avg amount (mg)	Conclusion
A	0,291	0,959	151,373	152,176	N
	0,289	0,957	152,253		
	0,293	0,961	152,901		
B	0,360	1,030	144,153	144,183	N
	0,380	1,050	144,408		
	0,377	1,047	143,987		
C	0,314	0,933	129,816	128,582	N
	0,299	0,986	136,380		
	0,256	0,868	119,549		
D	0,291	0,983	175,495	170,963	N
	0,289	0,967	173,108		
	0,293	0,924	164,285		
E	0,278	0,946	130,622	146,656	N
	0,268	0,936	128,960		
	0,624	1,299	180,386		
Entire Average amount (mg)				148,512	N

Conclusion :

N = Not in accordance with CPOTB regulations.

DISCUSSION

Preliminary Analysis of Sample Packaging

A total of five samples were pre-analyzed on the sample packaging. In addition to looking directly at the packaging, this analysis is carried out by checking the product on the official BPOM RI website, namely www.cekbpom.pom.id with product name, registration number, and packaging categories. Based on Table 1. it was found that all samples did not meet the requirements set for traditional medicinal products that could be circulated in Indonesia. Incompatibility of tag on sample packaging with applicable regulations is found in batch number, registration number, expiration date, herbal medicine logo and label.

In Table 1. it is known that all samples do not include batch numbers, while registration numbers are listed in all samples except sample C. The results of checking on the BPOM website also note that samples A, B, D, and E cannot be found in various categories so it can be concluded that the registration number listed on the packaging is fake. This is not by the provisions of PERMENKES No. 7 of 2012, which states that traditional medicinal products must have a distribution permit number. This is also in line with PerKB POM RI No. HK.00.05.41.1384 of 2005, which regulates the criteria and procedures for registering traditional medicines to be able to register traditional medicinal products at BPOM, various documents must be included, including claims for indications, the dosage of use, and batch number. If viewed from the results of the analysis obtained, it can be concluded that all samples are not officially registered with BPOM.

The batch number is a marker of the number of products made in one cycle so that they have uniform characteristics and quality, while the registration number is a form of approval that the product can be circulated in Indonesia. If these two things are not listed on the packaging, it can be concluded that the sample product cannot be circulated in Indonesia and may be subject to sanctions. The expiration date of traditional medicinal products is also regulated in PerKB POM RI No. HK. 03.1.23.06.10.5166 of 2010, with a definition in the form of a description of the time limit for traditional medicines that are suitable for consumption. Article 6 states that traditional medicines must include an expiration date on the label. In Table 1. it is found that all samples include expiration dates so they do not meet the applicable provisions.

The traditional medicine logo has been established in BPOM RI Decree No. 00.05.4.2411 of 2004 which regulates the grouping of traditional medicines so the shape of the logo and label attached to the packaging of traditional medicine is very important to explain the type of traditional medicine of a product. In Table 1. it is found that samples B, D, and E have the appropriate herbal medicine logos, sample A includes the herbal medicine logo but does not include the herbal medicine label, while sample C does not include either the herbal medicine logo or label.

The results of the initial identification on the packaging showed that the samples did not meet the applicable provisions. It can be concluded that the samples are products that should not be circulated in Indonesia. However, this is accompanied by the fact that these herbal products are products that are of interest to the public, requiring more attention to herbal products circulated in the community. BPOM RI as a regulator needs to supervise the circulation of these products.

Organoleptic Test

The organoleptic test was not only intended to determine the characteristics of the sample but also aimed to determine the similarity of the description of the sample with BKO diclofenac sodium. In Table 2. it is stated that all samples have the same shape, smell, and taste, namely in the form of a powder with a characteristic odor of herbal medicine and a bitter astringent taste. The five samples have different colors because the types of plants made for herbal products are also different. Herbal medicine can be made from various plant parts such as roots, leaves, stems, fruits, rhizomes, or other parts that give a certain color to the product. Samples C and E have a striking color difference because there are some parts of the powder that are not homogeneous in the form of a mixture of white, black, orange, and brown powders. According to the Indonesian Pharmacopoeia edition VI (2020) of the BKO diclofenac sodium, it is a white to almost white crystal powder (Depkes RI 2020). With the discovery of white powder in samples C and E, it can be indicated that both contain BKO diclofenac sodium.

Color Test

The color test is intended to determine the ratio of the reactions formed in the samples of herbal medicine and BKO diclofenac sodium when reacted with KMnO_4 . In this color test, all samples were tested with positive and negative controls. The positive control was a standard solution of diclofenac sodium in methanol while the negative control only contained solvent. Diclofenac sodium has an alkene group in its chemical structure and will react with KMnO_4 . The reaction mechanism between diclofenac sodium and KMnO_4 is as follows:

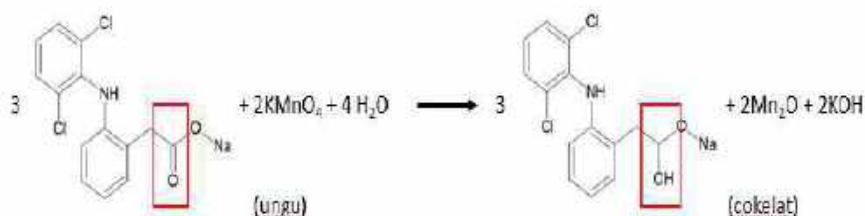


Figure 4. Reaction Mechanism of KMnO_4 with alkene group on diclofenac sodium

In Table 3. It is known that all study samples and positive controls reacted with KMnO_4 and produced a brown color change. These results concluded that all samples of herbal medicine had a double bond group which was oxidized by KMnO_4 . However, this color change also does not mean that the sample contains BKO diclofenac sodium because the alkene group is found in secondary metabolites of medicinal plants such as alkaloids, tannins, flavonoids, saponins, steroids, and so on. Therefore, the results of this color test will be continued to the next analysis, namely quantitative analysis using UV-Visible spectrophotometry to confirm the presence of BKO diclofenac sodium.

Analysis Method Verification

Determination of the Maximum Wavelength of Diclofenac Sodium

Determination of the maximum wavelength aims to determine the maximum sensitivity of diclofenac sodium in the UV-Visible wavelength range. Determination of the maximum wavelength requires a standard solution of diclofenac sodium 10 ppm and 20 ppm in methanol p.a. solvent. Both solutions were measured in the wavelength range of 200-400 nm. The results of the measurement of the maximum wavelength of diclofenac sodium at 292 nm at 10 ppm with an absorbance value of 0.304 and 20 ppm with an absorbance value of 0.568. At a wavelength of 292 nm, an absorption peak was formed which indicated that the compound detected by the spectrophotometer was diclofenac sodium. This wavelength was selected as the maximum wavelength of diclofenac sodium and used for subsequent analysis.

Linearity

Linearity is one of the parameters of the method verification test that was carried out to determine the ability of the spectrophotometric method to detect the concentration of diclofenac sodium in the sample. Linearity is expressed in the correlation coefficient (r) of the linear regression curve $y = a + bx$ with an ideal value of $a = 0$ and $r = +1$ or -1 (Riyanto 2016). The linearity test was carried out by measuring the absorbance of the standard diclofenac sodium solution at various concentrations of 10 ppm, 11 ppm, 12 ppm, 13 ppm, 14 ppm, and 15 ppm and replicated three times to obtain consistent absorbance results. In Figure 3. It is shown that the calibration curve formed from the variation in concentration gives an absorbance response according to Lambert's Beer's law, which is in the range of 0.2 - 0.8. The calibration curve for each replication gives the coefficient of determination (r^2) values, respectively, as follows: 0.9941; 0.9986; 0.9944. The calibration curve is declared to meet the linearity criteria because it has a value of R^2 0.99. With the fulfillment of the linearity criteria, the UV-Visible spectrophotometric method can be used as a method for the analysis of BKO diclofenac sodium in rheumatic herbal products. Based on these results, replication 2 gave a better r^2 value with the equation $y = 0.0981x - 0.6501$ so the linear equation in replication 2 was chosen as the basis for measurement in the next analysis.

Precision

Precision is a parameter of the method verification test or the accuracy carried out to determine the

suitability of repeated measurements on the same sample, in this case, the measurement of diclofenac sodium levels (Riyanto 2016). Precision is calculated based on the standard deviation or relative standard deviation (coefficient of variation) of the sample measurement results. Precision is applied according to the ICH procedure, which is a minimum of nine measurements (three concentrations with three replications) or a minimum of six measurements at one concentration (ICH 2005). The generally accepted precision criterion is 2%. The precision test was applied to three different concentrations of 10 ppm, 11 ppm, and 12 ppm and replicated three times. The results of the precision test at these concentrations showed the %RSD values successively as follows: 0.24%; 1.02%; and 0.70%. The %RSD value is declared to have entered the required precision criteria, namely 2%. With these precision results, it can be stated that the UV-Visible spectrophotometric method provides good precision and the method can be used for subsequent analysis.

Accuracy

Accuracy as a verification test parameter is intended to determine the degree of accuracy of the measurement results with the actual levels. The accuracy test is expressed in % recovery (% recovery). The accuracy test was applied based on the ICH procedure, namely the measurement of a minimum of three concentrations with three repetitions (replication). The accuracy test in this study used the addition method, in which a sample of herbal medicine was added with a standard amount of diclofenac sodium and the absorbance was measured, then the recovery rate was calculated using a predetermined regression equation. In addition method, the accuracy test is measured in the concentration range of 80%, 100%, and 120%. The comparison between the sample used and the standard added was 70: 30. The accuracy results in the range of 80%, 100%, and 120% respectively were 101.89%; 101.10%; and 101.92%. The average overall recovery is 101.64%. Acceptable accuracy criteria for an analyte concentration of 10 ppm are in the range of 80-110% (Riyanto 2016). Based on these results, it can be stated that the UV-Visible spectrophotometric method used in this study has an acceptable level of accuracy.

Quantitative Analysis

The amount of Diclofenac Sodium in the sample was determined based on a predetermined linear regression equation. The results of the calculation have known levels of diclofenac sodium (ppm) which is then converted into the form of amounts (mg). In Table 6. it is stated that samples A, B, C, D, and E were positive for diclofenac sodium in the range of 120 mg – 175 mg per package. The average amount of BKO obtained was 148,512 mg. The amount of BKO obtained has exceeded the maximum dose of diclofenac sodium per day, which is 150 mg. The tag of how to use the herbal medicine packaging varies between 2-3 times of consumption per day. Of course, this result is worrying because it can pose a health hazard such as overdose. All of the following analysis results are declared not to meet the requirements of PERMENKES No. 7 of 2012 that herbal medicine may not contain medicinal chemicals or psychotropic drugs and narcotics or other substances that are considered to be harmful to health. This is also in line with PerBPOM No. 32 of 2019, which establishes that traditional medicine manufacturers must ensure the safety and quality of traditional medicines. If this is not complied with, administrative sanctions can be imposed as established in PerKB POM RI No. 5 of 2016 such as written warnings, product withdrawals from circulation, and production cessation.

CONCLUSION

Based on the results of the study, the analysis of BKO diclofenac sodium in rheumatic herbal medicine products has been carried out qualitatively with organoleptic tests and color tests and quantitatively with UV-Visible spectrophotometric methods. The results of the study concluded that samples A, B, C, D, and E of rheumatic herbs circulating in Bekasi City were positive for BKO diclofenac sodium.

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DETERMINATION OF RETINOIC ACID LEVELS IN FACIAL WHITENING CREAM FOR SALE IN BEKASI CITY USING UV-Visible SPECTROPHOTOMETRY

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Abstract

Introduction: Retinoic acid is a substance used in the treatment of acne by prescription. Its use in cosmetics has been prohibited by the Food and Drug Supervisory Agency of the Republic of Indonesia in regulation Number 23 of 2019 (BPOM RI, 2019). The purpose of this study was to determine the validity of the UV-Visible spectrophotometry method and the levels of retinoic acid in the preparation of facial whitening creams sold in the Bekasi City

Method: This research is a non-experimental type of research and the sample is taken using the purposive sampling method. Qualitative analysis with a thin layer chromatography and quantitative analysis with UV-Visible spectrophotometry.

Results: Retinoic acid has a maximum wavelength of 355 nm. The results of the linearity test were shown by the correlation coefficient (r) = 0.9983, the accuracy-test obtained the retinoic acid recovery value of 100.70%, and the precision test with the RSD value of 1.84%, the LOD value of 0.434 ppm and the LOQ of 1.448 ppm. The results of the analysis showed that of the 12 samples studied, there were 3 positive samples containing retinoic acid with an average level of 2.39% in S9, 2.96% in S10, and 0.86% in S12.

Conclusion: The validity of the UV-Visible spectrophotometric method meets the requirements. There are 3 samples of facial whitening cream containing retinoic acid so it does not meet the requirements set by BPOM.

Key words: Retinoic acid, Face Whitening Cream, UV-Visible Spectrophotometry

INTRODUCTION

Whitening cream is a cosmetic product that has recently been in great demand by the public, especially women. Whitening creams that contain a mixture of chemicals to whiten the skin are sold freely in the *marketplace*. In addition, many irresponsible whitening cream manufacturers include a mix of chemicals and hazardous materials such as metallic mercury, K3 dye, and retinoic acid into their products (Wijaya, 2013).

In 2016, a public warning was issued by the Food and Drug Supervisory Agency of the Republic of Indonesia regarding cosmetics with a mixture of hazardous chemicals and 6% of them contain retinoic acid. In 2017, the use of retinoic acid in cosmetics increased to 10% and increased to 21% in 2018. The use of retinoic acid in cosmetics has been prohibited by the Head of the Food and Drug Supervisory Agency, further stated in regulation No. 23 of 2019 concerning technical requirements for ingredients cosmetics. (BPOM RI, 2019).

The use of retinoic acid can only be obtained with a doctor's prescription. Generally, doctors prescribe retinoic acid as an acne treatment with levels of 0.05% -0.1%. In addition to treating acne, retinoic acid is also often used to treat skin damaged by exposure to the sun and is used as a bleach. However, the use of retinoic acid can also cause dangerous side effects, namely in the first week of use it can cause the skin to become irritated and red, then feel hot and burn and dry and peel (Riyanto, 2014). Excessive use of retinoic acid is teratogenic for pregnant women (Kumar *et al.*, 2019). Research on the analysis of retinoic acid in cosmetics in the form of creams have been carried out by Ghina *et al.* (2015) in the city of Bandung using the TLC-UV-Visible spectrophotometry method. Another study conducted by Wardhani *et al.* 2019 with the analysis of retinoic acid in cosmetics in Klaten City using the UV-Visible spectrophotometric method, Agustina *et al.* thin layer chromatography method to analyze retinoic acid contained in night cream preparations at the Klaten market and Perdina Nursidika *et al.* (2019) using the TLC-UV-Visible Spectrophotometry method.

METHOD

Research

Design The design of this study was a non-experimental study with purposive sampling.

Population and Sample

The population in this study is a face whitening cream that is sold in the city of Bekasi with sales through the marketplaces X, Y, and Z. The inclusion criteria, namely whitening cream without notification number, has a price of less than Rp. 50,000, branded and much in demand by the public. The exclusion criteria were whitening cream that did not come after purchase and whitening cream that did not match the product ordered. The research was carried out at the Pharmaceutical Chemistry Laboratory, Mitra Keluarga College of Health, Bekasi in February-March 2022. The research variables included the type of independent variable, namely retinoic acid levels.

Tools and Materials

The tools used include analytical scales (Ohaus), spatula, watch glass, beaker glass (Iwaki Pyrex), measuring flask (Iwaki Pyrex), funnel (Iwaki Pyrex), volume pipette, capillary tube, dropper pipette, aluminum foil, stirring rod, Whatman No. 41 filter paper, UV lamp²⁴⁵, chromatography vessel, silica gel 60F²⁵⁴, UV-Vis spectrophotometer (Genesys 10S UV-Vis), cuvette, retinoic acid pro-analysis (Acros organics), facial whitening cream obtained online marketplace through) in Bekasi City, methanol pro-analysis (Emsure, ethanol absolute pro-analysis (Smart lab), acetone, n-hexane pro-analysis (Emsure) and phosphomolybdic acid pro-analysis (Emsure).

Qualitative Analysis

Performed by making the mobile phase first. The mobile phase for qualitative analysis of retinoic acid was a mixture of n-hexane-acetone solvent with a ratio of 6:4 v/v and made in a volume of 30 mL. Then the TLC plate was activated by heating it in the oven at 105^oC for 30 minutes. Previously, a lower boundary line of 1 cm and an elution boundary of 1.5 cm from above were drawn on the TLC plate to be activated. The mobile phase is inserted into a 10 x 10 cm chamber. The chamber is saturated using filter paper which is placed into the chamber. Leave until the solvent rises until the filter paper is completely wet. The standard solution and sample solution were smeared using a capillary tube on a heated TLC plate and allowed to dry. After the chamber is saturated, the TLC plate which has been stained with the sample solution is inserted into the chamber phase is allowed to rise until it approaches the elution limit. The TLC plate was removed and allowed to air dry observed under UV²⁵⁴ and after being sprayed with a 5% phosphomolybdic acid staining solution in ethanol (BPOM, 2019).

Preparation of Standard Solution of 1000 ppm Retinoic Acid

Made by weighing 0.1 g of standard retinoic acid then put into a glass beaker and dissolved with 100 mL of methanol (Suhartini *et al*, 2013).

Preparation of 500 ppm Retinoic Acid Standard Solution was

Made by taking 25 mL of 1000 ppm retinoic acid solution, then put into a 50 mL volumetric flask and added methanol to the mark (Suhartini *et al*, 2013).

Preparation of Standard Solution 100 ppm Retinoic Acid

Made by taking as much as 10 mL of 500 ppm retinoic acid solution, then put into a 50 mL volumetric flask and added methanol up to the mark.

Preparation of 50 ppm Retinoic Acid Standard Solution was

Made by taking 25 mL of 100 ppm retinoic acid solution, then put into a 50 mL volumetric flask and added methanol to the mark.

Determination of Maximum Wavelength

Made by pipetting 1 mL of 50 ppm retinoic acid solution and put into a 10 mL volumetric flask (concentration 5 ppm), then add methanol to the mark and homogenize. Measured absorption maximum at a wavelength of 200-400 nm using a blank. The blank used was methanol (Suhartini *et al*, 2013).

Preparation of Standard Curve and Linearity Test

Made by pipetting 0.2 mL, 0.3 mL, 0.4 mL, 0.5, and 0.6 mL (1 ppm concentration, 1.5 ppm, 2 ppm, 2.5 ppm, and 3 ppm) 50 ppm retinoic acid solution and put into a 10 mL volumetric flask, then add methanol to the mark and homogenize. The maximum absorbance was measured at a wavelength of 200-400 nm using a blank and replicated 3 times. The blank used was methanol (Suhartini *et al*, 2013).

Precision Test

Made by pipetting 0.2 mL of retinoic acid standard solution with a concentration of 1 ppm, then the absorbance was measured at the maximum wavelength and replicated six times. The data obtained is then calculated as standard deviation and relative standard deviation using Microsoft excel (Fauziah, 2016).

Accuracy Test

Performed using spiked-placebo recovery method. A number of analytes were added to a pharmaceutical preparation mixture that did not contain analytes (placebo) and then analyzed and compared with the actual analyte levels (added analytes). The absorbance results obtained are then calculated as the value of % recovery or recovery. A total of 3.0 g of cream samples that did not contain retinoic acid were prepared and then added with standard retinoic acid of 80.0 mg, 160.0 mg, and 240.0 mg so that the concentrations of 1 ppm, 2 ppm, and 3 ppm were obtained. The maximum absorption of the three concentrations of the solution was measured at a wavelength of 355 nm using a methanol blank and replicated every 3 times (triple).

Determination of LOD and LOQ

Limit of Detection (LOD) and Limit of Quantitation (LOQ) is calculated statistically by looking at the linear regression equation from the standard a curve that is formed. The absorbance results from the standard solution are then calculated and entered into the obtained linear regression equation (Fauziah, 2016).

Sample Preparation

Made by weighing the cream sample as much as 3.0 g. Then put it in a glass beaker and wrapped in aluminum foil. 10 mL of methanol was added and mixed until homogeneous using a vortex mixer for 5 minutes. Next, cool the homogenized cream sample in the refrigerator for 15 minutes and filter it using Whatman No. 41 filter paper in a 50 mL volumetric flask. The filtrate obtained was then added with methanol to the mark and homogenized. A total of 5 mL of the diluted filtrate was then pipetted and put into a 10 mL volumetric flask, then methanol was added to the mark and homogenized. 0.5 mL of the filtrate was pipetted and put into a 10 mL volumetric flask and then added methanol to the mark and homogeneous. A total of 0.25 mL of the filtrate was pipetted again and then added methanol to the mark and was homogeneous and then the absorbance was measured at a wavelength of 200-400 nm (Suhartini *et al*, 2013).

Determination of Retinoic Acid Levels

The determination of retinoic acid levels in the sample is calculated using the following formula:

$$\left(\frac{A_u}{A_s} \right) \left(\frac{C_s}{C_u} \right) \times 100$$

Description:

Au = Absorbance of the test solution/sample

As = Absorbance of standard solution

Cs = Content of retinoic acid in standard solution (mg/mL)

Cu = Content of retinoic acid in test solution (mg/mL)

RESULTS

Sampling

Facial whitening cream used as a sample was taken as many as 12 products. The sample used came from Bekasi City with online through 3 marketplaces and was selected based on inclusion and exclusion criteria. The cream samples were coded as samples S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11 and S12.

Qualitative Analysis

Performed using thin layer chromatography method by calculating the R_f value. The results of the qualitative analysis can be seen in **Table 1**.

Table 1. Results of qualitative analysis of retinoic acid on facial whitening cream

Sample Code	Color	Rf	Resolution	Conclusion
Standard	Dark green	0,66	Not detected	Positive
S1	Not detected	Not detected	Not detected	Negative
S2	Not detected	Not detected	Not detected	Negative
S3	Not detected	Not detected	Not detected	Negative
S4	Not detected	Not detected	Not detected	Negative
S5	Not detected	Not detected	Not detected	Negative
S6	Not detected	Not detected	Not detected	Negative
S7	Not detected	Not detected	Not detected	Negative
S8	Not detected	Not detected	Not detected	Negative
S9	Dark green	0,50	Not detected	Positive
S10	Dark green	0,51	2	Positive
S11	Not detected	Not detected	Not detected	Negative
S12	Dark green	0,54	2,8	Positive

Here are the results of the qualitative analysis using TLC:

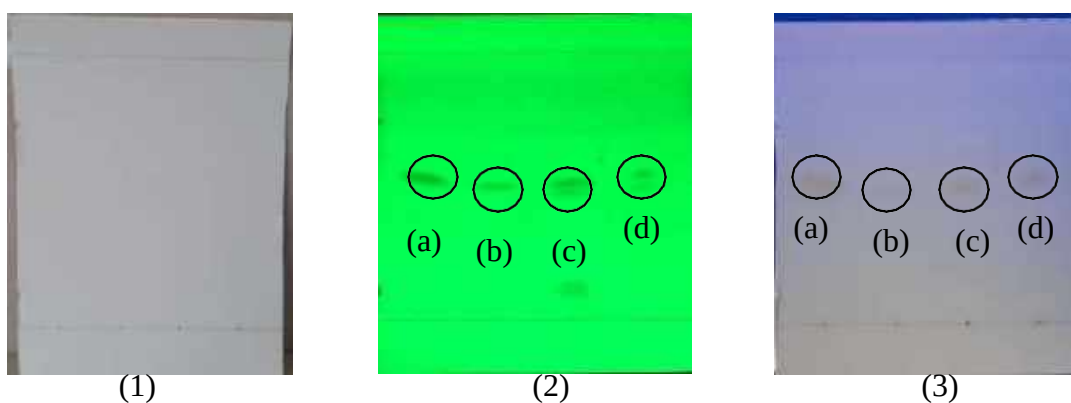


Figure 1. TLC Results Before Spraying

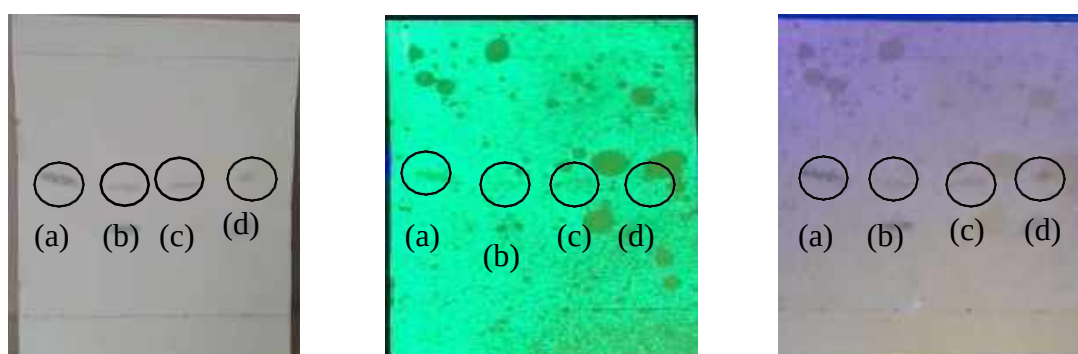


Figure 2. TLC results after spraying

Information:

- (1) = TLC result in visible light
- (2) = TLC result under UV light 245
- (3) = TLC result under UV light 366

- (a) = Standard stain of retinoic acid (Rf 0,66)
- (b) = sample stain S9 (Rf 0,50)
- (c) = sample stain S10 (Rf 0,54)
- (d) = sample stain S12 (Rf 0,51)

Based on the analysis it is known that 3 out of 12 of the sample tested positive for retinoic acid. The samples are samples with codes S9, S10 and S12. Positive results are indicated by the formation of the same color stain, namely dark green between the reference standard and the sample after spraying and the Rf value of the sample is close to the Rf value of the comparison standard retinoic acid.

Determination of the Maximum Wavelength The maximum

Wavelength of retinoic acid is 355 nm with an absorbance of 0.616. The following are the results of the maximum wavelength spectra obtained:

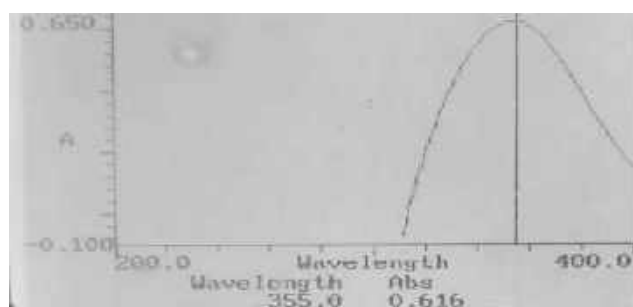


Figure 3. Maximum wavelength spectra of retinoic acid

Determination of the Retinic acid Standard Curve

Determination of the standard curve for retinoic acid using 5 concentration series, namely 1 ppm, 1.5 ppm, 2 ppm, 2.5 ppm and 3 ppm. The results of the standard curve of retinoic acid are shown in **Figure 4**.

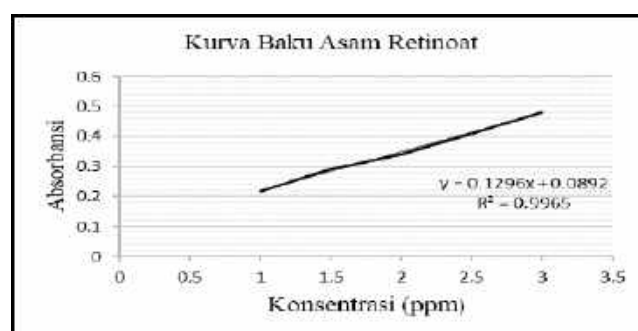


Figure 4. Standard curve of retinoic acid

Linearity Test

In this test, the regression equation obtained from the standard curve is $y = 0.1296x + 0.0892$ and the correlation coefficient (r) is 0.9983. This value already shows a good linearity relationship because it is close to 1.

Precision

Test The precision test that has been carried out gives the result of the %RSD value of 1.84%. The precision results have met the criteria for acceptable value requirements according to Gonzales and Herrador, namely the precision requirement for an analyte content of 1 ppm is less than 16% (Gonzales and Herrador, 2007). Other requirements according to the AOAC, precision can be accepted if it gives results in the form of coefficient of variation (KV) or relative standard deviation (RSD) <2%. Precision test results are shown in **Table 2**.

Tables 2. Precision test results

Replication	Actual concentration (ppm)	Absorbance	Level obtained (ppm)
1	1	0,225	1,048
2	1	0,224	1,040
3	1	0,226	1,056
4	1	0,223	1,032
5	1	0,227	1,063
6	1	0,220	1,009
Average			1,041
SD			0,019
%RSD			1,84

Accuracy Test

In the accuracy test carried out, the average value of % *Recovery* or recovery is 100.70%. These results have met the requirements for reacquisition. In theory, the accuracy requirement is if the recovery received is 80-110%. The results of the accuracy test are shown in **Table 3**.

Table 3. The results of determining accuracy

Standard addition concentration	Rep.	Absorbance	(%) Recovery	(%) Recovery average
1	1	0,221	102%	101,18%
	2	0,222	102%	
	3	0,218	99%	
2	1	0,358	103,70%	103,06%
	2	0,359	104,09%	
	3	0,352	101,39%	
3	1	0,478	100%	97,86%
	2	0,460	95,37%	
	3	0,471	98,20%	
Recovery average				100,70

Determination of LOD and LOQ The

Determination of LOD and LOQ gives the results of the LOD value of 0.434 ppm and the LOQ value of 1.448 ppm. These results can be seen in **Table 4**.

Table 4. Results of determining accuracy

Parameter	Result
SE of Intercept:	0,009
SD of Intercept:	0,019
LOD:	0,434
LOQ:	1,448

Determination of Retinoic Acid Levels

Determination of retinoic acid levels in face whitening cream preparations in samples S9, S10 and S12 the average grade value is 2.39% for sample S9, 2.96% for sample S10 and 0.86% for sample S12. The three samples did not meet the requirements set by the Regulation of the Food and Drug Supervisory Agency of the Republic of Indonesia regarding the content of retinoic acid in cosmetics. The following is a table of the results of the determination of retinoic acid levels:

Table 5. Retinoic acid levels in samples of facial whitening cream

Sample code	Rep.	Abs.	Sample weight (mg)	Retinoic acid level (%)	Average % b/b	SD	Desc.
S9	1	0,695	3000,0	2,493%	2,39%	0,172	Not eligible
	2	0,661	3000,0	2,353%			
	3	0,652	3000,0	2,316%			
S10	1	0,775	3000,0	2,822%	2,96%	0,221	Not eligible
	2	0,822	3000,0	3,016%			
	3	0,827	3000,0	3,036%			
S12	1	0,292	3000,0	0,835%	0,86%	0,035	Not eligible
	2	0,298	3000,0	0,859%			
	3	0,301	3000,0	0,872%			

DISCUSSION

Research on the determination of retinoic acid levels in facial whitening cream preparations circulating in the city of Bekasi with sales through the marketplace by TLC-UV-Vis spectrophotometry, which aims to determine the presence or absence of retinoic acid in the face whitening cream sample. This refers to the Regulation of the Head of the Food and Drug Supervisory Agency Number 23 of 2019 concerning the technical requirements of cosmetic ingredients. The regulations do not allow the presence of retinoic acid in cosmetics. In this study, the sample used is facial whitening cream which is sold in the city of Bekasi with sales through the marketplace. A total of 12 samples were obtained from 3 marketplaces after previously determining the sampling. A total of 4 samples were obtained from marketplace X, 5 samples were obtained from marketplace Y and 3 samples were obtained from marketplace Z.

Qualitative analysis using thin-layer chromatography (TLC) was carried out to identify retinoic acid contentor separating retinoic acid from other compounds contained in the face whitening cream sample. The mobile phase is determined by looking at the polarity of the analyte to be analyzed. In this study, the mobile phase used was a mixture of n-hexane-acetone (6:4) v/v which was made in a volume of 30 mL. The choice of the solvent mixture in the mobile phase is determined based on its polarity. Based on the calculations, the mobile phase in this study has a polarity index of 2.1 which indicates the mobile phase is a semi-polar solvent. The polarity index is an empirical quantity that is used to measure the attraction between molecules in a solute and the solvent molecules on the solubility parameters of the solvent in question in its pure state (Rubiyanto, 2016). The higher the polarity index, the more polar the solvent. While the stationary phase of silica gel GF₂₅₄ has more polar properties. Meanwhile, the analyte in this case retinoic acid has a low polarity. In the separation, the non-polar mobile phase will hold polar compounds in the polar stationary phase and bring non-polar compounds up to the top.

TLC plate activation was performed before cream sample analysis. This activation is carried out to activate the silanol and siloxane groups contained in silica and play a role in adsorption (Harmita, 2004). The surface of the silica gel is a siloxane group (Si-O-Si) and a silanol group (Si-OH) and is slightly acidic and polar so it can interact with solutes that are slightly polar to very polar. The -OH group in silanols will form hydrogen bonds with the -OH groups of retinoic acid (C₂₀H₂₈O₂) as shown in the figure below:

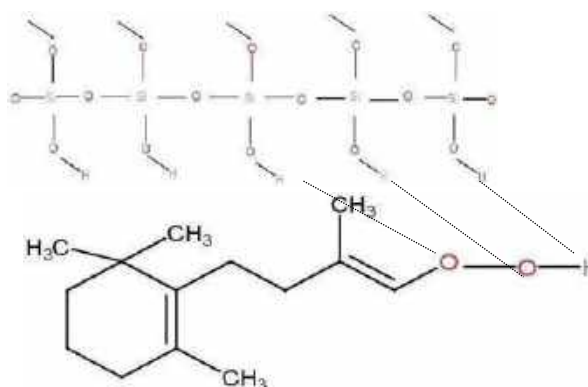


Figure 5. Interaction between silanol groups and retinoic acid

Activation in this study was carried out by drying the TLC plate in an oven at 105^oC for 30 minutes. In addition, it is necessary to saturate the chamber. This is because the interaction between the TLC plate sorbent and the solvent vapor molecules depends on the saturation of the chamber (Wulandari, 2011). Saturation is done by placing the filter paper vertically into the chamber containing the eluent. And let the eluent rise to move along the filter paper and wet the entire filter paper. The eluent used in this analysis is a mixture of n-hexane-acetone (6:4).

Retinoic acid will give blue to dark green spots by spraying phosphomolybdic acid when analyzed using TLC (Ministry of Health RI, 2020). Of the 12 samples of whitening cream analyzed in this study, 3 of them gave results in the form of dark green spots. The three samples are samples with codes S9, S10, and S12. The calculation of the R_f value was then carried out to determine the possibility that the sample with dark green spots was the analyte. The result of the R_f value obtained in the S9 sample is 0.50. The sample S10 gave three spots, namely spot A with an R_f of 0.12, spot B with an R_f value of 0.46, and spot C with an R_f value of 0.51. Meanwhile, the S12 sample gave two spots, namely spot A with an R_f of 0.47 and spot B of 0.54. Resolution calculations were carried out on sample spots S10 and S12. This is because samples S10 and S12 formed two stains so the resolution calculation was used to ensure that the spots formed were well separated. In the S10 sample between node A to node B, the resolution value is 2, while in the S12 sample, between node A and node B, the resolution value is 2.8. The separation of substances in samples S10 and S12 has given good results. This is to the requirements for the separation or resolution of the analyte with other substances, it will be said to be good if the value is > 1.5 (Harmita, 2004). A sample is declared positive if in the qualitative analysis it gives an R_f value equal to or close to the comparison R_f and the difference is less than 0.2 (Ministry of Health RI, 2020). In this study, it has met the requirements because the result of the difference in the R_f value of the sample with the comparison is less than 0.2, namely, the S9 sample has a different value of 0.16, the S10 sample has a different value of 0.12 and the S12 sample has a 0.15 difference value. From these results, samples S9, S10 and S12 were positive samples containing retinoic acid. In addition, positive results were also indicated by the color formed in the three samples after being given a phosphomolybdic acid spot viewer. Samples S9, S10, and S12 showed the same color as the standard retinoic acid, which was dark green.

Before validating the method, the maximum wavelength of retinoic acid was first measured. Determination of wavelength aims to determine the maximum absorbance of retinoic acid that can be read by a UV-Vis spectrophotometer. The maximum wavelength obtained is 355 nm. According to the Indonesian Pharmacopoeia VI edition, retinoic acid has a wavelength of 352 nm with a standard deviation of 3% or 10.65 nm so in the analysis of retinoic acid it will conform to the standard if the resulting wavelength ranges from 341.35 nm to 362.65 nm (Ministry of Health). RI, 2020). The maximum wavelength results in this study are in accordance with the theory where the shift that occurs is less than 3% (10.65 nm) or ranges from 341.35 nm to 362.65 nm. The shift in wavelength can be caused by differences in the analysis tools and the condition of the equipment and the differences that can occur during sample preparation.

Linearity is expressed as the ability of the analytical method to provide comparable results to the analyte concentration contained in the sample (Riyanto, 2014). Linearity can be expressed in the correlation coefficient obtained from the measurement results of several analyte concentrations. According to AOAC, the linearity parameter is good and acceptable if the value obtained is > 0.99 (AOAC, 2016). In this study, 5 concentration standard curves were used to determine linearity, namely the standard curve concentrations of retinoic acid 1 ppm, 1.5 ppm, 2 ppm, 2.5 ppm, and 3 ppm. The results obtained for linear regression are $y = 0.1296 + 0.0892x$ with a correlation coefficient (r) of 0.9982 and a coefficient of determination (r^2) of 0.9962. Based on this value, linearity shows a linear result between concentration and absorbance results.

Precision is defined as a measure that expresses the closeness of the test results to a homogeneous sample in a series of measurements that are repeated. According to Riyanto (2014), the precision test was carried out on a homogeneous mixture of at least six replications. Precision can be done as repeatability and reproducibility (Riyanto, 2014). Precision is expressed in the form of standard deviation which is then calculated the coefficient of variation of the standard deviation.

The precision carried out in this study is repeatability, where precision is carried out under the same conditions and repeated at short time intervals. In the precision test in this study, the standard deviation (SD) was 0.019 and the coefficient of variation (CV) or %RSD was 1.84%. This value has met the criteria for a good precision value, which is less than 2%. An acceptable precision criterion is if a method can give results in the form of coefficient of variation (CV) or relative standard deviation (RSD) $< 2\%$. Meanwhile, according to Gonzalez and Herrador, the precision required for the analyte content of 1 ppm is less than 16%. The precision results of this study have met the requirements of RSD Horwitz, namely 1.84% or less than 16% (Gustavo González & Angeles Herrador, 2007).

Accuracy is one of the parameters in method validation in the form of a value that shows the closeness between the test results and the actual levels and is expressed in percent recovery (%recovery) (Riyanto, 2014). In the accuracy results, the closer the measurement value to the actual level, the more accurate the method used. Based on the results of the recovery measurements at three concentrations of 1, 2, and 3 ppm after measurement with three replications, the average percent recovery was obtained at 100.70%. At a concentration of 1 ppm, the average yield of recovery is 101.11%, the concentration of 2 ppm is 103.06% and the concentration of 3 ppm is 97.86%. According to Harmita (2004), the error range in each analyte concentration is still acceptable if the analyte contained in the sample is < 1 ppm, which is 80%-110% (Harmita, 2004). The 80%-110% accuracy range is also an accuracy range that is declared good if the analyte concentration in the sample is 1 ppm based on the AOAC (Association of Official Analytical Chemists) (2016). The accuracy results in this study have met the acceptable accuracy requirements because they are in the 80%-110% range.

The limit of detection (LOD) is the lowest concentration of an analyte in a sample that still gives a value or is detected by an analytical method. While Limit of Quantitation (LOQ) or is the the lowest concentration of an analyte in a sample that can still be quantified. In this study, LOD and LOQ were determined by statistically calculating the linear regression line from the previously obtained calibration curve (Riyanto, 2014). From this measurement, it is known that the LOD or the smallest retinoic acid concentration that can still be measured by a UV-Vis spectrophotometer is 0.434 ppm while the LOQ or retinoic acid concentration that can still be measured and quantified using a UV-Vis spectrophotometer is 1.448 ppm.

Quantitative analysis was carried out to determine the level of concentration of retinoic acid contained in samples of facial whitening cream. In this study, quantitative analysis was carried out using the UV-Vis spectrophotometric method. Retinoic acid is a compound that has ring aromatics, conjugated double bonds, and auxochrome anions -O so that they can be detected through a UV detector (Nastiti, 2016). Retinoic acid has a chromophore group which is part of the molecule with the function of absorbing light in the UV-Vis region and has an auxochrome group that plays a role in the intensity of UV light absorption on the chromophore. The following is a picture of the chromophore and auxochrome groups present in retinoic acid:

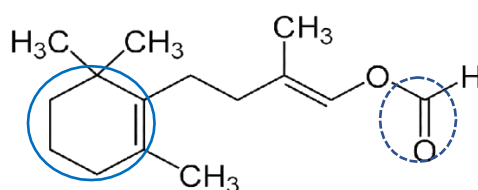


Figure 6. Chromophores and Auxochrome Groups of Retinoic Acid

Remarks:

=Chromophores

= Auxochromes

In the UV-Vis spectrophotometric analysis method, the molar extinction value (ϵ) is important. The value of a sample can be analyzed using UV-Vis spectrophotometry, one of which is if it has a value $> 1,000$ (Tati, 2017). This is because the absorbance obtained will be greater at a small analyte concentration. Analysis of retinoic acid has an extinction value (ϵ) of 36,288.32, which means that retinoic acid can be analyzed using spectrophotometry. Determination of retinoic acid levels in the preparation of facial whitening cream is determined by processing the absorbance measurement data from the prepared sample solution. The resulting data is calculated using the linear regression equation that has been obtained previously, namely $y = 0.1296 + 0.0892x$. In determining the levels, replication or repetition of each sample was carried out 3 times. Replication aims to determine repeatability. The prepared face cream samples were measured for absorbance using a UV-Vis spectrophotometer with a maximum wavelength of 355 nm. Of the 12 facial whitening creams that were sampled, 3 of them were samples that were graded. The samples of the facial whitening cream are samples that have codes S9, S10, and S12. This is because samples S9, S10, and S12 are samples that give positive results in the qualitative test using Thin Layer Chromatography (TLC). Based on the analysis, it shows that samples of facial whitening cream circulating in the city of Bekasi with sales through the marketplace analyzed in this study contain retinoic acid with an average level of 2.39%. These levels are 2.39% in the S9 sample, 2.96% in the S10 sample, and 0.86% in the S12 sample. The results of these levels indicate that the three samples of facial whitening cream analyzed using a UV-Vis spectrophotometer did not meet the requirements set by the Food and Drug Supervisory Agency of the Republic of Indonesia where in Regulation Number 23 of 2019 concerning technical requirements for cosmetic ingredients it is not allowed to contain retinoic acid in cosmetics.

CONCLUSION

Based on the research conducted, it was concluded that 3 of the 12 samples of facial whitening creams sold in Bekasi City online on the X, Y, and Z marketplaces were positive for retinoic acid with levels ranging from 0.86% - 2.96% and did not meet the requirements outlined in this article determined by BPOM. The analytical method used fulfills the validation requirements as indicated by the correlation coefficient (r) of 0.9983, %RSD of 1.84%, accuracy obtained with an average recovery of 100.70%, LOD value of 0.44 ppm, and the LOQ result is 1.45 ppm.

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DETERMINATION OF CURCUMIN LEVELS IN TAMARIND TURMERIC HERBAL ON MARKETPLACE X USING UV-VIS SPECTROPHOTOMETRY METHOD

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Abstract

Introduction: Turmeric contains chemical compounds, namely curcumin, demethoxycurcumin, bisdemethoxycurcumin, essential oils, resins, oleoresins, gums, fats, proteins, calcium, phosphorus, and iron. The content of curcumin in turmeric is 3-8%, higher than white turmeric and temulawak. The purpose of this study was to determine the levels of curcumin in the herbal turmeric acid sachet circulating in marketplace X. **Method:** This type of research is descriptive non-experimental by conducting qualitative analysis and quantitative analysis. Qualitative analysis of curcumin with organoleptic test and color test using 1% FeCl₃ reagent. Quantitative analysis of curcumin with UV-Vis Spectrophotometry.

Results: The results obtained in the qualitative analysis were that the five samples of the positive acid turmericherbal medicine contained curcumin while the results of the quantitative analysis of the maximum wavelength obtained were 426 nm. The linearity of the calibration curve is obtained by the regression equation $Y = 0.1436x + 0.0128$ with the correlation coefficient (r) = 0.9996 and the coefficient of determination (r^2) = 0.9993. The average % recovery was 102.34%. The average content of curcumin in sample A was 0.024%, sample B was 0.027%, sample C was 0.016%, sample D was 0.015% and sample E was 0.014%.

Conclusion: Based on the results of the research on determining the levels of curcumin in the herbal turmeric acid sachet circulating in marketplace X using the UV-Vis spectrophotometry method, it can be concluded thatthe curcumin levels obtained in the samples of herbal turmeric acid sachets with code A have an average level of 0.024%, at code B is 0.027%, code C is 0.016%, code D is 0.015%, and code E is 0.014%.

Keywords: Curcumin, turmeric herbal products, tamarind herbal medicine, traditional medicine, UV-Vis spectrophotometry.

INTRODUCTION

Indonesia is a tropical country that has many plants, some of which are known as medicinal plants (Ridla and Kristanto., 2016). In this modern era, not a few people still use traditional medicine. Traditional medicine is one of the community cultures that has considerable benefits in the development of public health. Traditional medicine is a form of active community participation in solving health problems and its role has been recognized by countries in improving health status (Puspitaningrum et al., 2021).

Traditional medicine can be said as ingredients or ingredients including plants, animals, minerals, preparations of sarin (galenic), or mixtures of these materials that have been used for treatment for generations and can also be used by the norms that apply to the community (Permenkes RI., 2016). People believe that herbal medicine has many benefits for the health of the body, including for the treatment of various diseases, maintaining a healthy body, and preventing disease because herbal medicine has fewer side effects (if used in the right way) compared to drugs from synthetic chemicals (Sumayyah and Salsabila., 2017).

Based on previous research conducted by Permatasari et al (2021) with samples of tamarind turmeric herbal using the TLC-Densitometry method, the results were that sample 1 gave a percentage of 0.004768% and sample 2 gave a percentage of 0.004111%.

The reason for doing this research is to see the levels of curcumin contained in the herbal turmeric acid sachet by using the UV-Vis spectrophotometric method. The advantage of using the UV-Vis spectrophotometry method is to analyze the levels of curcumin contained in the tamarind turmeric herbal medicine because UV-

Vis spectrophotometry can analyze many organic and inorganic substances, is selective, has high accuracy with a relative error of 1-3%, the analysis can be done with fast and precise (Rohmah et al., 2021).

MATERIALS AND METHOD

Research Design

The type of research design used is descriptive non-experimental.

Research Location and Time

This research was carried out at the STIKes Mitra Keluarga Bekasi Laboratory from February to March 2022.

Population and Sample

The population is the entire object of research while the sample is part of the population, meaning that the sample size must be sufficient to describe the population (Roflin et al., 2021). To determine the sample in this study can be calculated by the Slovin formula. There was 25 shop from 34 cosmetic shops in Marketplace X. From the 25 shop samples selected based on the inclusion criteria, 5 samples were obtained.

Tools and materials

The tools used in this study were UV-Vis spectrophotometry (Genesys 10S UV-Vis), analytical balance (Ohaus), filter paper, funnel (Iwaki Pyrex), micropipette (Socorex) and tip, stirring rod, spatula, glass cuvette, flask, measuring pipette (Iwaki Pyrex), dropper pipette, porcelain cup, measuring cup (Iwaki Pyrex), drop plate, beaker glass (Iwaki Pyrex), measuring pipette and ball pipette, wrapping.

The materials used in this study were standard curcumin (Biosynth), 1% FeCl₃, ethanol absolute pro-analysis (Emsure), and samples of tamarind turmeric herbal obtained in marketplace X.

Preparation of Sample Test Solution

The tamarind turmeric herb was weighed as much as 200 mg then replicated 3 times and put into a glass beaker, then added 10 mL of absolute ethanol, then stirred until dissolved. After dissolving, the sample solution was filtered and the filtrate was transferred to a 10 mL volumetric flask, then absolute ethanol was added to the mark (Daulay et al., 2019).

Qualitative Analysis

Organoleptic Test

Organoleptic examination of the samples of the tamarind herbal medicine included the shape, smell, color, and taste of each sample of the tamarind herbal medicine circulating in the marketplace X (Dewi and Rusita, 2017).

Color Test with 1% FeCl₃ Reagent

Samples were taken in as many as 4 drops and then placed on a drop plate. $\pm$ 7 drops of 1% FeCl₃ reagent were added. Observe the color changes that occur. A positive reaction gives a purple color indicating the presence of phenol (Prabowo et al., 2019).

Analysis Method Verification

Preparation of Curcumin Standard Solution

20 mg of curcumin was weighed and then put into a 100 mL volumetric flask, then dissolved in absolute ethanol up to the mark (200 ppm). The solution was pipetted as much as 12.5 mL from the standard solution, then put into a 50 mL volumetric flask, then added with absolute ethanol up to the mark (50 ppm) (Daulay et al., 2019).

Maximum Wavelength

An 4 mL of the standard solution was pipetted from 50 ppm, then put into a 10 mL volumetric flask and added with absolute ethanol up to the mark (20 ppm). The dilution was carried out from 20 ppm to 5 ppm with a 2.5 mL pipette from a concentration of 20 ppm then put into a 10 mL volumetric flask and added absolute ethanol

up to the mark (5 ppm). Then read at the maximum wavelength of curcumin with UV-Vis spectrophotometry at a wavelength of 400-800 nm (Daulay et al., 2019).

Calibration Curve Linearity

The solution is pipetted as much as 0.3 mL; 0.4 mL; 0.5 mL; 0.6 mL and 0.7 mL of a standard solution of 50ppm so that the results of the concentration of each solution are 1.5 ppm; 2 ppm; 2.5 ppm; 3 ppm and 3.5 ppm (3 times replication) and then the absorption was measured at 'maximum' respectively (Daulay et al., 2019).

Accuracy

In the accuracy test using the addition method (standard edition), the sample is analyzed then several analytes being examined are added to the sample, then mixed and reanalyzed (Riyanto., 2016). The sample solution was taken, then added with standard curcumin with a concentration of 80%, 90%, and 100%, which had previously been calculated from the concentration of the sample solution. So that the average standard solution taken is 2.18312 ppm (80%), 2.45601 ppm (90%), and 2.72890 ppm (100%). Three times replication for each concentration (Majumder et al., 2020).

Precision

The 50 ppm standard solution was taken at a concentration of 1.5 ppm, 2 ppm, and 2.5 ppm and then tested three times (Singh dan Avupati., 2017).

Quantitative Analysis

Preparation of Test Solution for Tamarind Turmeric Herbal Samples

An 4 mL of the solution was pipetted from the sample test solution, then put into a 10 mL volumetric flask, then dissolved with absolute ethanol up to the mark. It was replicated 3 times. The absorbance of the sample was measured using UV-Vis spectrophotometry with a predetermined wavelength of 426 nm (Daulay et al., 2019).

Data analysis

In this study, data analysis is descriptive in the form of tables, graphs, images, and statistical data processing using Microsoft Excel 2019.

RESULTS

Sampling

As many as 5 samples of the tamarind turmeric herbal product were used with various brands, namely A, B, C, D, and E obtained from marketplace X. The results of the markings on the tamarind turmeric herbal packaging can be seen in Table 5.1.

Table 1. The markings contained in the tamarind turmeric herbal packaging

The markings on the packaging	Sample				
	A	B	C	D	E
Product name	+	+	+	+	+
Manufacturer's name and address	+	+	+	+	+
Composition	+	+	+	+	+
Batch number	+	+	+	+	+
Registration number	+	+	+	+	+
Expiration date	+	+	+	+	+
Efficacy	+	+	+	+	+
Serving method	+	+	+	+	+

Information :

+ = there is a marking on the packaging

Qualitative Analysis

Organoleptic Test

An organoleptic examination was carried out to determine the characteristics of the sample by covering shape, odor, and color. Organoleptic was carried out before sample preparation, which aims to determine the initial characteristics of the herbal samples used (Herdini., 2019). The results of the organoleptic test can be seen in Table 2. and Figure 1.

Table 2. Organoleptic Test on Herbal Samples

Sample Code	Color	Shape	Odor	Taste
A	Yellow	Powder	The smell of herbal medicine	Sweet
B	Very light yellow	Powder	The smell of herbal medicine	A little sweet
C	Chocolate	Powder	The smell of herbal medicine	Sweet slightly bitter
D	White with yellow powder	Powder	The smell of herbal medicine	Sweet
E	Light yellow	Powder	The smell of herbal medicine	Sweet

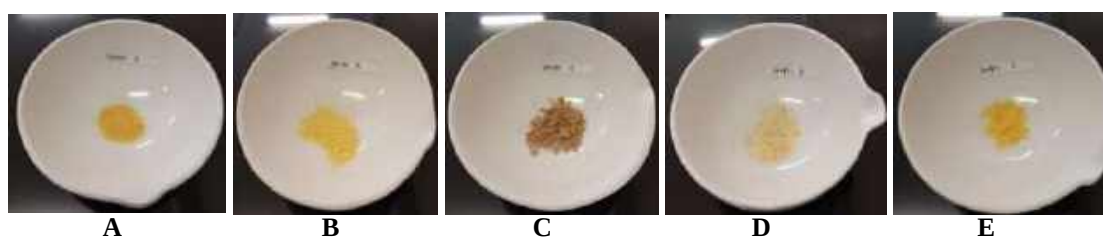


Figure 1. Organoleptic Test

Color Test

The color test using 1% FeCl_3 aims to determine the presence of phenol groups in the sample. A purple color change occurs because FeCl_3 reacts with the hydroxyl group ($-\text{OH}$) contained in phenol compounds (Putri et al., 2018). The results of the color test using 1% FeCl_3 reagent can be seen in Table 3. and Figure 2.

Table 3. Color Test

Sample Code	Color	Results
Control +	Dark purple	+
Control -	Yellow	-
A	Purple	+
B	Purple	+
C	Purple	+
D	Purple	+
E	Purple	+

Information :

Positive control : 1% FeCl_3 reagent + curcumin standard

Negative control : solvent + standard curcumin

(+) : positive contains curcumin

(-) : negative contains curcumin

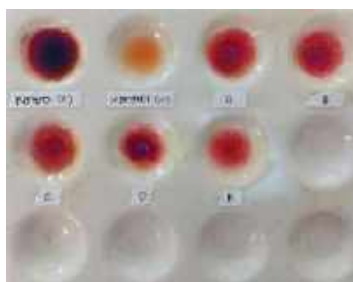


Figure 2. Color Test

Analysis Method Verification

Maximum Wavelength of Curcumin

The maximum wavelength of curcumin obtained is 426 nm with an absorbance of 0.699 at a concentration of 5 ppm. The results of the maximum wavelength of curcumin can be seen in Figure 3.

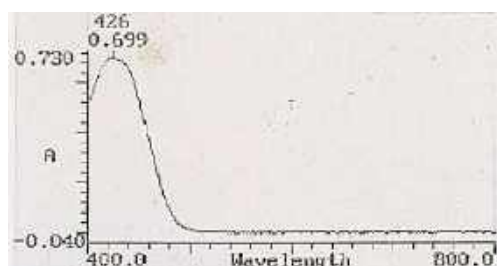


Figure 3. Maximum Wavelength of Curcumin

Linearity of Curcumin Calibration Curve

The standard curve of curcumin was made using 5 series of concentrations, namely 1.5 ppm; 2 ppm; 2.5 ppm; 3 ppm and 3.5 ppm and obtained $y = 0.1436x + 0.0128$ with a value of $R^2 = 0.9993$. The results of the linearity of the curcumin calibration curve can be seen in Figure 4.

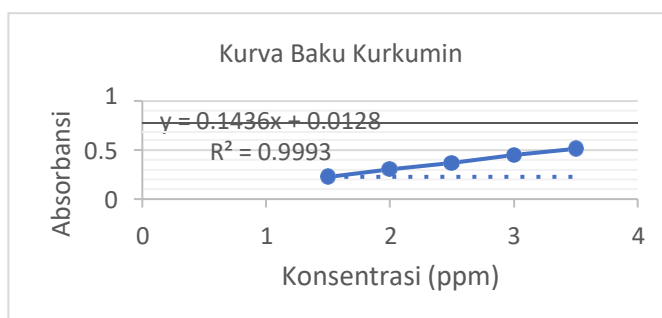


Figure 4. Linearity of Curcumin. Calibration Curve

Accuracy

The results of % Recovery obtained in determining the accuracy of 102.34% have met the requirements of % Recovery, namely 80 - 110%. This proves that the method used has met the accuracy requirements with a good accuracy value. Accuracy results can be seen in Table 4.

Table 4. Accuracy

Range (%)	% Recovery	Average % recovery
80%	100,48%	101,97%
	103,99%	
	101,44%	
90%	102,92%	102,64%
	100,37%	
	104,63%	
100%	102,33%	102,41%
	101,31%	
	103,61%	
Average overall recovery (%)		102,34%

Precision

The results of %RSD obtained in the determination of precision that is 1.70%, 0.67%, and 0.69% have met the requirements of %RSD which is less than 2%. This can prove that the method used has met the requirements of precision with a good repeatability value. Precision results can be seen in Table 5.

Table 5. Precision

Concentration	Replication	Absorbance	Concentration (Ppm)	Average	SD	RSD
1,5	1	0,234	1,540390	1,551996	0,02636	1,70%
	2	0,233	1,533426			
	3	0,240	1,582173			
2	1	0,323	2,160167	2,155525	0,01450	0,67%
	2	0,320	2,139276			
	3	0,324	2,167131			
2,5	1	0,395	2,661560	2,682451	0,01842	0,69%
	2	0,400	2,696379			
	3	0,399	2,689415			

Determination of Curcumin Levels in Tamarind Turmeric Herbal

The results of determining the levels of curcumin in the tamarind turmeric herbal medicine circulating in marketplace X are in sample A with an average of 0.024%, sample B with an average of 0.027%, sample C with an average of 0.016%, sample D with an average of 0.015%, and sample E with an average of 0.014%. The results of the determination of curcumin levels in the tamarind turmeric herbal medicine can be seen in Table 6.

Table 6. Determination of Curcumin Levels in Tamarind Turmeric Herbal

Sample Code	Absorbance	Number of Samples (mg)	Sample Content (%b/b)	Average Grade (%b/b)
A	0,360	0,048	0,0243	0,024
	0,355	0,048	0,0238	
	0,353	0,047	0,0236	
B	0,409	0,055	0,0275	0,027
	0,406	0,055	0,0273	
	0,399	0,054	0,0268	
C	0,234	0,031	0,0154	0,016
	0,241	0,032	0,0159	
	0,238	0,031	0,0157	
D	0,220	0,029	0,0144	0,015
	0,233	0,031	0,0153	
	0,240	0,032	0,0158	
E	0,219	0,029	0,0144	0,014
	0,217	0,028	0,0142	
	0,215	0,028	0,0141	

DISCUSSION

Sampling

The sample used in this study was tamarind turmeric herbal medicine with 5 different brands (A, B, C, D, and E) circulating in marketplace X by the inclusion criteria, namely having a BPOM distribution permit number, stating the efficacy, composition, method of presentation and expired, star and star+ products and prices below 50,000.

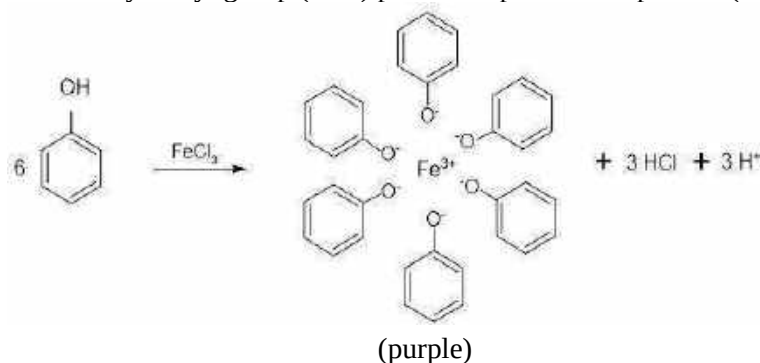
Based on table 1 the results of the marking contained in the samples of tamarind turmeric herbal after checking through the website Cekbpom.pom.go.id, regarding BPOM numbers, all samples were registered with BPOM numbers. Checks are also carried out on the herbal medicine packaging regarding the efficacy, composition, method of presentation, and expiration, all samples are by what is stated on the package (efficacy, composition, method of presentation, and expiration). The reason for taking samples of herbal medicine that has a BPOM distribution permit number is because this tamarind turmeric herbal medicine is safe for consumption and is guaranteed by the existence of a BPOM distribution permit number. In addition, the samples taken were also listed with the composition so that researchers would know that this tamarind turmeric herb contains turmeric so that it can be carried out for research on curcumin levels. The samples were taken also have efficacy, presentation method, expiration date, star and star+ products, and a price of <50,000 so that the public knows the benefits of consuming the tamarind turmeric herbal medicine for the body in a good and correct way to make it easier for people to consume the herbal medicine before the expiration date. The public can already consume tamarind turmeric herbal at an affordable price and it is one of the most popular products (BPOM RI., 2020).

Organoleptic Test

Based on table 2, the organoleptic test results of the tamarind turmeric herbal medicine with sample code A are yellow, sample B is very light yellow, sample C is brown, sample D is white with yellow fine powder, and sample E is light yellow. All samples have the same dosage form, namely powder as well. All samples also have the same characteristic odor, namely the typical smell of herbal medicine. All samples have a sweet taste but sample B only has a slightly sweet taste and sample, C has a sweet taste accompanied by a slightly bitter taste. According to the Indonesian Herbal Pharmacopoeia Edition II (2017) which states that the color of turmeric is yellow to brown and has a distinctive odor and a slightly bitter taste, slightly spicy so that over time it will cause a thick taste on the tongue. From the test results organoleptic regarding color, smell, and taste showed results by the Indonesian Herbal Medicine Faculty, Edition II.

Color Test

The color test in this study used a 1% FeCl₃ reagent to identify the phenolic compounds contained in the samples of tamarind turmeric herbal medicine circulating in marketplace X. Based on table 5.3 the results of the curcumin color test using 1% FeCl₃ reagent, purple complex compounds were obtained in five samples because FeCl₃ reacts with the hydroxyl group (-OH) present in phenol compounds (Putri et al., 2018).



To obtain accurate results, the five samples were checked using a UV-Vis spectrophotometer. Based on previous research conducted by (Prabowo et al., 2019), The sample used was positive for phenol.

Verification Method

Maximum Wavelength of Curcumin

The occurrence of electronic excitation that can obtain maximum absorbance results, is the definition of maximum wavelength. The reason for the measurement of the maximum wavelength is that at the maximum wavelength, the maximum sensitivity of the analysis is obtained because it has the largest change in absorbance at each concentration unit. Determination of the maximum wavelength aims to determine the wavelength with the maximum absorbance value on UV-Vis spectrophotometry of the curcumin solution used and can also be used to measure the absorbance of the sample (Noviyanto., 2020).

Checking the maximum wavelength of curcumin was carried out in the range of 400 - 800 nm which is included in the visible range (visible light). This study uses the Visible range because the curcumin solution is a colored solution that can be seen by the human eye. The light that can be seen by the human eye is light with a wavelength of 400-800 nm (Munandar., 2017).

The maximum wavelength of curcumin in this study was 426 nm with a concentration of 5 ppm using ethanol absolute pro analysis as a solvent. The results obtained in this study are different from previous studies conducted by (Majumder et al., 2020) by obtaining the maximum wavelength of 429 nm. This difference can be caused by a hypochromic shift, namely a shift in the absorbance to a shorter wavelength region due to substitution or solvent effects (Dachriyanus., 2004).

Calibration Curve Linearity

To find out whether the variables to be tested have a linear relationship or not significantly, is the purpose of the linearity test (Setiawan dan Yosepha., 2020). Based on the Lambert-Beer law, the absorption or absorption

will be directly proportional to the concentration of the irradiated solution. The linearity relationship parameter test used is the correlation coefficient (r) and the coefficient of determination (R) in the linear regression analysis $y = bx + a$. y is instrument response, b is the slope, x is analyte concentration and a is the intercept. If the value achieved is $a = 0$ and $r = +1$ or -1 , it can be said that the result has an ideal and perfect linear relationship, the $+$ and $-$ signs depend on the direction of the line. The positive sign ($+$) indicates a positive correlation indicated by the direction of the line sloping to the right, while the negative sign ($-$) indicates a negative correlation which is indicated by the direction of the line sloping to the left (Riyanto., 2016).

Based on Figure 4. Curcumin Calibration Curve was made using 5 concentration series, namely 1.5 ppm; 2 ppm; 2.5 ppm ; 3 ppm, and 3.5 ppm by obtaining the equation results, namely $Y = 0.1436x + 0.0128$. Determination of the curcumin calibration curve at that concentration gave absorbance results by Lambert-Beer's law, namely 0.2 - 0.8. In this study, the correlation coefficient (r) obtained is 0.9996 and the coefficient of determination (R^2) is 0.9993. It can be interpreted that the value of r is close to 1 indicating that the curcumin calibration curve in this study is linear and there is a relationship between the concentration of the curcumin solution and the absorption value.

Determination of Accuracy

To measure the accuracy between the measured value and the actual value obtained, an accuracy test method can be used. This accuracy testing method can carry out tests or analyses to obtain the correct value after it is done repeatedly. The closer the analysis replication value is to the actual sample, the more accurate the method used is (Riyanto., 2016). The accuracy test was carried out based on the ICH Guideline (2005) with three variations of concentration with three repetitions. This study uses the addition method with variations in concentration, namely 80%, 90%, and 100%. This study uses the addition method (addition of raw materials).

The average % recovery results obtained in this study were 102.35%. This percent recovery gives acceptable results because it has met the accuracy requirements, which is in the range of 80 – 110% (Riyanto., 2016). It can be seen that the UV-Vis spectrophotometric method gives good accuracy results.

Precision Determination

Precision is measured as standard deviation or standard deviation of repeatability (coefficient of variation). In this study, the type of precision used is repeatability. Repeatability is the accuracy of the method if it is carried out repeatedly by the same analyst under the same conditions and in short time intervals (Riyanto., 2016).

This study uses the method based on the ICH Guideline, namely three concentrations with three replications. The SD value obtained from a concentration of 1.5 ppm is 0.02636, a concentration of 2 ppm is 0.01450, and a concentration of 2.5 is 0.01842 and the % RSD results obtained from a concentration of 1.5 ppm is 1.70%, from the concentration of 2 ppm is 0.67%, and from a concentration of 2.5 ppm, it is 0.69%. Based on the results obtained, it can be interpreted that the precision test in this study has met the requirements of less than 2% so it can be said that the method has a good repeatability value.

Determination of curcumin levels in the tamarind turmeric herbal medicine

Determination of sample levels of curcumin is the last stage carried out in this study. Determination of sample levels of curcumin in herbal turmeric acid sachets was carried out by measuring the absorbance of the previously prepared sample solution using UV-Vis spectrophotometry at the maximum wavelength that was obtained, which was 426 nm. Determination of curcumin levels was carried out to determine the levels of curcumin contained in herbal turmeric acid sachets. The curcumin level obtained in sample A has an average of 0.024%, sample B has an average of 0.027%, sample C has an average of 0.016%, sample D has an average of 0.015% and sample E has an average of 0.014%.

The results of this study are the same as the results of previous studies conducted by Permatasari et al (2021) with samples of herbal tamarind turmeric using the TLC-Densitometry method giving the results of curcumin levels, namely in sample 1 giving a percentage yield of 0.004768% and in sample 2 giving a percentage result levels of 0.004111%.

CONCLUSION

Based on the results of the research on determining the levels of curcumin in the herbal turmeric acid sachet circulating in marketplace X using the UV-Vis spectrophotometry method, it can be concluded that the curcumin levels obtained in the samples of herbal turmeric acid sachets with code A have an average level of 0.024%, at code B is 0.027%, code C is 0.016%, code D is 0.015%, and code E is 0.014%.

SUGGESTION

From the results of the research that has been done, the suggestions that can be given by researchers are that further researchers can analyze curcuminoid compounds or other derivatives (bisdemethoxycurcumin and demethoxycurcumin) and can use more specific methods, namely to conduct validation research on analytical methods using the HPLC (High Performance Liquid) method. Chromatography).

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FORMULATION AND EVALUATION OF TRANSPARENT SOAP EXTRACT OF KENCUR (*Kaempferia galanga* L.)

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Abstract

Introduction: Soap is a cosmetic with cleaning power to clean dirt from the skin. The content in kencur has antibacterial benefits such as flavonoids, polyphenols and saponins. The research design used was experimental laboratory, then the purpose of this study was to evaluate the physical stability of the transparent solid soap formulation of kencur extract with concentrations of 0.5%, 1%, and 1.5%. The method of making soap is by heating to a final temperature of not more than 70°C. **Result:** Testing the characteristics of transparent solid soap through several tests such as organoleptic test, pH test, water content test, foam strength test, free alkali content test and free fatty acid content test. The results obtained from the three formulas obtained appropriate results for the five tests except that free fatty acid levels were not detected. **Conclusion:** The comparison of the three formulations that got the best results was F3 with an average water content of 5.78%, the most stable average pH range was 9.6, the average foam height was 10cm, and the free alkali content was 0.10%, namely the lowest.

Keywords: transparent solid soap, kencur extract, coconut oil

INTRODUCTION

Solid soap itself is one of the cosmetic and pharmaceutical industry products that are often used by Indonesian people in everyday life to sterilize germs / microbes, dirt attached to the skin of the human body. If the skin is not cleaned, then these germs will trigger skin inflammation and other diseases (Widyasanti et al., 2016) that, the results of testing the chemical properties of transparent soap produced are in accordance with SNI 06-3532-1994, so that the manufacture of transparent solid soap using palm oil base can be applied by the public. Transparent solid soap or glycerin soap is an excellent moisturizing soap and is beneficial for people with dry or flaky skin. This soap dissolves easily so it doesn't leave a film on the skin like other soaps. This glycerin absorbs water and air and is a key factor in keeping skin soft and healthy, and one of the best soaps for delicate and sensitive skin. Based on previous research, Megantara & Soleh, (2019) stated that kencur extract has several compounds such as essential oils, saponins, flavonoids, polyphenols which are known to have many benefits, kencur can also inhibit *Streptococcus pyogenes* and *Staphylococcus aureus* bacteria with a concentration of 0.2%, 0.4%, 0.6%, 0.8%, and 1%. The best results were obtained with 1% kencur extract with an inhibition zone of 5.44 mm for *Streptococcus pyogenes* and 5.56 mm for *Staphylococcus aureus* (Belgis et al., 2021). Likewise with research from Susanti & Antikasari, (2018) which states that kencur can inhibit the growth of *Escherichia coli* bacteria in concentrations of 30%, 45%, 60%, 75%, then the results of the largest inhibition zone at 75% concentration are 31 mm. . Based on the research of Imanda et al., (2021) also stated that solid soap with kencur extract successfully passed 3 tests that had been carried out, namely the pH test, high foam test and organoleptic test. In this study the authors used palm oil as a soap-making base to make transparent solid soap with kencur extract, the researchers found that kencur had never been used as an extract from transparent solid soap, so the authors were interested in formulating and evaluating transparent soap preparations from kencur extract with made three concentration variations of 0.5%, 1% and 1.5%.

METHOD

A. Stability Testing of Physical Properties of Transparant Solid Soap

1. Flavonoid Test

A total of 0.5 g of extract was put in a test tube, add 5 mL of ethanol and then heated for 5 minutes. A total of 10 drops of concentrated HCl was put into a test tube and added 0.2 grams of Mg

powder. The appearance of a brownish red color indicates the presence of flavonoids (Kala'Rante et al., 2020).

2. Saponin Test

A total of 0.5 grams of extract was added with 10 mL of distilled water and then shaken vigorously for about 1 minute. Then let stand for 10 minutes and observe the foam or foam formed. The presence of saponin compounds in the sample was indicated by the formation of a stable foam for 10 minutes with a height of 1-3 cm (Kala'Rante et al., 2020).

3. Polyphenol Test

0.5 grams of extract was put into a test tube. Added 5 drops of 5% FeCl₃ solution and shaken vigorously. The formation of a blue-black color after the addition of 5% FeCl₃ indicates the presence of phenolic compounds (Mentari, 2018).

B. Transparent Soap Preparation Formula

Tabel 1. Formulasi Pembuatan Sabun Padat Transparan (Widyasanti et al., 2016)

Composition	Replication			Function
	I (g)	II (g)	III (g)	
Kencur Extract	1,5	3	4,5	Active substance
Palm Oil	60	60	60	Oil Phase
Stearic Acid	21	21	21	Emulsifier
NaCl	0,6	0,6	0,6	Tonicity Agent
NaOH 30%	60,9	60,9	60,9	Buffer Agent
Sugar	45	45	45	Humectants
Glycerin	39	39	39	Preservative
Coco-DEA	3	3	3	Foam stability
Fragrance Oil	0,3	0,3	0,3	Deodorizer
Etanol 96%	45	45	45	Solvent
Aquadest	23,7	22,2	20,7	Additives

C. Manufacturing of Transparent Solid Soap

The process of making transparent solid soap uses the hot method using a water bath as the medium. Pure coconut oil which has been placed in a glass beaker is heated in a water bath. The stearic acid is put into a glass beaker containing virgin coconut oil, then mix and shake until homogeneous. After that, add 30% NaOH liquid and add other supporting materials such as 96% ethanol, glycerin, sugar syrup (sugar + distilled water first), coco-DEA, NaCl, and aromatic oil. The mixture is stirred until it is completely mixed, lower the temperature to $\pm 50^{\circ}\text{C}$ before adding the extract. Stir again until the mixture is completely mixed, then pour it into the soap mold and let it sit for 1 day at room temperature. (Widyasanti et al., 2016)

D. Transparent Solid Soap Characteristic Test

1. Organoleptic observations

Organoleptic tests include observations of shape, color changes, and odor changes (Susanti & Guterres, 2018).

2. pH test

The pH test was tested by weighing 1g of soft soap dissolved in 9ml of distilled water. Then the pH marker is dipped and the pH value of soft soap is observed, sourced from SNI 06-3532-1994 (2016) soap pH is 9-11 (Susanti & Guterres, 2018).

3. The Foam Strenght Test

The foam strength test was tested by weighing 10g of soft soap dissolved in aquadest. After that, the solution was shaken vigorously and the size of the foam formed after shaking for 30 seconds and 60 seconds was measured (Susanti & Guterres, 2018).

4. Water Contant Test

The soft soap is weighed 1 gram, after that it is put into a tool called a Moisture analyzer after which it is waited for the results of the water content to be recorded.

5. Fatty Acid Content Test

5g of solid soap was dissolved with aquadest, then bromthymol blue was added and titration was carried out using 0.1 N NaOH until the color changed from blue to yellow (Susanti & Guterres, 2018).

6. Free Alkali Content Test

5g of solid soap was weighed into a measuring cup, then aquadest was added and a phenolphthalein marker was added. After the solution turns red, then it is titrated with 0.1 N HCl solution until the red color disappears (Susanti & Guterres, 2018).

RESULTS

A. Qualitative Test of Kencur Rhizome Extract

Tabel 1. Result Of Qualitative Test of Kencur Rhizome Extract

Sample Name	Condition	Parameter	Result	Analysis Techniques
Kencur Extract	Thick Extract	Saponin	Positive	Stable foam is formed
		Flavonoid	Positive	Formation of yellow-orange color
		Polifenol	Negative	No bluish black color is formed

B. Organoleptic Observation Transparent Solid Soap Kencur Extract

Tabel 2. Result of Organoleptic Observation

Formula 1 (Kencur Extract 0,5%)

Replication	Color	Scent	Texture
1	Transparent brown	Aromatic	Solid
2	Transparent brown	Aromatic	Solid
3	Transparent brown	Aromatic	Solid

Formula 2 (Kencur Extract 1%)

Replication	Color	Scent	Texture
1	Transparent brown	Aromatic	Solid
2	Transparent brown	Aromatic	Solid
3	Transparent brown	Aromatic	Solid

Formula 3 (Kencur Extract 1,5%)

Replication	Color	Scent	Texture
1	Brown	Aromatic	Solid
2	Brown	Aromatic	Solid
3	Brown	Aromatic	Solid

Description :

Formula 1 : Transparent solid soap kencur extract with a concentration of 0.5%

Formula 2 : Transparent solid soap kencur extract with a concentration of 1%

Formula 3 : Transparent solid soap kencur extract with a concentration of 1.5%

C. pH Test Transparent Solid Soap Kencur Extract

Tabel 3. Result of pH Test

Replication	Formula 1	Fomula 2	Formula 3
1	9,7	9,5	9,3
2	9,9	9,5	9,7
3	9,8	9,7	9,7
Average $\pm$ SD	9,8 $\pm$ 0,1	9,6 $\pm$ 0,1	9,6 $\pm$ 0,2

Description :

Formula 1 : Transparent solid soap kencur extract with a concentration of 0.5%

Formula 2 : Transparent solid soap kencur extract with a concentration of 1%

Formula 3 : Transparent solid soap kencur extract with a concentration of 1.5%

D. Foam Strength Test of Transparent Solid Soap Kencur Extract

Tabel 4. Result of Foam Strength Test

Replication	Formula 1	Fomula 2	Formula 3
1	10,50 cm	11,00 cm	11,00 cm
2	11,00 cm	12,00 cm	9,00 cm
3	10,00 cm	11,50 cm	10,00 cm
Average $\pm$ SD	10,50 $\pm$ 0,5 cm	11,50 $\pm$ 0,5 cm	10,00 $\pm$ 1 cm

Description :

Formula 1 : Transparent solid soap kencur extract with a concentration of 0.5%

Formula 2 : Transparent solid soap kencur extract with a concentration of 1%

Formula 3 : Transparent solid soap kencur extract with a concentration of 1.5%

E. Water Content Test Transparent Solid Soap Kencur Extract

Tabel 5. Result of Water Content Test

Replication	Formula 1	Fomula 2	Formula
1	13,48 %	9,44 %	5,68 %
2	12,56 %	9,96 %	5,99 %
3	10,46 %	10,21 %	5,66 %
Average $\pm$ SD	12,17 $\pm$ 1,55 %	9,87 $\pm$ 0,39 %	5,78 $\pm$ 0,19 %

Description :

Formula 1 : Transparent solid soap kencur extract with a concentration of 0.5%

Formula 2 : Transparent solid soap kencur extract with a concentration of 1%

Formula 3 : Transparent solid soap kencur extract with a concentration of 1.5%

F. Alkali Free Test Solid Soap Transparent Kencur Extract

Tabel 6. Result of Alkali Free Test

Replication	Formula 1	Fomula 2	Formula 3
1	0,14 %	0,12 %	0,07 %
2	0,11 %	0,09 %	0,09 %
3	0,11 %	0,11 %	0,08 %
Average $\pm$ SD	0,12 $\pm$ 0,02 %	0,11 $\pm$ 0,01 %	0,08 $\pm$ 0,01 %

Description :

Formula 1 : Transparent solid soap kencur extract with a concentration of 0.5%

Formula 2 : Transparent solid soap kencur extract with a concentration of 1%

Formula 3 : Transparent solid soap kencur extract with a concentration of 1.5%

G. Fatty Acid Test Solid Soap Transparent Kencur Extract

Tabel 7. Result of Fatty Acid Test

Replication	Formula 1	Fomula 2	Formula 3
1	0%	0%	0%
2	0%	0%	0%
3	0%	0%	0%
Average $\pm$ SD	0%	0%	0%

Description :

Formula 1 : Transparent solid soap kencur extract with a concentration of 0.5%

Formula 2 : Transparent solid soap kencur extract with a concentration of 1%

Formula 3 : Transparent solid soap kencur extract with a concentration of 1.5%

DISCUSSION

This research formula makes three variations of the formula, namely the concentration of kencur extract F1 (kencur extract 0.5%), F2 (kencur extract 1%), F3 (kencur extract 1.5%). The existence of three variations in the formula is intended to determine the differences that occur in several test variations such as organoleptic test, pH test, water content test, foam height test, free alcation level test, and free fatty acid content test.

In this study, qualitative tests were carried out, including flavonoid test, polyphenol test and saponin test. The results of the phytochemical screening obtained from the kencur extract that we studied were positive for saponins with the result that a stable foam was formed. The next result obtained was positive for flavonoids with the formation of an orange-orange colored solution, this reaction occurred because flavonoid compounds were reduced with Mg and HCl so as to produce red, yellow or orange colors (Sulistyarini et al., 2020). Furthermore, the researchers conducted a polyphenol test but did not get the results in accordance with the journal, namely positive polyphenols, there was no change in the solution to blackish blue which indicated the absence of polyphenol compounds in kencur. This is presumably because the difference in where the kencur plant grows, including location, soil type, climate, fertility level, and sunlight intensity, affects the number of secondary metabolites contained in plants that grow in a certain area with other areas (Yani & Dirmansyah, 2021). Polyphenols were not detected in this kencur rhizome extract but this did not make the kencur compound non-antibacterial, because kencur also has flavonoids as one of the active chemical substances that are able to form complex compounds with bacterial extracellular proteins that damage bacterial cell membranes. Flavonoids are also able to inhibit nucleic acid synthesis and inhibit the energy metabolism of bacterial cells. Likewise with saponins, saponins are active compounds that have antibacterial activity. Saponins work by interfering with the surface tension of bacterial cells, so that bacterial cells easily leak and lyse, so that in the absence of polyphenolic compounds, kencur remains useful as an anti-bacterial (Hayati et al., 2017).

Organoleptic observations at room temperature, for formulation 1 after making 3 replications with a concentration of 0.5%, the results were transparent solid soap of dark brown color with a distinctive aromatic odor and solid form. At room temperature, for formulation 2 by doing 3 times replication with the formulation of 1% kencur rhizome extract, good results were obtained, namely in a dark brown solid form with a distinctive aromatic odor and transparent results. At room temperature, for formulation 3 by doing 3 times replication with a concentration of 1.5%, the results obtained good solid soap with dark brown color and a distinctive odor but there is no transparency in the solid soap formulation 3 due to the darker kencur extract which causes the more extract or the higher the percentage of the formula causes the soap to become thicker. dark and not transparent.

The pH test was carried out to determine the acidity and basicity of the transparent solid soap preparation. It showed that the pH of the transparent solid soap of kencur extract had different results from formula one replication one to formulation three replications three. For the results obtained by formula one, namely 9.7, 9.9, and 9.8 respectively from replications 1 to 3, the results obtained have entered the pH range of transparent solid soap requirements with an average of 9.8, namely 9-11. For the next formula two results were obtained, namely 9.5, 9.5, 9.7 sequentially from one to three replications, for the data from formula two

also entered the pH range of transparent solid soap with an average of 9.6, namely 9-11. For the last formula three, the results obtained are 9.3, 9.7, 9.7 which have entered the requirements also with an average of 9.6 such as formulations one and two. The high and low pH of soap is influenced by the saponification process during soap making. The high pH value of soap resulted from the hydrolysis reaction in the saponification process. This can be overcome by adding excess fat or oil. However, the addition of fat or oil will reduce the hardness of the soap (Setiawati & Ariani, 2019).

Testing the water content of solid bath soap is something that needs to be done because the water content will affect the quality of the soap that has been made. The amount of water content can affect the solubility of soap in water when used. If the water content in the soap is too high, it will cause the soap to shrink easily and become uncomfortable when used. The results obtained from this water content test are for formula one 13.48%, 12.56%, 10.46% with an average of formulation one, namely 12.17% which has entered the soap water content requirement, then for formulation two namely 9.44%, 9.96%, and 10.21% with an average of 9.87% which have entered the range of water content requirements. Furthermore, the last three formulations obtained results, namely 5.68%, 5.99%, and 5.66% with an average value of 5.78% which had entered the requirements. The higher the water content in the soap causes the soap to be softer, on the contrary if the water content in the soap is getting smaller it can cause the soap to become hard. Soap with levels exceeding 15% if stored in an open state and in contact with air will cause a decrease in the weight and dimensions of the soap (Elmitra & Noviyanti, 2020).

The foam strength test aims to determine the amount of foam and the stability of the foam produced by transparent solid soap in 3 formulations (Elmitra & Noviyanti, 2020). The results obtained in this test varied from formulations of one replication one to formulations of three replications of three, for formulation one the results obtained were 10.50 cm, 11.00 cm, 10.00 cm respectively from one to three replications with an average value of 10, 50 cm which has been included in the requirements, namely the height of the foam ranges from 13-220 mm or 1.3-22 cm. For the second formulation, the results were 11.00 cm, 12.00 cm, and 11.00 cm respectively for one to three replications with an average of 11.50 cm which had entered the requirements. For the last formula, namely formulation three, the results were 11.00 cm, 9.00 cm, and 10.00 cm respectively from one to three replications with an average of 10.00 cm which also entered the requirements of the foam height. The results of the height of the foam may vary due to the strength of the shaking during the test.

Free alkali test is carried out to see the amount of base that is not bound by fatty acids (Dimpudus et al., 2017). Excess free alkali that is not up to standard can cause skin irritation. Excess alkali can be caused by the addition of excess alkali in the soap making process (Sukawaty et al., 2016.) The free alkali test has a requirement that is not more than 0.14%, so to obtain this percentage the researcher is required to calculate the result of milliliters of 0.1N HCL solution used during the titration in 3 replications to be converted into a percentage (Susanti & Guterres, 2018). The results of the calculation of the percentage obtained by the researchers from formula one replication one to three replication formula three, namely, formula one 0.11%, 0.09%, and 0.14% respectively from replication one to three with an average of 0.12% which states that formula one enters the water content test requirements. Furthermore, for formula two, namely 0.12%, 0.13%, 0.13% sequentially from one to three replications with an average of 0.13% which entered the water content test requirements. Furthermore, for formula three, namely 0.08%, 0.14%, and 0.07% respectively, from one to three replications with an average of 0.10% which entered the requirements of the free alkali content test. There was no difference between all the transparent solid soap formulations of kencur rhizome extract from formulations one to three. The free alkali in the transparent solid soap is caused by the excessive amount of NaOH in the saponification process (Susanti & Guterres, 2018). In a perfect saponification reaction, KOH will bind to the oil phase, but if KOH is not completely bound it will form free alkali. It can be seen that the free alkali content of all transparent solid soap formulations of kencur extract entered a good range or entered the requirement of a maximum of 0.14% so that it can be proven that the soap produced by the researchers will not cause skin irritation.

Free fatty acid testing aims to determine the free fatty acid content contained in the oil. A good oil to be used as a raw material for soap is an oil that has a high Free Fatty Acid (FFA) content (Sukawaty et al., 2016.) A good free fatty acid in soap is < 2.5%. This test is carried out by titrating the same as free alkali but using a different solution, namely 0.1 N NaOH and a blue bromthymol indicator or marker with a color change from blue to yellow (Susanti & Guterres, 2018). The requirement for a good free fatty acid test level

is below 2.5%, before obtaining the data, the researcher is required to calculate the number of mL of NaOH used in one titration and then calculate the results in order to obtain the percentage results in accordance with the requirements (Susanti & Guterres, 2018). However, in this experiment the researchers did not detect the presence of free fatty acids in the transparent solid soap that had been made, when testing the three concentrations of the preparation there was no change between the soap solution that had been given the bromthymol blue indicator and 0.1N NaOH solution. This condition occurs because the free fatty acids in the oil have reacted with bases to form soap and glycerol (Hardian et al., 2018).

CONCLUSION

1. Rhizome of kencur (*Kaempferia galanga* L.) can be used as an active ingredient in making transparent solid soap with various concentrations of 0.5%, 1%, and 1.5%. The three formulas are good formulas because they are included in the requirements for evaluating organoleptic tests, pH tests, water content tests, alkaline-base tests, and free fatty acid levels.
2. In this study the best formulation according to the researcher is formulation number 3 because in the water content formula 3 has the best results, namely with an average of 5.78%, foam strength with an average of 10cm, as well as the highest pH. good and stable compared to the other two formulas, namely 9.6 and the free alkali content with an average of 0.10% which is the best result of the three formulations because the smaller the free alkali content, the better the soap, and 0% yield for the free alkali content. free fatty acids because no fatty acids were detected in this formulation.

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FORMULATION AND EVALUATION OF NUTMEG LEAF EXTRACT (*Myristica fragrans* Houtt.) TRANSPARENT SOLID SOAP

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Abstract

Introduction: Soap is a cosmetic that can act as a means of skin beauty, by keeping the skin supple, smooth and not dry. Soap is also known as a body cleansing tool to minimize the onset of symptoms or diseases that can grow on the skin area. The purpose of this study was to formulate and evaluate the transparent soap of nutmeg leaf extract with three variations of the formula, namely the concentration of nutmeg leaf extract 0,5%, 1%, and 1,5%. **Method:** The evaluation test treatment for the preparation was in the form of organoleptic tests, pH, foam strength, water content, free alkali and free fatty acids to show soap with the best evaluation test results in table data according to SNI standards. **Results:** The results showed that each formulation produced stable dosage forms with various extract concentrations of 0,5%, 1%, and 1,5%. **Conclusion:** So, the best results were obtained in formulation 1, with the pH test getting the most stable results with an average of 9.6, the highest foam height with an average of 127 mm, the value of water content does not exceed the requirements, namely on an average of 12,6 %, free alkali with the lowest value with an average of 0,08%, and free fatty acid test with an average of 0%, which means that free fatty acid content is not detected.

Key words : Extract, *Myristica fragrans* Houtt., Nutmeg Leaf, Transparent Solid Soap

INTRODUCTION

Soap is a cosmetic product that can play a role in maintaining skin beauty and skin health. This is starting to become a very important aspect with the increasing market demand for beauty and skin health products, one of which is transparent solid soap products (Ainiyah & Utami, 2020). Transparent soap as a charming and transparent appearance, with a softer density, making the skin more moist, and made using natural ingredients to nourish the skin and can prevent premature aging. The use of transparent solid soap is very suitable for use among teenagers and adults, especially for skin beauty use (Ramadian *et al.*, 2019). Factors that affect the transparency of soap lies in the content of alcohol, sugar, and glycerin which have a role to clarify soap (Widyasanti *et al.*, 2016). Transparent solid soap is made with the help of active substances from safe natural ingredients, the natural ingredients used are nutmeg leaves which contain secondary metabolites in the form of alkaloids, flavonoids, triterpeneoids and tannins (Anggriani *et al.*, 2018). Nutmeg leaves can act as an antibacterial in the content of flavonoid and terpenoid compounds. Antibacterial can make soap as a cleaning tool that can minimize the onset of symptoms or diseases that grow on the skin area (Arrizqiyani *et al.*, 2018). Pratiwi, 2019 say in the manufacture of liquid soap using the active substance of nutmeg leaf extract with a concentration of 0,5% found the inhibition zone of *S. aureus* bacteria on agar media of 10-20 mm. It is stated that nutmeg leaf extract can function in inhibiting bacteria. The greater the concentration of nutmeg leaf extract, the better it will be in inhibiting bacteria (Moningka *et al.*, 2020). Based on several previous studies and seeing the advantages of transparent solid soap preparations, researchers are interested in conducting research on the formulation and evaluation of transparent solid soap from 96% ethanol extract of nutmeg leaves (*Myristica fragrans* Houtt.) using various extract concentrations of 0,5%, 1%, and 1,5%.

METHOD

A. Qualitative Test of Nutmeg Leaf Extract

1. Alkaloid Test

The extract was taken as much as 0,1 g and added 5 ml of chloroform and 3 drops of ammonia. Divided into 3 soaps, each added with Mayer, Wagner, and Dragendorff reagents. The alkaloids in the

sample will be characterized by the presence of a white precipitate in the *Mayer* reagent, an orange precipitate in the *Dragendorff* reagent, and a brown precipitate in the *Wagner* reagent (Pratiwi *et al.*, 2019).

2. Flavonoid Test

0,1 g of extract was taken and put into a test tube, 0,1 g of magnesium powder and 0,4 ml of amyl alcohol were added to the test tube. Add 4 ml of alcohol and mix until homogeneous. A positive reaction is indicated by the formation of a red, yellow or orange color (Pratiwi *et al.*, 2019).

3. Tanin Test

The extract was put into a test tube. Add FeCl_3 until a green-blue color is formed. The color is a marker of the presence of tannin compounds in the sample (Pratiwi *et al.*, 2019).

4. Triterpenoid Test

0,1 g of the extract sample was taken, then 3 drops of concentrated HCl and 1 drop of concentrated H_2SO_4 were added. A positive trirepennoid is indicated by the formation of a red color (Pratiwi *et al.*, 2019).

B. Transparent Solid Soap Preparation Formula

Table 1. Transparent Solid Soap Formulation of Nutmeg Leaf Extract (Widyasanti *et al.*, 2016)

No	Material Composition	Formula			Function
		I(g)	II(g)	III(g)	
1.	Nutmeg Leaf Extract	1	2	3	Active Substance
2.	Coconut Oil	40	40	40	Oil Phase
3.	Stearic Acid	14	14	14	Emulsifier
4.	NaCl	0,4	0,4	0,4	Tonicity Agent
5.	NaOH 30%	40,6	40,6	40,6	Buffer Agent
6.	Glycerin	26	26	26	Preservative
7.	Coco-DEA	2	2	2	Foam Stability
8.	<i>Fragrance Oil</i>	0.2	0.2	0.2	Fragrance
9.	Sugar	30	30	30	Humectants
10.	Ethanol 96%	30	30	30	Solvent
11.	Aquadest	15,8	14,8	13,8	Solvent

C. Preparation of Transparent Solid Soap

The process of making soap using water bath media with the hot method. Pure coconut oil that has been placed in a glass beaker is heated through a water bath. Add stearic acid, then stir until homogeneous. After homogenization, the 30% NaOH solution was added and other supporting materials such as 96% ethanol, glycerin, sugar syrup (sugar + liquefied aquadest), coco-DEA, NaCl and fragrance oil. Stirring is done until the dough looks mixed evenly. The addition of nutmeg leaf extract with the temperature of the dough lowered first until it reaches 70°C. After that, the mixture was stirred until the extract was completely mixed. Then it is poured into the prepared silicone soap mold. Let the dough rest at room temperature for 24 hours (Widyasanti *et al.*, 2016).

D. Transparent Solid Soap Characteristic Test

1. Organoleptic Observations

Organoleptic tests include observations of shape, color changes, and odor changes (Susanti & Guterres, 2018).

2. pH Test

The pH test was tested by weighing 1 g of soft soap dissolved in 9 ml of distilled water. Then the pH marker is dipped and the pH value of soft soap is observed, the pH of soft soap is 9-11 (Susanti & Guterres, 2018).

3. Foam Strength Test

The foam strength test was tested by weighing 10 g of soft soap dissolved in aquadest. After that, shake vigorously and measure the amount of foam that is formed after shaking for 30 seconds and 60 seconds (Susanti & Guterres, 2018).

4. Water Content Test

The water content test was carried out using a moisture analyzer. The tool is first turned on, then press the mode button and select the mode you want to use. Open the cover on the device so that the display status will change. Insert it into an empty aluminum pan that has been cleaned, making sure the pan is in the correct position. The cover on the tool is closed again until the towering is done automatically. When it is tare, open it again and add 1g of soap sample then close the tool again. The appliance will start heating the soap sample until drying is complete. If the display indicator is green, the sign has shown constant results to determine the weight of the sample after drying (Febriani *et al.*, 2021).

5. Free Alkali Content Test

5g of solid soap was dissolved using 10 ml of distilled water, then bromthymol blue was added and titration was carried out using 0,1 N NaOH until the color changed from blue to yellow. The test was carried out 3 times replication. Determination of free fatty acid content can be calculated using the formula:

$$\frac{V \times N(BS) \times BM}{W \times 1000} \times 100\%$$

Information :

V : Volume of NaOH during titration
 N : Normality of NaOH
 BM : Molecular weight of free fatty acids
 W : Weight of soap preparation (Susanti & Guterres, 2018).

6. Free Fatty Acid Content Test

5g of solid soap is weighed into a measuring cup. Then 10 ml of distilled water was added and phenolphthalein marker was added. After the solution shows a pink color, it is titrated using 0.1N HCl solution until the red color change disappears. Determination of free fatty acid content can be calculated using the formula:

$$\frac{V \times N(BS) \times BM}{W \times 1000} \times 100\%$$

Information :

V : Volume of HCl during titration
 N : Normality of HCl
 BM : Molecular weight of free fatty acids
 W : Weight of soap preparation (Susanti & Guterres, 2018).

RESULTS

A. Qualitative Test of Nutmeg Leaf Extract

Phytochemical screening carried out in this study was in the form of tests such as alkaloids, flavonoids, tannins and triterpenoids. The results of some of these tests yielded positive results in all four tests. The results of phytochemical screening can be seen in full in table 2.

Table 2. Qualitative Test Results of Nutmeg Leaf Extract

Sample Name	Keadaan Sampel	Parameter	Hasil	Teknik Analisi	
Nutmeg Leaf Extract	Thick Extract	Alkaloid	Mayer	Positive	White Precipitate
			Wagner	Positive	Brown Precipitate
			Dragendroff	Positive	Orange Precipitate
		Flavonoid		Positive	Make Orange Color
		Tanin	FeCl ₃ 1%	Positive	Make Blackish Green Color
		Triterpenoid		Positive	Brownish Red Precipitate

B. Stability Test

1. Transparent Solid Soap Evaluation Test Results

Table 3. Results of Organoleptic Observations of Transparent Solid Soap

Formula	Replication	Color	Odor	Appearance
F1	1	Light Brown	Aromatic	Solid
	2	Light Brown	Aromatic	Solid
	3	Light Brown	Aromatic	Solid
F2	1	Light Brown	Aromatic	Solid
	2	Light Brown	Aromatic	Solid
	3	Light Brown	Aromatic	Solid
F3	1	Dark Brown	Aromatic	Solid
	2	Dark Brown	Aromatic	Solid
	3	Dark Brown	Aromatic	Solid

Information :

F1 : Transparent solid soap with 0,5% nutmeg leaf extract concentration

F2 : Transparent solid soap with 1% nutmeg leaf extract concentration

F3 : Transparent solid soap with 1,5% nutmeg leaf extract concentration

If seen in table 3, the results obtained from organoleptic tests of transparent solid soaps with the three formulations using various extract concentrations. The results in Formula 1 and Formula 2 get a light brown color from transparent soap, with a solid form and a distinctive aromatic aroma. Likewise, the third formulation produces transparent soap with a distinctive aromatic aroma and solid shape but with a darker color. This is due to the increasing concentration of the extract used.

2. Transparent Solid Soap pH Test Results

Table 4. pH Test Results

Replication	F1	F2	F3
1	9,9	9,7	9,7
2	9,4	9,8	9,7
3	9,5	9,5	9,6
Average $\pm$ SD	9,6 $\pm$ 0,26	9,7 $\pm$ 0,15	9,7 $\pm$ 0,56

Information :

F1 : Transparent solid soap with 0,5% nutmeg leaf extract concentration

F2 : Transparent solid soap with 1% nutmeg leaf extract concentration

F3 : Transparent solid soap with 1,5% nutmeg leaf extract concentration

Table 4 shows the results of the pH test on transparent solid soap preparations of nutmeg leaf extract (*Myristica fragrans* Houtt) from each formulation with an average pH value in the range from 9.6 to 9.7.

3. Transparent Solid Soap Foam Strength Test Results

Table 5. Foam Strength Test Results

Replication	F1	F2	F3
1	120 mm	120 mm	120 mm
2	110 mm	130 mm	110 mm
3	150 mm	90 mm	120 mm
Average $\pm$ SD	127 $\pm$ 2,08 mm	113 $\pm$ 2,08 mm	117 $\pm$ 0,58 mm

Information :

F1 : Transparent solid soap with 0,5% nutmeg leaf extract concentration

F2 : Transparent solid soap with 1% nutmeg leaf extract concentration

F3 : Transparent solid soap with 1,5% nutmeg leaf extract concentration

The results on the foam strength test of the transparent solid soap preparations of nutmeg leaf extract (*Myristica fragrans* Houtt) can be seen in table 5. The results show that each formulation has foam height in the foam height value in the range of 113-127 mm.

4. Transparent Solid Soap Water Content Test Results

Table 6. Water Content Test Results

Replikasi	F1	F2	F3
1	14,7%	10%	5,7%
2	12,6%	9,4%	6,3%
3	10,5%	10%	5,7%
Rata-Rata ± SD	12,6 ± 2,12%	9,8 ± 0,34%	5,9 ± 0,36%

Information :

F1 : Transparent solid soap with 0,5% nutmeg leaf extract concentration

F2 : Transparent solid soap with 1% nutmeg leaf extract concentration

F3 : Transparent solid soap with 1,5% nutmeg leaf extract concentration

The results of the water content test of transparent solid soap preparations of nutmeg leaf extract (*Myristica fragrans* Houtt) can be seen in table 6. The results of each formulation get an average value of water content in the range of 5,9 – 12,6%.

5. Transparent Solid Soap Free Alkali Content Test Results

Table 7. Free Alkali Content Test Results

Replication	F1	F2	F3
1	0,07%	0,11%	0,11%
2	0,10%	0,14%	0,10%
3	0,08%	0,12%	0,08%
Average ± SD	0,08 ± 0,02%	0,12 ± 0,02%	0,10 ± 0,02%

Information :

F1 : Transparent solid soap with 0,5% nutmeg leaf extract concentration

F2 : Transparent solid soap with 1% nutmeg leaf extract concentration

F3 : Transparent solid soap with 1,5% nutmeg leaf extract concentration

Table 7 shows the results in the form of free alkali data which is still contained in the transparent solid soap preparation of nutmeg leaf extract (*Myristica fragrans* Houtt). The results showed that the free alkali content of each formulation obtained a concentration value with an average range of 0,08 – 0,12%.

6. Transparent Solid Soap Free Fatty Acid Content Test Results

Table 8. Free Fatty Acid Content Test Results

Replication	F1	F2	F3
1	0%	0%	0%
2	0%	0%	0%
3	0%	0%	0%
Average ± SD	0%	0%	0%

Information :

F1 : Transparent solid soap with 0,5% nutmeg leaf extract concentration

F2 : Transparent solid soap with 1% nutmeg leaf extract concentration

F3 : Transparent solid soap with 1,5% nutmeg leaf extract concentration

The results of the free fatty acid test on transparent solid soap preparations of nutmeg leaf extract (*Myristica fragrans* Houtt) can be seen in table 8. The results obtained in each formulation get a free fatty acid content value of 0%, which means that free fatty acids are no longer found. contained in soap preparations.

DISCUSSION

In this study, three transparent solid soap formulations were made with various extract concentrations of 0,5%, 1% and 1,5%. It aims to obtain a formula with the best results seen from the physical stability of the preparation. This research was carried out in several stages, namely preparation of nutmeg leaf samples, nutmeg leaf extraction, phytochemical screening of nutmeg leaf extract, formulation and evaluation of transparent solid soap preparations of nutmeg leaf extract. The evaluation carried out in the form of organoleptic test, pH test, foam strength test, water content test, free alkali test and free fatty acid test. Nutmeg leaf extract was made using the maceration method starting from nutmeg leaf simplicia powder which was macerated with 96% ethanol for 3x24 hours. The maceration method was chosen because it has the advantages of simple, easy-to-reach and relatively inexpensive equipment and working methods. Secondary metabolic compounds such as flavonoids are easily damaged when using high-temperature heating, so this maceration method was chosen to avoid damage to compound

components on heating. The choice of the 96% ethanol solvent was based on the degree of safety and ease of evaporation. Its properties are able to dissolve almost all substances, whether polar, semipolar, nonpolar, and can attract flavonoid and phenolic compounds optimally (Ramadhani *et al.*, 2020).

Phytochemical screening of the thick extract aims to determine the presence of secondary metabolites in the sample using color reagents to see what is contained in the extract preparation (Ramadhani *et al.*, 2020). Anggriani (2018) say that nutmeg leaves contain secondary metabolites in the form of alkaloids, flavonoids, triterpeneoids and tannins. The alkaloid test was carried out by dissolving the extract using HCl and adding three reagents. The use of HCl aims to extract the alkaloid content of the sample. The addition of three reagents (*Mayer*, *Wagner*, and *Dragendorff*) to identify the presence of alkaloid compounds was characterized by a precipitation reaction (Iskandar, 2020). The precipitation reaction occurs because the nitrogen atom has a lone pair of electrons on the alkaloids replacing the iodine ion in the reagent (Nurlani & Situmorang, 2021).

Alkaloid test using *Mayer's* reagent got positive results in the presence of a white precipitate. White-yellow precipitate on the addition of *Mayer's* reagent because nitrogen in the alkaloids will react with metal ions K^+ from $K_2[HgI_4]$ (potassium tetraiodomercurate (II)) to form potassium-alkaloid complexes which precipitate. The alkaloid test using *Wagner's* reagent got a positive result in the form of a brown precipitate. *Wagner's* reagent is a mixture of iodine (I_2) with KI. The brown precipitate comes from the bond between the nitrogen atom (N) which has a lone pair of electrons on the alkaloid to the metal ion K^+ to form a potassium-alkaloid complex compound. Positive alkaloid test with the addition of *Dragendorff's* reagent will form an orange precipitate. *Dragendorff* reagent makes nitrogen in alkaloids form coordinate covalent bonds with K^+ which is a metal ion so that an orange precipitate can be formed (Wahyuni & Marpaung, 2020).

The flavonoid test was carried out by adding magnesium (Mg) powder and concentrated HCl. Positive results of flavonoids with the formation of an orange color. The addition of concentrated HCl breaks the glycoside bonds of flavonoids, the glycoside bonds with flavonoids in the extract must be broken first by reducing the bond with magnesium powder, thus forming an orange color indicating the presence of flavonoids in the sample (Iskandar, 2020). The tannin test got the results that formed a blackish green color. Positive in the tannin test will be indicated by the appearance of a blue green blackish color (Pratiwi *et al.*, 2019). The extract was dissolved using aquadest, because tannins are polar phenolic compounds. The addition of $FeCl_3$ which reacts with the tannin compound will find a mechanism for the formation of a green-blue color as a sign of the presence of a phenol group contained in the sample, so that the identification results show that it contains positive tannins. In the triterpenoid test, the sample was dripped with concentrated HCl and concentrated H_2SO_4 , producing a brownish red color. The addition of concentrated HCl was used to attract the triterpenoid content in the sample, and the addition of concentrated sulfuric acid to the triterpenoid compound resulted in the formation of a red color that occurred (Iskandar, 2020).

In soap making, a saponification reaction will occur which is the process of breaking the triglyceride chain in the form of oil or fat using alkaline compounds. The alkaline compound used is in the form of NaOH base where NaOH is suitable to produce solid soap products, and can be seen in the characteristics of NaOH which is difficult to dissolve or not easily soluble in water. This saponification process will produce a product in the form of solid soap and a by-product in the form of glycerol (Agustini & Winarni, 2017).

Organoleptic test is one of the physical parameters to see changes in color, shape, and odor changes in transparent solid soap preparations (Susanti & Guterres, 2018). In formula 1 using 0,5% extract, it is obtained transparent soap with a light brown solid form with a distinctive aromatic odor derived from fragrance oil. Formula 2 using 1% extract produces a transparent soap in the form of a solid with a light brown color with a distinctive aromatic odor. In formula 3 using 1,5% extract, you get solid soap with a distinctive aromatic smell, and dark brown in color with less visible transparency, this is because the more extracts used, the darker the soap will be and the transparency will also look better (Pratiwi *et al.*, 2019).

The pH test is one of the important parameters used to determine the resulting soap preparation. The results from the average pH range of 9,6-9,7 are known to get the results in table 4 in accordance with the

requirements of SNI 06-3532-1994 soap pH ranges from 9-11 (BSN, 2016). Soaps that have a pH value that is too high or too low can increase the absorption capacity of the skin, which can cause skin irritation such as sores, itching and peeling (Fanani *et al.*, 2020). The high and low pH of soap can be influenced by the saponification process and the composition of the formula used when making soap. When you find a high pH value, it can be overcome by adding fat or oil. The amount of alkali present in soap can also affect the level of pH. The more alkaline content, the higher the pH of the soap (Setiawati & Ariani, 2021).

The foam strength test aims to determine the amount of foam produced by transparent solid soap preparations. This test is carried out by measuring the height of the foam produced on each soap. The results in table 5 show that the strength of the foam obtained has met the requirements of the foam height, namely 13 - 220 mm according to the requirements of SNI 06-3532-1994 (BSN, 2016). High - low foam can be experienced due to a factor, namely when shaking the soap. The characteristics of the foam are influenced by the presence of materials that function as surfactants or foam stabilizers, the foam stabilizer used in this formulation is the role of Coco – DEA (Rosi *et al.*, 2021).

The water content test is carried out to find out how much water content is in solid soap preparations. The results in table 6 find quite good results, from each replication and the average results do not exceed the required limit of water content, which is 15% in accordance with the requirements of SNI 06-3532-1994 (BSN, 2016). The water content is said to affect the level of hardness of the soap preparation. The higher the water content, the softer the soap will be. Vice versa, the lower the water content produced, the harder the soap will be. In addition, the amount of water in soap can affect the storage of soap. If it produces soap with a high moisture content or more than 15% in open storage and in contact with air, it can cause the soap to shrink from the weight of the soap. Soap preparations with a good water content value will increase their storage period or are relatively long, and will be more efficient in use because soap will not dissolve easily in water (Elmitra & Noviyanti, 2020).

Free alkali test was carried out to determine the presence of excess free alkali. The results in table 7 show that the levels of free alkali still meet the requirements of the free alkali test in accordance with the requirements of SNI 06-3532-1994, which is not more than 0,14% (BSN, 2016). Free alkali is an alkali that does not react with fatty acids during the formation of soap preparations. Free alkali has a maximum content of 0,14% in soap. The analysis on free alkali aims to find out how much or how much alkali metal is not saponified, because it is feared that it will have an impact on the quality of the soap produced (Sa'diyah *et al.*, 2018). Alkali is known to have a hard nature, so that if the free alkali level produced is too high it can irritate the skin, and can cause the skin to become dry. This is because sodium hydroxide is hygroscopic so it can cause absorption of skin moisture quickly (Fanani *et al.*, 2020).

The free fatty acids in soap are part of the fatty acids that are not bound to the alkali. The results of the evaluation of the free fatty acids in table 8 did not state that the presence of free fatty acids was detected. This is indicated by the absence of a color change that is produced. As happened in the study of Hardian *et al.*, (2016) which stated that the assessment of the free fatty acid levels in transparent solid soap was considered undetectable. It is suspected that the free fatty acid content is considered non-existent because the free fatty acid in the preparation has reacted with the base to form soap and glycerol. The free fatty acid test on soap preparations is not expected to produce a high value of not more than 2,5% in accordance with the requirements of SNI 06-3532-1994. Because it will reduce the binding power of soap to dirt, oil, fat or sweat (BSN, 2016). Temperatures that are too high and heating times that are too long can cause damage and will form free fatty acids again, so that the levels of free fatty acids will be higher (Tobing *et al.*, 2021). High free fatty acids can also affect the formation of foam, the resulting foam will be less because there are still free fatty acids that do not react with NaOH in the soap making process (Prasetiyo *et al.*, 2020). Free fatty acids are associated with the odor produced in soap, if the free fatty acids exceed the standard soap will smell rancid and can inhibit the process of cleaning the skin surface by soap (Fanani *et al.*, 2020).

CONCLUSION

Nutmeg leaf extract (*Myristica fragrans* Houtt) can be formulated into transparent solid soap preparations with various extract concentrations of 0,5%, 1% and 1,5% by getting the best dosage results in Formula 1. an average of 9.6, the highest foam height with an average of 127 mm, the water content corresponding to an average of 12,6%, the lowest free alkali content with an average of 0,08%, and the free fatty acid content that is no longer detected.

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THE EFFECT OF STORAGE TEMPERATURE ON VITAMIN C LEVELS IN PACKED RED GUARANTEE DRINKS SOLD IN BEKASI CITY

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Abstract

Red guava packaged drinks contain vitamin C. Vitamin C or ascorbic acid has properties that are soluble in water and can be oxidized. Factors that oxidize vitamin C, such as temperature and pH. The purpose of this study was to determine the effect of storage temperature on vitamin C levels in packaged red guava drinks sold in the city of Bekasi. The method in this research is experimental design. The analysis carried out in this research is qualitative analysis and quantitative analysis. Qualitative analysis using KMnO₄ color test and quantitative analysis using UV-Vis spectrophotometry at a wavelength of 289 nm. The highest levels of vitamin C were found at a heating temperature of 30°C and the lowest levels at a temperature of 50. In sample A with a temperature treatment of 30 it had an average level of 169.348 mg/mL; temperature 40°C 154.312 mg/mL; temperature 50 126,051 mg/mL, in sample B temperature 30°C 141,268 mg/mL; temperature 40°C 115.906 mg/mL; temperature 50°C 95,616 mg/mL, in sample C temperature 30°C 85,471 mg/mL; temperature 40°C 74,420 mg/mL, temperature 50°C 61,014 mg/mL. Data processing and analysis used in this study, namely One Way ANOVA obtained sig.0.00 (p<0.05). The results of this study indicate that temperature affects vitamin C levels in guava packaged drinks.

Keywords: Ascorbic Acid, Guava, UV-Vis Spectrophotometry, Packaged Drinks, Psidium guajava L.,

INTRODUCTION

Human life today is almost all the time as in instant things, consuming packaged drinks that contain lots of vitamins. Over time, more and more factories are producing fruit juice-based drinks containing guava seeds (Simanjuntak, 2021). However, the process of storage, distribution, and sale makes these packaged drinks exposed to high temperatures and sunlight (Wulandari 2017).

Red guava packaged drinks contain vitamin C. The compound contained in vitamin C is ascorbic acid. Foods and fruits that contain vitamin C will be damaged by the oxidation process of the air, especially if they contain. Storage carried out at low temperatures (refrigerator) and cooking that does not result in discoloration of foods containing vitamins (Wulandari 2017).

Previous research on the effect of storage temperature and heating time has been carried out. Simanjuntak (2021) suggested that watermelon juice will decrease vitamin C levels with heating because vitamin C has unstable properties and is easily oxidized. The results obtained from the heating temperatures used are 30°C, 40°C, 50°C, 65°C, and 80°C indicating a good temperature for watermelon juice at 30°C and stating that temperature can affect the decrease in concentration in watermelon juice.

In a study conducted by Wasallwa (2016), it was stated that the infusion process with a time of 120 minutes at a temperature of 37°C produced the highest concentration results. The results obtained can be seen that the temperature and time of immersion affect the process of dissolving vitamin C contained in banana peels to dissolve in water.

Various studies have been conducted to determine the effect of temperature on vitamin C levels. So far, research with this title has never been done. Research on the effect of temperature on vitamin C levels has been carried out but the use of red guava packaged drinks in the city of Bekasi has never been done. Researchers are interested in knowing the effect of temperature on vitamin C levels in packaged red guava drinks sold in the city of Bekasi.

METHOD

A. Research design

This research is included in quantitative research with the research design used is experimental.

B. Research Location and Time

This research was conducted at the STIKes Mitra Keluarga Laboratory, Bekasi City, West Java Province from March to April 2022.

C. Population and Sample

The population in this study were packaged drinks sold at Naga Swalayan, Bekasi City, West Java Province. Samples in this study were packaged red guava drinks sold in Bekasi City, West Java Province.

D. Tools and materials

The tools used include analytical balance (Ohaus), UV-Vis spectrophotometry (Genesys 10S UV-Vis), oven, measuring flask (Iwaki Pyrex), test tube, test tube rack (Iwaki Pyrex), measuring cup, beaker glass, paper filter, funnel, dark glass vial, dark vial, dropper, measuring pipette, tip and micropipette. The materials used included samples of guava packaged drinks, ascorbic acid (BBI Life Sciences), KMnO₄ (e merck), aquadest pro analysis.

E. Preparation Sampling

The vitamin C sample consisted of three guava packaged drinks. Guava packaged drinks were treated with heating using an oven at temperatures (30°C, 40°C, and 50°C) for 30 minutes each and the packaged drinks were filtered using filter paper before assaying.

F. Determination Precision

Tests were carried out at concentrations of 2 ppm, 4 ppm and 6 ppm with 3 times in a row. Then the absorbance was measured at its maximum wavelength. Accuracy is determined from the standard deviation (SD) and % RSD (Nasution et al. 2019).

G. Determination Accuracy

Determination of accuracy in this study was carried out by the standard addition method. The comparison between the sample and the standard was 70:30 with a range of 80%, 100%, and 120%. Absorption from was measured using the maximum wavelength, and replicated 3 times (Yunita et al. 2020).

H. Qualitative Test

A total of 3 mL of samples without temperature treatment and 3 mL of samples that have been treated with temperature (30°C, 40°C, and 50°C), each added 3 mL of 0.1% KMnO₄ solution. Samples containing vitamin C turned purple in KMnO₄ and the solution turned brown (Karaginani, 2019).

I. Production of KMnO₄ 0.1%

A total of 0.5 grams of KMnO₄ was added to 50 ml of distilled water in a beaker and boiled for 15 minutes then filtered (Maulida et al, 2020).

Quantitative Analysis

J. Preparation of 100 ppm Vitamin C mother liquor

Ascorbic acid is weighed as much as 50 mg, put into a 500 mL volumetric flask, added 500 mL of distilled water, shaken until dissolved, added with distilled water to the mark line, and shaken until homogeneous (100 ppm) (Simanjuntak, 2021).

K. Determination of the Maximum Absorption Wavelength of Vitamin C

Larutan Solution 10 mL of ascorbic acid standard mother liquor was pipetted, put into a 100 mL volumetric flask, added with distilled water to the mark line, and shaken until homogeneous. Ascorbic acid standard solution was measured absorbance at a wavelength of 200 nm to 800 nm (aquadest was used as a blank) (Dewi, 2019).

L. Determination of Linearity of Ascorbic Acid

standard liquor was pipetted in an amount of 0.2 mL; 0.4 mL; 0.6 mL; 0.8 mL; and 1 mL, were added separately to a 10 mL volumetric flask, added with distilled water up to the marking line, and shaken until homogeneously mixed (a standard series solution of ascorbic acid was obtained with a concentration of 2.0 ppm; 4.0 ppm; 6.0 ppm; 8.0 ppm; and 10 ppm).) and replicated three times.

Ascorbic acid standard series solution was measured absorption at the maximum wavelength, and the regression equation was obtained, namely $y=bx+a$ (Simanjuntak, 2021).

M. Determination of Vitamin C Levels in Packaged Beverage Samples

Packaged drinks were treated with heating temperatures with variations in heating temperatures (30°C, 40°C, and 50°C) for 30 minutes each. Packaged guava drink was determined for vitamin C content by pipetting as much as 1 mL, put into a 50 mL volumetric flask, added with distilled water until the line marks, shaken until homogeneously mixed and repeated 3 times for each treatment. The mixture was filtered using a funnel and filter paper, the filtrate was collected, and the absorption was measured at the maximum wavelength (Simanjuntak, 2021).

N. Processing and data analysis

Vitamin C levels were calculated using a linear regression equation that obtained from ascorbic acid standard curve data. Data is processed using Microsoft Excel 2010 program. The results of the excel data will then be tested statistics using the Product Services Solution (SPSS) 21 statistical program using the One way ANOVA method (One-way analysis of variance) with 95% confidence level or $\alpha = 0.05$

RESULTS

A. Qualitative Test

The qualitative test was carried out in this study aimed to determine the presence or absence of vitamin C contained in the sample of packaged drinks. Qualitative test carried out is color test with KMnO_4 reagent. A positive sample indicates that it contains Vitamin C if the sample is purple which disappears into a brown color (Kurniawati & Evi 2019). The results of the qualitative test with KMnO_4 0.1% without temperature treatment are presented in table 1 and the results of the qualitative test with KMnO_4 0.1% with temperature treatment are presented in table 1.

Table 1. Qualitative Test Results with KMnO_4 0.1%

Treatment	Sample Code	Color	Results
Control		Purple	-
No Temperature	A	Chocolate	+
	B	Chocolate	+
	C	Light brown	+
Temperature 30°C	A	Chocolate	+
	B	Chocolate	+
	C	Light brown	+
Temperature 40°C	A	Chocolate	+
	B	Chocolate	+
	C	Light brown	+
Temperature 50°C	A	Chocolate	+
	B	Chocolate	+
	C	Light brown	+

Information :

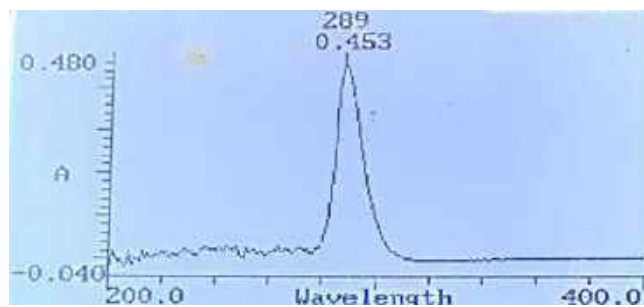
+ = Positive contains Vitamin C

- = Negative contains Vitamin C

B. Ascorbic Acid Maximum Wavelength Determination

Ascorbic acid standard was determined with a concentration of 10 ppm. The maximum wavelength obtained in this study is 289 nm. The determination of the maximum length is shown in Figure 1

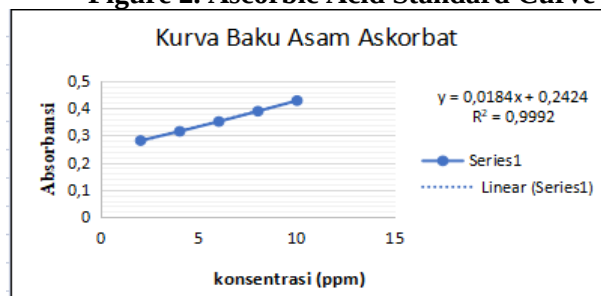
Figure 1. Maximum Wavelength



C. Determination of the Quercetin Standard Curve

In this study, the determination of the standard curve for ascorbic acid was carried out with a series of concentrations of 6,7,8,9 and 10 ppm. The results obtained are in the form of a linear equation, namely $y = 0.0338x + 0.4442$ with an r^2 value of 0.993 in addition, there is an SD value of 0.0133 and an RSD of 0.29%. The standard curve for quercetin can be seen in Figure 2.

Figure 2. Ascorbic Acid Standard Curve



D. Determination of Vitamin C Levels

Determination of levels in guava packaged drinks, each sample was treated with heating temperatures of 30°C, 40°C, 50°C and samples that were not treated.

Table 2. Determination of Vitamin C Kadar Levels

Treatment	samplel	Replication	Absorbance	Vitamin C Level (mg/mL)	Average Vitamin C Level (mg/mL)
No temperature	A	1	0,59	188,913	192,536
		2	0,601	194,891	
		3	0,599	193,804	
	B	1	0,548	166,087	167,174
		2	0,552	168,261	
		3	0,550	167,174	
	C	1	0,53	156,304	156,123
		2	0,527	154,674	
		3	0,532	157,391	
Temperature 30°C	A	1	0,555	169,891	169,348
		2	0,55	167,174	
		3	0,557	170,978	
	B	1	0,527	142,717	141,268
		2	0,522	141,087	
		3	0,53	140,000	
	C	1	0,471	85,652	85,471
		2	0,473	81,848	
		3	0,479	88,913	
	A	1	0,505	154,674	

Temperature 40°C	B	2	0,502	151,957	154,312
		3	0,500	156,304	
		1	0,457	116,630	
	C	2	0,454	115,000	115,906
		3	0,456	116,087	
		1	0,421	73,152	
	A	2	0,415	72,065	74,420
		3	0,419	78,043	
		1	0,400	124,239	
Temperature 50°C	B	2	0,393	125,326	126,051
		3	0,406	128,587	
		1	0,377	97,065	
	C	2	0,375	93,804	95,616
		3	0,386	95,978	
		1	0,352	59,565	
	A	2	0,357	62,283	61,014
		3	0,355	61,196	
		1			

E. Data Analysis Processing

Table 3. Results of Tests of Normality

		Kolmogorov-Smirnov ^a			Shapiro Wilk		
Suhu		Statistic	df	Sig.	Statistic	df	Sig.
Kadar	Suhu 30°C	.209	9	.200*	.855	9	.084
	Suhu 40°C	.204	9	.200*	.861	9	.099
	Suhu 50°C	.185	9	.200*	.927	9	.449

Table 4. Results of Homogeneity of Variance.

Levene Statistic	df1	df2	Sig.
1.947	2	24	.165

Table 5. One Way Anova Test Results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6594.802	2	3297.401	45.056	.000
Within Groups	1756.413	24	73.184		
Total	8351.214	26			

DISCUSSION

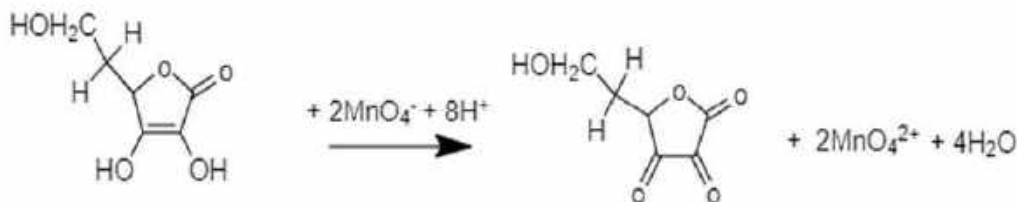
Qualitative Test

Qualitative analysis of Vitamin C was carried out by color test using KMnO₄ reagent. The function of KMnO₄ as an oxidizing agent that undergoes reduction (Kurniawati & Evi 2019). In the qualitative test, two different treatments were used, namely the sample without being treated with temperature and the sample being treated with temperature. Samples without temperature treatment and samples treated with temperatures of 30°C, 40°C and 50°C, in all three samples (A, B, C) the same results were obtained, namely the purple color slowly disappeared and there was a brown precipitate in the solution, it can be concluded that all samples tested positive for vitamin C.

The permanganate ion accepts electron ions that are released from vitamin C so that the KMnO₄ color is lost. The addition of potassium permanganate reagent to the sample resulted in a change in the color

of KMnO_4 which was initially brownish purple and turned into a brown precipitate indicating the formation of manganese ions (Kurniawati & Evi 2019). The following equation for the reaction of Vitamin C with KMnO_4 is presented in Figure 3:

Figure 3. Reaction of Vitamin C with KMnO_4 (Putri and Dyna 2019)



Ascorbic Acid Wavelength Determination

The maximum wavelength is a substance that provides the highest absorption (Dewi, 2019). The reason for the measurement at the maximum wavelength is that the change in absorbance for each unit of concentration is greatest at the maximum wavelength, so that maximum analytical sensitivity will be obtained (Rosalina, 2018). Determination of the wavelength in this study was carried out by measuring the absorbance of vitamin C at a wavelength between 200 nm - 400 nm in UV light.

The purpose of measuring the maximum wavelength in this study is to determine the optimum absorption of vitamin C and the obtained wavelength will be used to measure the absorbance of the sample. Vitamin C can be measured using UV-Vis spectrophotometry at a wavelength of 200-400 nm because vitamin C has a chromophore molecular structure that can absorb UV rays (Dewi, 2019).

The maximum wavelength of Vitamin C in this study was 289.0 nm with a concentration of 10 ppm with aquadest as a solvent. The wavelength obtained in this study is different from other literature, namely 265.0 nm. This is due to the bathochromic shift or red shift. The bathochromic effect or red shift is a change in wavelength absorption towards a larger wavelength, this occurs due to differences or changes in solution and the presence of certain substituents/auxochromes on the chromophore (Suhartati, 2017).

Determination of Ascorbic Acid Standard Curve

The linearity test was carried out to determine the standard capability so that there was a linear relationship between the analyte concentration and the detector response (Apriliyani et al. 2018). Linearity test is included in the validation of the method used to prove that these parameters meet the requirements for use in research. Based on Figure 5.3, the standard curve of ascorbic acid was made using 5 concentration series, namely 2,4,6,8, and 10 ppm, obtained $Y = 0.0184x + 0.2424$. The intercept value (a) represents the effect of the matrix. The greater the value, the greater the effect of the matrix on the sample measurement. The slope value (b) indicates the sensitivity of a method, namely the effect of changes in concentration on the resulting signal. The greater the value, the greater the sensitivity value of a method. The concentration value to be determined (x) is the measured analyte concentration (Sri, Nurbayanti, and Bahan 2019). Determination of the standard curve of ascorbic acid was carried out at the concentration series because it produced absorbances that met a good absorbance range, namely 0.2-0.8. This linear regression equation was used to calculate the vitamin C content in the guava packaged beverage sample. In this study, the correlation coefficient value (r) = 0.9996 and the coefficient of determination (r^2) = 0.9992 indicates that the value of r which is close to 1 has a linear standard curve and there is a relationship between the concentration of ascorbic acid solution and the absorption value.

Determination of Vitamin C Levels

In sample A with a temperature treatment of 30°C decreased by 12.04%, at a temperature of 40°C by 16.58%, and a temperature of 50°C by 39.49%. In sample B with a temperature treatment of 30°C decreased by 7.69%, at a temperature of 40°C by 24.88%, and a temperature of 50°C by 35.79%. In sample

C at a temperature of 30°C the concentration decreased by 19.26%, at a temperature of 40°C by 24.14%, and at a temperature of 50°C 36.18%. The results of this study are in accordance with previous research conducted by simanjuntak (2021) that the highest vitamin C levels were from the 30°C temperature treatment. This is because vitamin C has properties that are easily damaged, mostly due to heating temperatures, the results show that the higher the temperature, the lower the concentration of vitamin C produced.

Data Analysis Processing

Data processing in this study used the One Way Anova test. Based on the results of the Shapiro Wilk normality test of the three temperatures, the p value > 0.05 means that the data is normally distributed. Based on the output of SPSS "Test Homogeneity of Variance" obtained a significance value of $0.165 > 0.05$, it can be concluded that the data is the same or homogeneous so that the assumption of homogeneity in the One Way Anova test is fulfilled. One Way Anova test results sig. $0.000 < 0.05$ indicates that the red guava packaged drink has a significantly different average vitamin C content. In the Multiple Comparison output at temperatures of 30°C, 40°C, and 50°C, the sig. ($p < 0.05$) so that the decision H_0 is rejected and H_1 is accepted, which means that there is a significant difference.

CONCLUSION

Based on the research, it was found that the temperature of 30°C had the highest level and the lowest level was at a temperature of 50°C. The results of the One Way Anova test obtained the probability value $<$ (significance level) which is $0.00 < 0.05$ so it can be concluded that there is a significant difference in the effect of the average value of vitamin C levels in each temperature treatment group.

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Formulation and Evaluation of Red Ginger Extract Transparent Solid Soap (*Zingiber Officinale* Var. *Rubrum*)

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Abstract

Introduction: Soap is a cosmetic that is useful for cleaning the skin and can also be useful for other health such as anti-microbial. The content of red ginger which has antibacterial properties such as gingerols and flavonoids. The purpose of this study was to obtain the best formulation of transparent solid soap preparations red ginger extract. **Method:** Transparent solid soap is made through a saponification process using 3 different concentrations of red ginger extract of F1 (0,5%), F2 (1,0%) and F3 (1,5%) with evaluation including organoleptic test, pH test, water content test, foam strength test, free alkali content test and free fatty acid content test. **Results:** The evaluation results obtained from the three formulas obtained suitable results for the five tests except that the free fatty acid levels were not detected. The comparison of the three formulations that get the best results is F3 with an average water content of 5.78%, the most stable average pH range is 9.6, the highest average foam height is 10cm, and the free alkali content of 0.10% is the lowest. **Conclusion:** The conclusion of this study is that F1, F2, and F3 meet all the requirements for making red ginger extract transparent solid soap.

Keywords: *Transparent solid soap, red ginger extract, stability test.*

INTRODUCTION

Soap is a form of cosmetic that can be used in daily life to clean and remove dirt that sticks to the skin (Widyasanti et al., 2017). Soap with certain specifications combined with other ingredients can prevent or reduce skin diseases caused by bacteria and fungi (Sukawaty, et al., 2016). One type of soap that has its own charm is transparent solid soap. Transparent solid soap is a new innovation in cosmetic products as a body cleanser and looks attractive because it looks transparent, the foam released is very soft, and more shiny than other soaps (Purwanto et al., 2019). Transparent solid soap is used on the skin which is useful as a cleansing agent and can function as an antidote to free radicals and prevent infection and microbes when combined with other active ingredients (Sukeksi et al., 2018). Virgin coconut oil (VCO) is very useful in soap making because it can moisturize the skin, brighten the skin and is anti-inflammatory. Sodium hydroxide is used as an alkaline solution for making hard soap, as well as adding other ingredients to improve the quality of soap (Munarsih & Rini, 2019). Based on the research of Widyasanti et al., (2016) using coconut oil as a base in making transparent solid soap and showing good results in testing chemical properties and in accordance with SNI 06-3532-1994, so that it can be developed and applied to the community. Transparent solid soap is a soap that can be combined with natural ingredients and can clean and protect the skin. In making soap, it is necessary to add natural antibacterial ingredients. One of the natural antibacterial ingredients that are often found is red ginger extract, which is antibacterial. This material is used very little in soap making, which makes further research necessary (Elmitra et al., 2019). Several studies on transparent solid soap have been carried out such as the research of Widyasanti et al (2016) using white tea extract as the active substance and Akbar's research (2019) using apple extract as the active substance, but there has been no research on making transparent solid soap with ginger extract. red. Based on research by Redi Aryanta (2019), red ginger extract contains many chemical compounds such as flavonoids, essential oils, phenolics, and tannins which are known to have many properties, such as anti-bacterial. Based on Rizkita's research (2017) using ginger extract with a concentration of 10% and Apriliana et al's research (2020) using ginger extract with a concentration of 1% and also in the research of Awanis & Mutmainnah (2016) using red ginger extract at a concentration of 5% has inhibited the growth of *Streptococcus pyogenes* bacteria with an inhibition zone of 11.25mm. Based on the research of Putra et

al (2017) stated that liquid soap with red ginger extract had good results in organoleptic tests, namely the preparation was brown, had a distinctive red ginger smell, and the consistency of the preparation was not too stiff, then the pH test obtained an average pH of 9.2. In this study, researchers used palm oil as the oil phase and red ginger extract as the active ingredient in making transparent solid soap. Researchers have never found any research on red ginger extract as an active substance in the manufacture of transparent solid soap, so the authors are interested in making a transparent solid soap formulation from red ginger extract with three concentration variations of 0.5%, 1.0%, and 1.5%.

METHOD

A. Qualitative Test Of Red Ginger Extract

1. Phenolic Test

0,1g of extract was added 10 ml of distilled water, then filtered and then added reagent iron (III) chloride (FeCl₃) 1% 5 ml. If it causes a dark blue color or black results, the results show a positive tannin content (Rahmadani et al., 2018).

2. Triterpenoid Test

2ml of the sample was added to 20 ml of ether mixed, then filtered, the filtrate was then added with Lieberman Burchard. Positive results contain triterpenoids in the presence of a brownish green/red color (Rahmadani et al., 2018).

3. Flavonoid Test

Added as much as 0.5 g of simplisa used in a cup and then added 2 mL of 7% ethanol and stirred vigorously, then added 0.5 g of magnesium powder and 3 drops of concentrated HCl. If a yellow to red color is formed, it indicates the presence of flavonoids (Rahmadani et al., 2018).

B. Transparent Solid Soap Preparation Formula

Table 1. Transparent Solid Soap Preparation Formula (Widyasanti et al., 2019).

No	Ingredient (g)	Formula			Function
		I	II	III	
1.	Red Ginger Extract	1	2	3	Active substance
2.	Coconut oil	40	40	40	Oil phase
3.	Stearic acid	14	14	14	Emulsifier
4.	NaCl	0,4	0,4	0,4	Tonicity Agent
5.	NaOH 30%	40,6	40,6	40,6	Buffer Agent
6.	Ethanol 96%	30	30	30	Solvent
7.	Sugar	30	30	30	Humectants
8.	Glycerin	26	26	26	Preservative
9.	Coco-DEA	2	2	2	Foam Stability
10.	Fragrance Oil	0,2	0,2	0,2	Fragrance
11.	Aquadest	15,8	14,8	13,8	Solvent

C. Preparation of Transparent Solid Soap

Making transparent solid soap using the hot method with a water bath as the medium. Pure coconut oil which has been placed in a beaker on a hot plate with a temperature of $\pm 80^{\circ}\text{C}$. Put the stearic acid into a beaker glass containing virgin coconut oil, then stir until homogeneous. After adding NaOH 30% solution and adding other supporting ingredients, namely, ethanol 96%, glycerin, sugar syrup, coco-DEA, NaCl, and fragrance oil. Stir until completely mixed, then the dough is lowered to $\pm 50^{\circ}\text{C}$ to add red ginger extract to it. re-mixing the perfectly mixed extract, after that it gets into the silicone concrete and let it sit for 24 hours at room temperature (Widyasanti et al., 2016).

D. Transparent Solid Soap Characteristic Test

1. Organoleptic Observations

Organoleptic tests include observations of shape, color changes, and odor changes of transparent solid soap (Susanti & Guterres, 2018).

2. pH Test

The pH test was tested by weighing 1g of solid soap dissolved in aquadest. Then the pH marker is

dipped and the pH value of transparent solid soap is observed, the pH of transparent solid soap is 8-11 (Susanti & Guterres, 2018).

3. Foam Strength Test

The foam strength test was tested by putting 10 gram of solid soap into a 100mL measuring cup and then dissolving it with distilled water. After that it was shaken vigorously and the size of the foam that was formed after shaking 30 seconds and 60 seconds was measured, the requirements for good foam strength were 13 - 220mm (Susanti & Guterres, 2018)

4. Water Content Test

Weigh as much as 1 gram of solid soap and then put it in a container inside the Moisture Analyzer, then the work starts with a temperature of 105°C and works automatically until the results are visible. The requirements for the water content test are <15% (Susanti & Guterres, 2018).

5. Free Alkali Content Test

5 gram of solid soap was weighed into a measuring cup, then aquadest was added and phenolphthalein marker was added. After the solution turns red, then it is titrated with 0.1 N HCl solution until the red color disappears (Susanti & Guterres, 2018).

6. Free Fatty Acid Content Test

5 gram of solid soap was dissolved with aquadest, then bromthymol blue was added and titration was carried out using 0.1 N NaOH until the color changed from blue to yellow. The test was carried out 3 times.

RESULTS

A. Transparent solid soap evaluation test results

Evaluation test of physical properties of transparent solid soap which includes organoleptic examination, pH, foam strength, water content, free alkali content and free fatty acid content.

A. Qualitative Test Of Red Ginger Extract

Table 1. Qualitative Test Of Red Ginger Extract

Sample Name	Sample Condition	Parameter	Results	Analysis Techniques
Red Ginger Extract	Thick Extract	Phenolic	Positive	Form a bluish black color
		Flavonoid	Positive	Form a red color
		Triterpenoid	Positive	Form a brown or violet

B. Results of Organoleptic Observations of Transparent Solid Soap

Table 2. Organoleptic Test Results Observation Table

Formula	Replication	Color	Odor	Shape
F1	1	Brownish Yellow	Typical red ginger	Solid
	2	Brownish Yellow	Typical red ginger	Solid
	3	Brownish Yellow	Typical red ginger	Solid
F2	1	Brownish Yellow	Typical red ginger	Solid
	2	Light brown	Typical red ginger	Solid
	3	Light brown	Typical red ginger	Solid
F3	1	Brownish red	Typical red ginger	Solid
	2	Light brown	Typical red ginger	Solid
	3	Light brown	Typical red ginger	Solid

Information:

F1 : Transparent solid soap with 0.5% red ginger extract concentration

- F2 : Transparent solid soap with 1.0% red ginger extract concentration
 F3 : Transparent solid soap with 1.5% red ginger extract concentration

2. Transparent Solid Soap pH Test Results

Table 3. Observation Table of pH Test Results

Replication	F1	F2	F3
1	9,6	9,9	9,7
2	9,7	9,9	9,6
3	9,4	9,4	9,7
Rata-rata ± SD	9,6 ± 0,2	9,7 ± 0,3	9,7 ± 0,1

Information:

- F1 : Transparent solid soap with 0.5% red ginger extract concentration
 F2 : Transparent solid soap with 1.0% red ginger extract concentration
 F3 : Transparent solid soap with 1.5% red ginger extract concentration

3. Transparent Solid Soap Foam Strength Test Results

Table 4. Foam Strength Test Results Observation Table

Replication	F1(cm)	F2(cm)	F3(cm)
1	17 cm	13 cm	13 cm
2	14 cm	16 cm	11 cm
3	14,5 cm	12 cm	13 cm
Rata-rata ± SD	15,2 cm ± 1,6	13,7 cm ± 2,1	12,3 cm ± 1,2

Information:

- F1 : Transparent solid soap with 0.5% red ginger extract concentration
 F2 : Transparent solid soap with 1.0% red ginger extract concentration
 F3 : Transparent solid soap with 1.5% red ginger extract concentration

4. Transparent Solid Soap Water Content Test Results

Table 5. Water Content Test Results Observation Table

Replication	F1 (%)	F2 (%)	F3 (%)
1	10,64%	9,42%	5,66%
2	11,11%	10,21%	6,86%
3	10,61%	10,46%	9,96%
Rata-rata ± SD	10,78% ± 0,3	10,03% ± 0,5	7,49% ± 2,2

Information:

- F1 : Transparent solid soap with 0.5% red ginger extract concentration
 F2 : Transparent solid soap with 1.0% red ginger extract concentration
 F3 : Transparent solid soap with 1.5% red ginger extract concentration

5. Transparent Solid Soap Free Alkali Content Test Results

Table 6. Free Alkali Content Test Results Observation Table

Replication	F1 (%)	F2 (%)	F3 (%)
1	0,11%	0,08%	0,08%
2	0,12%	0,12%	0,10%
3	0,12%	0,12%	0,12%
Rata-rata ± SD	0,12% ± 0,01	0,11% ± 0,02	0,10% ± 0,03

Information:

- F1 : Transparent solid soap with 0.5% red ginger extract concentration

- F2 : Transparent solid soap with 1.0% red ginger extract concentration
 F3 : Transparent solid soap with 1.5% red ginger extract concentration

6. Transparent Solid Soap Free Fatty Acid Content Test Results

Table 7. Free Fatty Acid Content Test Results Observation Table

Replication	F1 (%)	F2 (%)	F3 (%)
1	0%	0%	0%
2	0%	0%	0%
3	0%	0%	0%
Rata-rata $\pm$ SD	0%	0%	0%

Information:

- F1 : Transparent solid soap with 0.5% red ginger extract concentration
 F2 : Transparent solid soap with 1.0% red ginger extract concentration
 F3 : Transparent solid soap with 1.5% red ginger extract concentration

DISCUSSION

Transparent solid soap formulation in this study consisted of an active substance, a base and an excipient. The base consists of coconut oil and NaOH as soap formers with a saponification process. Excipients consist of stearic acid as emulsifier, NaCl as tonicity agent, granulated sugar as humectant, glycerin as preservative, Coco-DEA as soap stability, Fragrance oil as fragrance, Ethanol and Aquadest as solvent. The active substance used in this transparent solid soap is red ginger extract.

Soap making in this study was carried out using a saponification reaction, namely the reaction between oil (palm oil) with an alkaline solution (NaOH 30%) by breaking the triglyceride chain with alkali which will produce a product in the form of soap (Sukeksi et al., 2018).

This research was conducted by making 3 variations of the formula, namely the concentration of red ginger extract F1 (red ginger 0.5%), F2 (red ginger 1.0%), and F3 (red ginger 1.5%) to see the effect on the test. evaluation to be carried out. The evaluation tests carried out were organoleptic test, pH test, foam strength test, water content test, free alkaline test, and free fatty acid content test..

Organoleptic test is intended to observe the physical appearance of a preparation which includes texture, smell, and color (Dimpudus et al., 2017). In this study, organoleptic tests were carried out when all formulations had been made, namely by observing the changes in color, odor, and texture of the transparent solid soap that had been made. Based on the three formulas that have been made, there are several differences in the color of the soap. In formula 1, using 0.5% red ginger extract, the result is a transparent brownish yellow color, with a solid form, and a distinctive red ginger odor that is not too strong because fragrance oil has been added as a fragrance agent for the preparation. Based on the three replications that have been made in formula 1, all three have the same shape, color, and smell. In formula 2, using 1.0% red ginger extract, different results were obtained in replication 1 compared to replications 2 and 3. The results of replication 1 showed a transparent brownish-yellow color, with a solid shape, and a distinctive red ginger odor. However, in replications 2 and 3, there were differences in the color of the preparations, namely light brown like caramel and a very minimal level of transparency, with a solid form, and a distinctive red ginger smell. The color that is not too transparent is obtained due to the addition of glycerin and sugar which affects the transparency of the soap (Suhadi & Rasmito, 2019). In formula 3, using 1.5% red ginger extract, the results were quite different in replication 1 compared to replications 2 and 3. The results of replication 1 showed a transparent brownish red color, with a solid shape, and a distinctive red ginger odor. , but in replications 2 and 3, there was a difference in color compared to replication 1. The results obtained from replications 2 and 3 showed a light brown color with a thin level of transparency, solid shape, and a characteristic red ginger odor. Different colors are obtained due to the influence of the temperature during manufacture which is too hot, so the desired form of transparency looks thin. A color that is not too transparent is obtained due to the influence of the addition of glycerin and sugar which have properties that can make the soap transparent (Suhadi & Rasmito, 2019). In the results of this study, it is known that the more extracts used, the darker the color of the transparent solid soap, as seen in formula 3 where the color becomes brownish red and dark brown. At the time of

manufacture, it is also necessary to pay attention to the temperature used, because each material has a different boiling point so that it can affect the shape, color, and smell of the preparations that have been made..

The degree of acidity or pH is a chemical parameter to determine whether the transparent solid soap produced is acidic or basic (Prasetyo et al., 2020). The pH test was carried out to determine the safety level of transparent solid soap on the skin, the pH of human skin has a range of 4.5-6.5. The pH value of the preparation should not be too acidic because it can cause skin irritation and should not be too alkaline because it can cause scaly skin (Noviardi et al., 2019). The pH test is carried out by means of a pH measuring device / pH meter must be calibrated first using a buffer solution of pH 4 and 6.86, then after calibration the electrode is dipped into a transparent solid soap preparation that has been dissolved and the pH value will be read on the pH meter screen. (Noviardi et al., 2019). Based on the test results, the pH results were quite different but not significant. In testing formula 1 using 3 replications, the pH results in the range of 9.4-9.7, in formula 2 using 3 replications, the pH results in a range of 9.4-9.9, and in formula 3 using 3 replication, the results obtained pH with a range of 9.6-9.7. The pH value of the preparation can also be influenced by the amount of emulsifier used. The more stearic acid used will make the pH value lower which will make the pH more acidic, and also the concentration of NaOH used can affect the level of basicity of the pH which will make the pH higher/alkaline. Measurement of pH in each formula has obtained good results, according to SNI 06-3532-1994 the pH of soap ranges from 8-11 (BSN, 2016). This transparent solid soap produced in this study has a pH that meets the requirements and is safe to use on the skin..

One of the important parameters in determining the quality of soap is the foam formed. Foam strength is expressed as the resistance of a bubble to maintain its size or rupture the film layer of the bubble. In this study we used coco-DEA as a foam stabilizer, coco-DEA functions as a foam stabilizer that is able to maintain foam stability (Prayadnya et al., 2017). Foam is a gas trapped between a thin layer of liquid containing a number of soap molecules that are absorbed in the thin layer, in bubbles, the surfactant's hydrophobic group will lead to the gas, while the hydrophilic part will lead to the solution and the bubbles will come out of the liquid body (Sukeksi et al. , 2018). The purpose of the foam stability test is to determine the stability of the foam as measured by the height of the foam in a test tube with a certain scale and time frame and the ability of the surfactant to produce foam (Murti et al., 2018). According to Rusli et al (2019), the criteria for good foam strength are if within 5 minutes the foam stability range is obtained with a height of 9.5 cm. Test results on foam strength for F1 at replication 1 (17cm), replication 2 (14cm), and replication 3 (14.5cm), with an average value of 15.2cm. For F2 at replication 1 (13cm), replication 2 (16cm), and replication 3 (12cm), with an average value of 13.7cm. And the last for F3 in replication 1 (13cm), replication 2 (11cm), and replication 3 (13cm), with an average value of 12.3 cm. Based on these results, the foam strength test on red ginger extract solid soap has been included in the requirements, which is in the range of 13 mm-220 mm. Soap foaming is influenced by several factors, namely the presence of surfactants (sugar), foam stabilizers (coco-DEA), and other soap constituents such as the oil used. The most stable results were seen in F3, from the three replications, it almost had the same level of foam stability, namely 11 cm – 13 cm..

The water content of soap has a relationship with the durability of soap from soap, because soap is not easily soluble in water (Surilayani et al., 2019). The water content of soap affects the characteristics of soap, the more water contained in the soap, the more the soap will shrink when used (Surilayani et al., 2019). In addition, the water content in soap affects the hardness of the soap produced, the higher the water content, the lower the hardness of the soap. The results of testing the water content in formula 1 using 3 replications ranged from 10.61% - 11.11%, for formula 2 using 3 replications it ranged from 9.42% - 10.21%, and for formula 3 using 3 replications. ranged from 5.66% - 9.96%. The water content in soap, apart from the water added during the soap making process, also comes from the ingredients used in the manufacturing process that are hygroscopic, such as glycerin and sugar (Surilayani et al., 2019). The materials used can cause the water content of transparent solid soap to be higher. The water content of solid soap according to BSN (2016) is less than 15% while the water content of transparent solid soap preparations red ginger extract has met the requirements, which is less than 12%. The best results were obtained in formula 3, because of the three replications, the water content was below 10%, because the smaller the moisture content, the better the preparation.

Free alkali is an alkali contained in soap that is not bound as a compound. Excess levels of free alkali in soap can be caused by high concentrations of alkali or excess in the soaping process. Soaps that contain high alkalinity can cause skin irritation, and usually soaps with high alkaline levels are used as laundry soap (Zalfiatri et al., 2018). The free alkali level test is intended to determine the level of alkali in soap that is not bound during the soap making process (Susanti & Guterres, 2018). The free alkali content in the resulting transparent solid soap should not exceed 0.14% for potassium soap (BSN, 2016). Determination of free alkali levels was carried out using the titration method with 0.1N HCl. Based on the test results that have been obtained, for F1 with three replications, namely, replication 1 (0.11%), replication 2 (0.12%), and replication 3 (0.12%), the results obtained have met the requirements of SNI, namely <0.14% and in F1 already got good results. In F2 with the three replications, the results obtained are, replication 1 (0.08%), replication 2 (0.12%), and replication 3 (0.12%), the results obtained have met the requirements of SNI, namely <0.14% and in F1 already get good results. In F3 with the three replications, the results obtained are, replication 1 (0.08%), replication 2 (0.10%), and replication 3 (0.14%), the results obtained have met the requirements of SNI, namely <0.14% and in F1 already get good results. The free alkali in the transparent solid soap is caused by the excessive amount of NaOH in the saponification process (Susanti & Guterres, 2018). The saponification process is the hydrolysis of fats into fatty acids and glycerol under alkaline conditions. In a perfect saponification reaction, NaOH will bind to the oil phase but if NaOH is not completely bound it will form free alkali, this is caused by incomplete mixing. The results of testing the free alkali levels in transparent solid soap with red ginger extract have obtained results that meet the SNI requirements, namely <0.14%, so the soap is safe to be applied to the body.

Free fatty acids are fatty acids that are left in the soap preparation, but are not bound as sodium compounds in the soap, free fatty acids are still left in the soap because they do not experience a perfect saponification reaction (Sukawaty et al., 2016). This test was carried out to determine the presence of excess free fatty acids contained in soap preparations. The levels of free fatty acids in soap can also be influenced by the levels of free fatty acids in palm oil used in the manufacture of transparent soap. High free fatty acids can reduce the binding capacity of soap to dirt, oil, fat, or sweat (Zalfiatri et al., 2018). Testing the free fatty acid levels in transparent solid soap red ginger extract obtained 0% results in each formula, because during the titration process using 0.1N NaOH there was no visible color change, so it was known that there was no free fatty acid content in this formulation, this was due to acid The free fat in the oil has reacted with the base to form soap and glycerol (Hardian et al., 2018). The free fatty acid content according to SNI 01-3741-2002 is less than 2.5%, if the fatty acid content is too high it will affect the process of emulsifying soap with dirt. The free fatty acid content of soap can be influenced by the fatty acid content of the oil used and the amount of base used in the soap making process. According to Hardian (2018), the use of NaOH at high concentrations will react with oil thereby reducing oil and increasing the amount of soap formed.

CONCLUSION

From the research that has been done, it can be concluded:

1. Red Ginger Extract (*Zingiber Officinale* var. *Rubrum*) can be formulated into transparent solid soap with various concentrations of red ginger extract, namely 0.5%, 1.0%, and 1.5%. The three formulas are good formulas because they are included in the requirements of each evaluation test that has been carried out.
2. The physical stability of transparent solid soap red ginger extract (*Zingiber Officinale* var. *Rubrum*) obtained the best preparation at F3 because the results in the pH test and foam strength were very stable from the three replications, namely 9.7 and 12.3 cm, the water content test obtained average results. 7.49% average which is the best result among the three formulas, and the free alkali content test gets an average value of 0.10%, this result is the best result of the three formulas because the smaller the water content and the free alkaline content, the results are stated the better, the last one in the free fatty acid test, getting 0% results in each formula because it is known that there are no free fatty acids in this formulation.

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QUALITATIVE SCREENING OF FITOCHEMICAL COMPOUNDS OF PANDAN FRAGILE LEAF EXTRACT (*Pandanus amaryllifolius* Roxb) GROWING IN DEPOK WITH VARIOUS TYPES OF SOLVENTS AND EXTRACTION METHODS

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Abstract

Introduction: Pandan Wangi contains many substances or compounds that are efficacious as medicine so it is also used as traditional medicine. One of the properties of Pandan Wangi is as an antibacterial and antifungal which is thought to come from the content of alkaloids, flavonoids, saponins, tannins. Therefore, it is necessary to conduct research on the screening of phytochemicals contained in Pandan Wangi leaf extract using water, ethanol (96%), and solvents. *n*-hexane by maceration and soxhlet extraction methods.

Method: This research is an experimental study using maceration and soxhlet extraction methods

Results: Yield results obtained by maceration and soxhlet extraction methods on water extract of Pandan Wangi leaves were 18.6% and 10.8%, 96% ethanol extract was 14.18% and 3.72%, *n*-hexane extract was 3.72 % and 3.24%.

Conclusion: The results of phytochemical screening on Pandan Wangi leaves with water and 96% ethanol as solvents indicate that Pandan Wangi leaves contain secondary metabolites in the form of alkaloids, flavonoids, saponins, tannins and steroids/terpenoids.

INTRODUCTION

Medicinal plants are one of the sources of biopharmaceuticals due to their chemical biosynthetic capabilities. One way to develop traditional medicine is to know in advance the chemical active components contained in medicinal plants and the results will be used for their phytochemical profiles. The use of plants as traditional medicinal ingredients requires scientific research to find out the truth of their efficacy so that they are not in doubt and can be accounted for. This will further encourage people to use plants or medicinal plants as raw materials for medicine (Atikah, 2018).

One of the medicinal plants whose leaves have the potential as biopharmaceuticals is Pandan Wangi (*Pandanus amaryllifolius* Roxb). The leaf part of the fragrant pandan plant has benefits, namely as a food additive. In particular, this leaf is used to give a green color and aroma to food. (Faraset al, 2014). In addition, fragrant pandan also has several pharmacological activities based on its extract solvent, including antibacterial, antidiabetic, anticancer, and antioxidant. (Prameswari and Widjanarko, 2014).

Related to the phytochemical content of Pandan Wangi leaves, it has been reported by Suryani, et al (2017), with the maceration method that the Pandan Wangi leaf plant contains several groups of compounds such as alkaloids, flavonoids, phenolics, terpenoids, saponins and steroids extracted with ethanol (95%) fragrant pandan leaves. In a study conducted by Utami and Rosa (2021) also reported that Pandan Wangi leaves contain alkaloids, flavonoids and tannins which were extracted with ethanol solvent (70%) by maceration method.

As for getting wider information from fragrant pandan leaves, researchers are interested in conducting phytochemical screening on fragrant pandan leaves (*Pandanus amaryllifolius* Roxb) with various solvents such as water, 96% ethanol, and *n*-hexane with maceration and soxhlet extraction methods.

METHOD

1. Research design

The design of this research is experimental. Experimental research design is a design that refers to how to collect data, analyze, interpret, then provide answers to problems (Sekaran & Bougie, 2016).

2. Research Time and Place

This research was conducted at the STikes Mitra Keluarga laboratory in East Bekasi, this research was carried out in February- March 2022.

3. Population and Sample

The sample in this study was Pandan Wangi leaves taken from the Mini Herbal Plantation, Depok City as much as 6 kg.

4. Research variable

Independent variables in the form of yield percentage and phytochemical screening of Pandan Wangi leaf extract (*Pandanus amaryllifolius* Roxb) with extraction methods, namely maceration and soxhletation.

5. Research Materials and Tools

- Tool

The tools used in this study were oven, analytical digital scale (Acuplus, China), beaker glass (Pyrex, USA), erlenmeyer (Pyrex, USA), dropper, test tube (Pyrex, USA), test tube rack, spatula, porcelain cup, filter paper, glass funnel, mask, measuring cup, gloves, rotary vacuum evaporator (IKA-RV-3V), water bath 6 Hole (HH, China), label paper, scissors, plastic bag, mattress thread.

- Ingredient

The materials used in this study were Pandan Wangi Leaves, aquadest, ethanol (96%), n-hexane, hydrochloric acid (HCl), Mayer's reagent (HgCl₂, KI), Wagner's reagent (Iodine, KI), Dragendorff's reagent (KI, KI), bismuth sub nitric + glacial acetic acid), powder Mg, FeCl₃ 1%, H₂SO₄.

How Research Works

1. Sample Setup

a. Sample selection

Fragrant Pandan leaves (*Pandanus amaryllifolius* Roxb) which is green or yellowish green with a length of about 40-80 cm and a width of about 3-5 cm as much as 6 kg. The age of the plants taken was <3 months old at around 17.00 WIB at the Mini Herbal Garden, Depok City.

b. Identification (Determination) Sample

In this study, the determination was made at the Biological Research Center for Biological Research Organization, Cibinong, Bogor Regency. Determination Test aims to ensure that the sample used is the original sample and to avoid errors in collecting research samples. Determination of Pandan Wangi leaves to ensure that the plant is the plant to be studied (Puspitasari et al, 2019).

c. Plant Organoleptic Identification

Organoleptic tests carried out on Pandan Wangi leaf powder products included: color, taste and smell.

d. Wet Washing and Sorting

Wash samples of adhering dirt such as soil, pests, other plants and others under running water. Make sure the sample is clean of impurities and drain the sample from water adhering to the sample by aerating.

e. chopping

To simplify and assist the drying process, the samples that have been sorted are wet and drained, then chopped using a knife or other cutter to form thin slices, if the sample taken is a leaf, then the chopping process is not required.

f. Drying

The chopped samples were dried in an oven at a temperature of 40°C.

g. Storage

Dried simplicia is stored in a tightly closed container and free from air humidity.

2. Extraction of Phytochemical Compounds

a. Maceration

The maceration method was carried out by soaking the simplicia powder, weighed 50 grams, put it in a glass container and added 96% ethanol solution, and soaked n-hexane for 24 hours. then filtered with filter paper to separate the filtrate and residue. The filtrate obtained was then concentrated using *rotary evaporator* at 50°C.

b. Soxhletation

Soxhlet apparatus was installed, then 50 grams of sample was wrapped in filter paper, tied with thread, inserted into the soxhlet apparatus, input 96% ethanol solvent, and 400 mL n-hexane into the soxhlet flask. Perform soxhletation at a temperature of 70°C until the cycle drops are colorless or for approximately 5 hours. The liquid extract obtained was then concentrated using *rotary evaporator* at 50°C.

3. Extract Evaporation

In this evaporation, the evaporation of Pandan Wangi leaf extract (*Pandanus amaryllifolius Roxb*) by using a water bath.

4. Extraction of Phytochemical Compounds

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6. Qualitative Phytochemical Screening

1) Alkaloids Check

The submission is made by taking 0.1 grams of thick extract of Pandan Wangi leaves which have been extracted with water, ethanol (96%), and ethanol as solvents. *n*-hexane into 4 different test tubes. After that, each extract was added with 5 drops of Dragendroff and Wagner reagent. If each solution forms an orange precipitate, it is positive that it contains alkaloids. While the positive Wagner reagent there is a brown precipitate. Furthermore, the alkaloid test using Mayer's reagent was carried out by taking each 0.1 gram of thick Pandan Wangi leaf extract which had been extracted with water, ethanol (96%), and *n*-hexane into 4 different test tubes. After that, each extract was added with 3 drops of concentrated hydrochloric acid and 5 drops of Mayer's reagent. If each solution forms a white precipitate, the positive sample contains alkaloids (Hidayah., 2017).

2) Flavonoid Examination

The test was carried out by taking 0.1 grams of Pandan Wangi leaf extract each which had been extracted with water, ethanol (96%), and *n*-hexane is then heated for approximately 5 minutes. After being heated, each was added with 0.1 grams of Mg metal and 5 drops of concentrated HCl. If each solution forms a yellow orange to red color, then it is positive that it contains flavonoids (Hidayah., 2017).

3) Saponin Check

The test was carried out by taking 0.1 grams of Pandan Wangi leaf extract each which had been extracted with water, ethanol (95%), and Hexane was then added with warm aquadest and then shaken vertically for 10 seconds. A positive result of the presence of saponins will cause foam for 10 minutes (Hidayah, 2017).

4) Tannin Check

Take 0.1 gram of each extract and add 3 drops of 1% FeCl. A positive result of the presence of tannins will be indicated by a blackish green, green or turquoise color (Hidayah, 2017).

5) Steroid and Terpenoid Examination

Take 0.1 gram of extract then add 3 drops of concentrated HCL and 1 drop of concentrated H₂SO. Positive results of terpenoids are indicated by the formation of a red or purple color and if a green color is formed, it indicates a positive steroid (Cahyaningsih et al., 2019).

Data analysis

The data analysis technique used in this research is descriptive quantitative data analysis which is done by interpreting the data obtained in the form of tables and figures. The variables measured in this study were to calculate the percentage yield of Pandan Wangi leaf extract and the content of secondary metabolites from the Pandan Wangi plant (*Pandanus amaryllifolius* Roxb) such as, alkaloids, flavonoids, saponins, tannins and steroids and terpenoids.

RESULTS

A. Macroscopic Test of Fragrant Pandan Plants

In the observation of the macroscopic test Pandan Wangi (*Pandanus amaryllifolius* Roxb.) aims to determine the morphology of Pandan Wangi leaves such as roots, stems and leaves. Morphological examination of Pandan Wangi in this study by comparing this study with previous studies by Mursyida, et al (2021) as a comparison parameter and can be seen in Figures 5.1 and 5.2 and in Table 5.1.



Figure 5.1. Measurement of Fragrant Pandan Leaves (Mursyida, et al 2021)

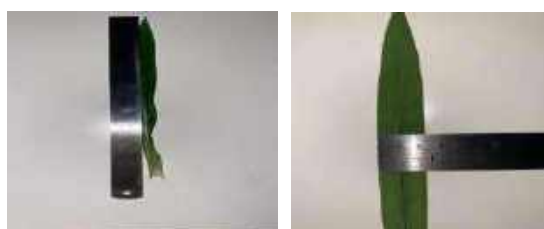


Figure 5.2. Fragrant Pandan Leaf Measurement (Personal Documentation)

Table 5.1 Macroscopic observation parameters

Parameter	Observation Result	Previous Research
long	25 cm	20 cm
Wide	3 cm	3-5 cm
Color	Green	Yellowish green
Leaf shape	Elongate	Elongate
Leaf tip	Pointed	Pointed
Leaf edge	Flat	Flat

Surface texture	Fine	Fine
Leaf bone	Parallel	Parallel

Pandan Wangi has fibrous roots, supporting roots that can support other plants. The roots of Pandan Wangi leaves have a length of 30-40 or even more, on the stems of Pandan Wangi it has a brownish color (Mursyida, et al (2021)). In observations made, the roots of Pandan Wangi leaves have roots that are fibrous, supportive and brown in color. It can be seen in Fig. Figures 5.3 and 5.4.



Figure 5.3. Fragrant Pandan Leaf Root (Mursyida Fatty, et al 2021)



Figure 5.4. Fragrant Pandan Leaf Root (Personal Documentation)

On the Pandan Wangi stem with plants with sweet roots, cylindrical stems, and soft and many branches. Pandan Wangi is a shrub or shrub that can provide shade around the Pandan leaf plant (Mursyida, et al (2021)). In observations made also the Pandan Wangi stem has a cylindrical stem shape, and many branches. It can be seen in Figure 5.5 and 5.6.



Figure 5.5. Fragrant Pandan Trunk (Mursyida, et al 2021)



Figure 5.6. Fragrant Pandan Leaf Stem (Personal Documentation)

B. Organoleptic Test

Organoleptic testing on Pandan Wangi leaf powder is seen from 4 parameters, namely shape, color, taste and smell as seen in table 5.7 below:



Figure 5.7. Fragrant Pandan Powder (Personal Documentation)

Organoleptic test is a sensory assessment or sensory assessment that utilizes the five senses of humans to make observations. The results of the organoleptic test of Pandan Wangi leaves included: shape, color, taste and smell which were observed directly. The results of observations in this study in Table 5.2 and Figure 5.7 show that the sample is in the form of a fine powder, green in color, has a distinctive aromatic odor and has no taste, while according to the MMI Literature Standard (Materia Medika Indonesia) in 1995, it shows that the sample is in the form of a fine powder, green in color. , characteristically aromatic odor and has no taste. So, it can be concluded that the simplicia is true Pandan Wangi leaves.

C. Yield percentage

The calculation of the percentage yield of the thick extract of Pandan Wangi leaves using three different types of solvents produces a different percentage of yield, seen in table 5.3 below.

Table 5.3. Yield of Fragrant Pandan Leaf Extract

Method	Extract Weight			Yield Value (%)		
	Water	Ethanol	n-hexane	Water	Ethanol	n-hexane
		96%			96%	
Maceration	9.3	7.09	1.86	18.6	14.18	3.72
Soxhletation	5.4	1.86	1.62	10.8	3.72	3.24

Based on table 5.3, the weight gain of aqueous extract, 96% ethanol and n-hexane from the maceration method were 9.3 grams, 7.09 grams and 1.86 grams, respectively, while the weight gain of aqueous extract, 96% ethanol and n-hexane from the soxhletation method were 5.4 grams, 1.86 grams, and 1.62 grams, respectively. The yields obtained from the maceration method with water, 96% ethanol and n-hexane as solvents yielded percentages of 18.6%, 14.18%, and 3.72%. The percentage of yield obtained from the soxhletation method was 10.8%, 3.72%, and 3.24%.

D. Phytochemical Screening Results

After the thick extract was obtained, phytochemical screening was carried out including alkaloids, flavonoids, saponins, tannins and steroids/terpenoids. The results of the phytochemical screening are in Tables 5.4 and 5.5.

Table 5.4. Phytochemical Screening of Fragrant Pandan Leaf Extract by Maceration Method

Phytochemical Screening	Reagent	Water	ethanol 96%	n-hexane	Description
Alkaloids	Mayer	++	+++	-	White precipitate
	Dragendroff	+	+++	-	Orange precipitate
	Wagner	++	+++	-	Brown precipitate

Flavonoids	Mg + Hcl concentrated	++	++++	-	Yellow
Saponins	Aquadest	+	++++	-	Stable foam
Tanins	FeCl ₃ 1%	++	++++	-	Blackish green
Steroids dan terpenoids	Hcl pekat + H ₂ SO ₄ concentrated	++	++++	-	Green
		Steroids	Steroids		

Table 5.5. Phytochemical Screening of Fragrant Pandan Leaf Extract by Soxhletation Method

Phytochemical Screening	Reagent	Water	ethanol 96%	n-hexane	Description
Alkaloids	Mayer	++	++++	-	White precipitate
	Dragendroff	+	+++	-	Orange precipitate
	Wagner	++	++++	-	Brown precipitate
Flavonoids	Mg + Hcl concentrated	++	++++	-	yellow
Saponins	Aquadest	+	++++	-	Stable foam
Tanins	FeCl ₃ 1%	++	++++	-	Blackish green
Steroids dan terpenoids	Hcl pekat + H ₂ SO ₄ concentrated	++	++++	-	green
		Steroids	Steroids		

Information :

- (+) = not enough
- (++) = currently
- (+++)= many
- (++++)= very many
- (-) = there isn't any

Comparison of the results of phytochemical screening on aqueous extracts and aquadest extracts is presented in table 5.6. This comparative data is used to show that there are differences in the results of phytochemical screening in Pandan Wangi leaf extract.

Tabel 5.6. Comparison of Screening Result of Maceration Extract Method

Screening Test	Water	Aquadest (Prameswari,dkk 2019)
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	-
Tanins	+	+
Steroids	+	TD

Tabel 5.6. Comparison of Screening Result of Soxhletation Extract Method

Screening Test	Water	Aquadest (Prameswari,dkk 2019)
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	-
Tanins	+	+
Steroids	+	TD

Information :

TD : Not Done

The data obtained in tables 5.3 and 5.4 are complemented by Figure 5.8, namely **maceration method water extraction** seen in samples of Pandan Wangi leaves (*Pandanus amaryllifolius* Roxb) at

the time of phytochemical screening.

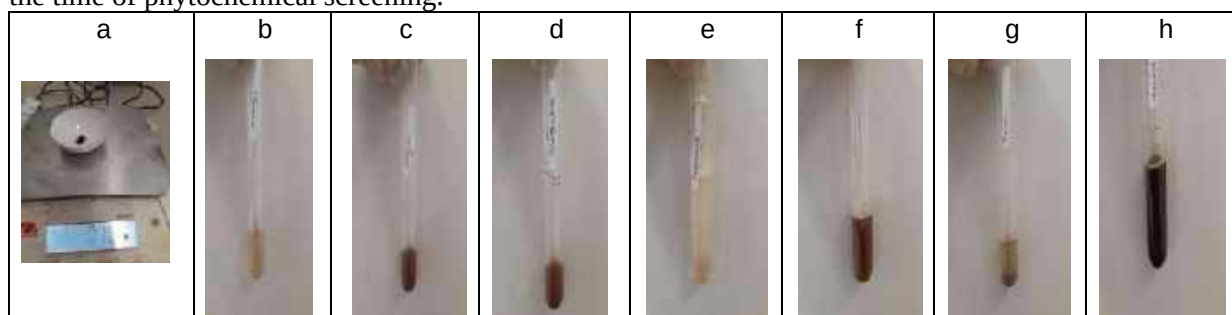


Figure 5.8. The results of the alkaloid test before and after the reagent was given: b. Mayer, c. Wagner, d. Dragendorff, e. Flavonoids, f. Saponins, g. Tannins, h. Steroids

Information :

a = Before
b, c, d, e, f, g, h = After

The data obtained in tables 5.3 and 5.4 are complemented by Figure 5.9, namely **water extraction method soxhletation** seen in samples of Pandan Wangi leaves (*Pandanus amaryllifolius* Roxb) at the time of phytochemical screening.

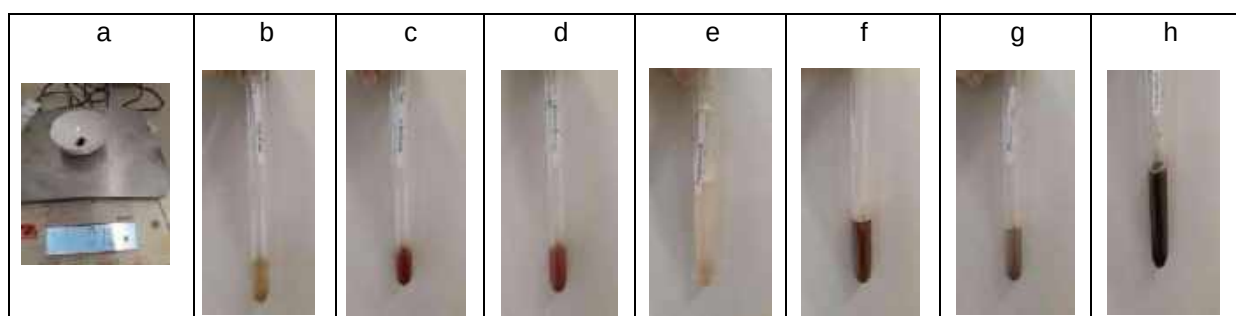


Figure 5.9. The results of the alkaloid test before and after the reagent was given: b. Mayer, c. Wagner, d. Dragendorff, e. Flavonoids, f. Saponins, g. Tannins, h. Steroids

The data obtained in tables 5.3 and 5.4 are complemented by Figure 5.10, namely **extraction of ethanol 96% maceration method** seen in samples of Pandan Wangi leaves (*Pandanus amaryllifolius* Roxb) at the moment Phytochemical screening was carried out.

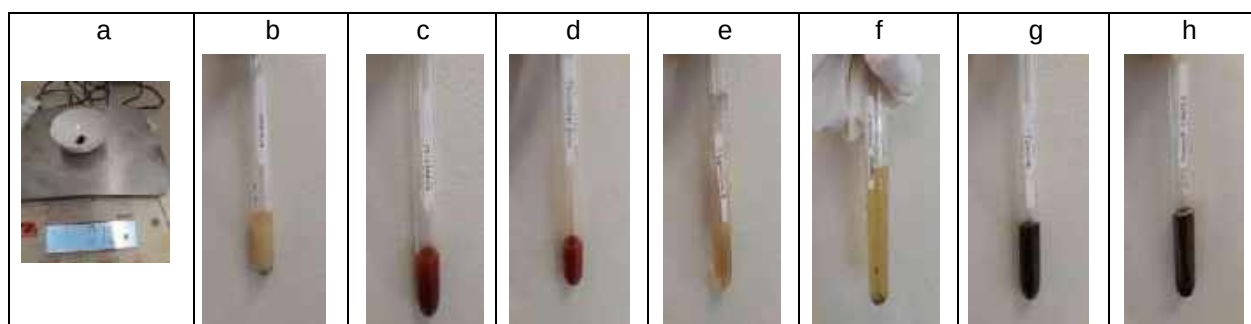


Figure 5.10. The results of the alkaloid test before and after the reagent was given: b. Mayer, c. Wagner, d. Dragendorff, e. Flavonoids, f. Saponins, g. Tannins, h. Steroids

The data obtained in tables 5.3 and 5.4 are complemented by Figure 5.11, namely **extraction of**

ethanol 96% soxhletation method seen in samples of Pandan Wangi leaves (*Pandanus amaryllifolius* Roxb) at the time of phytochemical screening.

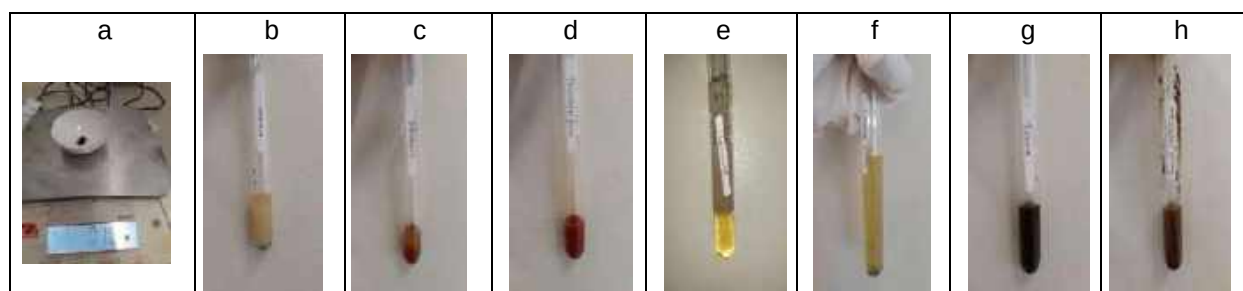


Figure 5.11. The results of the alkaloid test before and after the reagent was given: b. Mayer, c. Wagner, d. Dragendroff, e. Flavonoids, f. Saponins, g. Tannins, h. Steroids

The data obtained in tables 5.3 and 5.4 are complemented by Figure 5.12, namely **extraction of n-hexane maceration method** seen in samples of Pandan Wangi leaves (*Pandanus amaryllifolius* Roxb) at the time of phytochemical screening.

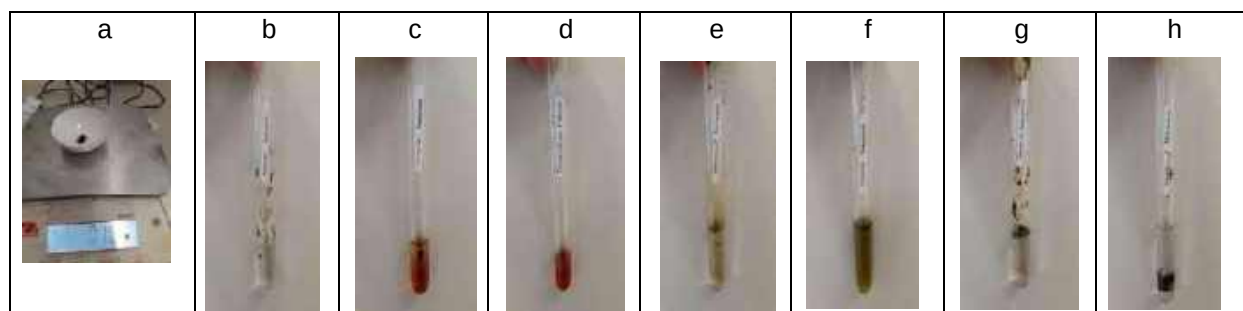


Figure 5.12. The results of the alkaloid test before and after the reagent was given: b. Mayer, c. Wagner, d. Dragendroff, e. Flavonoids, f. Saponins, g. Tannins, h. Steroids

The data obtained in tables 5.3 and 5.4 are complemented by Figure 5.13, namely **n-hexane extraction method soxhletation** seen in samples of Pandan Wangi leaves (*Pandanus amaryllifolius* Roxb) at the time of phytochemical screening.

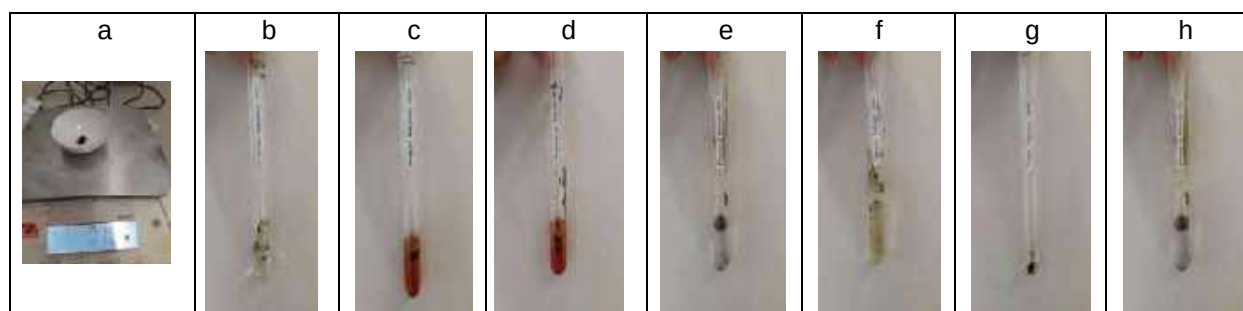


Figure 5.13. The results of the alkaloid test before and after the reagent was given: b. Mayer, c. Wagner, d. Dragendroff, e. Flavonoids, f. Saponins, g. Tannins, h. Steroids

DISCUSSION

In this study, the sample used was Pandan Wangi (*Pandanus amaryllifolius* Roxb) which grows in the Depok area, West Java. The sampling was in the Depok area because there is a plantation of Pandan Wangi leaves in that area, and the area is a highland area that has good moist soil, although Pandan Wangi leaves can grow anywhere just. The plant parts used in this study were

leaves collected in February 2022. The sampling criteria for this study included plant age, selection of fresh green or yellowish green leaves.

Collected and Determined at the Biological Research Center for Biological Research Organization, Cibirong, West Jawa.

A. Macroscopic Examination

Macroscopic examination is an examination carried out directly with the naked eye without the addition of certain reagents or substances. This test may include a morphological examination and an organoleptic test, in this study a morphological examination and an organoleptic test aims to determine a truth and to describe the shape, color, taste and smell.

1. Morphological Examination of Fragrant Pandan Leaves

Morphology is an examination to see the outer part of the physical form and body structure of a plant. In this study, morphological properties were tested on Pandan Wangi leaves such as size, leaf color, leaf shape, leaf tip, leaf edge, leaf surface texture and leaf bone which can be seen in table 5.1. Fragrant Pandan (*Pandanus amaryllifolius* Roxb.) is a low shrub that is only about 2 meters high. The shape of the leaves is elongated and the tips of the leaves are tapered and the stems are spreading and have fibrous roots, tunjang and brownish in color.

The results of the morphological test on Pandan Wangi leaves in this study showed that Pandan Wangi leaves had a length of 25 cm and a width of 3 cm, the leaves were green, had an elongated leaf shape, pointed leaf tips, flat leaf edges, smooth surface texture and parallel leaf veins. . In a previous study conducted by Mursyida Fatty, et al (2021) showed that Pandan Wangi leaves had a length of 20 cm and a width of about 3-5 cm, the leaves were yellowish green, had an elongated leaf shape, pointed leaf tips, flat leaf edges, surface texture. smooth and parallel leaf reinforcement which can be seen in Table 5.1, Figures 5.1 and 5.2.

2. Organoleptic Test

Organoleptic test is a sensory research or sensory assessment that utilizes the five senses of humans to make observations. The organoleptic test usually includes the shape/texture, color, smell and taste of the object being observed.

The results of observations in this study in Table 5.2 and Figure 5.7 show that the sample is in the form of a fine powder, green in color, has a distinctive aromatic odor and is tasteless after being compared with the literature standard according to Materia Medika Indonesia in 1995, indicating that Pandan Wangi powder is in the form of a fine powder, green in color, characteristic odor, aromatic and tasteless.

B. Yield Percentage

Yield is the ratio of the amount of extract produced from plant extraction, the resulting yield indicates the value of the extract produced is increasing. The yield data obtained in table 5.3 of the three solvents and carried out by maceration and soxhletation methods that meet the requirements of the Herbal Pharmacopoeia, namely not less than 10% is water solvent with maceration and soxhletation methods as much as 18.6% and 10.8% and ethanol solvent 96% with the maceration method as much as 14.18%.

The solvents used in this study were water, 96% ethanol and n-hexane. Water (H₂O) is a universal solvent because more substances dissolve in water than other chemicals. This has to do with the polarity of each water molecule. The polarity index of water itself is 10.2 (Ayu, 2017). The polarity index is an index that expresses the comparison of the resistance value of an insulating material measured over a certain period of time. Generally, the polarization index compares resistance in minutes and in tens of minutes. Ethanol or often also referred to as alcohol is a transparent, flammable, colorless, volatile liquid, with the chemical formula C₂H₅OH. Ethanol is a semipolar solvent because it can dissolve both polar and nonpolar compounds. Ethanol has a polarity index of 4, 3 and the boiling point at atmospheric pressure is 78.32°C (Ayu, 2017). While the solvent n-

hexane is a straight chain alkane hydrocarbon which has 6 carbon atoms with the molecular formula C_6H_{14} . The isomer of n-hexane is not reactive and is widely used as an inert solvent in organic reactions because n-hexane is nonpolar. N-hexane is a good solvent for extracting nonpolar compounds.

C. Phytochemical Screening

Phytochemical screening is a method used to determine the components of active compounds contained in a sample, including the chemical structure and chemical composition of a plant. Testing for alkaloids was carried out using the extract which was reacted with Mayer reagent, Dragendorff reagent and Wagner reagent. From the results of this study got positive results which can be seen in table 5.3. In Meyer's reagent the result obtained is a white precipitate is formed, in Dragendorff's reagent the result is the results obtained are a red orange precipitate is formed and for Wagner's reagent a brown precipitate is formed.

Based on the data obtained in tables 5.4 and 5.5, it is known that the Pandan Wangi leaf extract (*Pandanus amaryllifolius* Roxb) contains phytochemical compounds such as alkaloids, flavonoids, saponins, tannins and steroids which are characterized by color changes or reactions that occur. Data obtained from Pandan Wangi leaf extract using water as a solvent with maceration and soxhletation methods showed less significant results, because the changes when added reagents were not so clear the presence of phytochemical compounds, because the water used in this study used boiled water instead of distilled water. The reason the boiled water did not show significant results was because the boiled water was contaminated during boiling so it did not react with these phytochemical compounds. In the study of Prameswari et al, (2019), phytochemical screening using aquadest solvent has shown the presence of alkaloid compounds, flavonoids, tannins and polyphenols which can be seen in table 5.6. The positive results of steroids in water and aquadest showed differences, this was presumably due to the presence of minerals in the water which interfered with the chemical reaction process.

The data obtained using 96% ethanol solvent with maceration and soxhletation methods showed the content of phytochemical compounds clearly. However, the difference between maceration and soxhletation methods on alkaloid compounds, namely Mayer, Wagner and Dragendorff reagents with the maceration method showed that they were not as much as in the soxhletation method. According to research by Wahyuni, et al (2018), phytochemical screening has been carried out which shows the presence of steroid, terpenoid and phenolic compounds with 96% ethanol solvent.

The data obtained using n-hexane solvent with maceration and soxhletation methods showed negative or no phytochemical compounds contained, because at the time of testing, the n-hexane extract when added with reagents did not indicate the presence of these phytochemical compounds. According to research conducted by Suryani, et al (2017), it shows the presence of phenolic compounds and steroids.

CONCLUSION

Based on the results obtained, it can be concluded that there are significant differences between the three solvents used and the yield of the maceration method is greater than the soxhlet method, namely water solvent of 18.6%, 95% ethanol solvent of 14.18% and n-hexane solvent of 3.72%. And secondary metabolites found in Pandan Wangi leaves from maceration and soxhletation methods with water and 96% ethanol as solvents were positive for alkaloids, flavonoids, saponins, tannins, steroids and terpenoids. However, it is different from n-hexane solvent, which is negative containing alkaloids, flavonoids, saponins, tannins, steroids and terpenoids using maceration and soxhletation methods.

SUGGESTION

It is suggested that further research can be carried out using different solvents and further research on

different extraction methods can be carried out. For further research, it is not recommended to use plain water that has been boiled, but to use distilled water as a solvent.

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TEST BIOACTIVITY OF ANTIBACTERIAL COMPOUND EXTRACT OF RED BETLE LEAF (*Piper crocatum*) AGAINST *Propionibacterium acnes* BACTERIA.

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Abstract

Introduction: Skin disease is a disease on the outermost part of the body, which can be caused by chemicals, sunlight, bacteria, viruses, fungi, weak immune systems, and personal hygiene factors. Acne (*Acne vulgaris*) is a skin disease caused by blockage of dead skin cell follicles, excess oil production, and inflammation caused by acne-causing bacteria including *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* in sebaceous follicles. The purpose of this study was to determine the effect of Red Betel leaf extract (*Piper crocatum*) on the growth of *Propionibacterium acnes* with concentrations of 70%, 80%, 90%, and 100%.

Method: This study used an experimental design, to test the inhibition of red betel leaf extract against *Propionibacterium acnes* bacteria with concentrations of 70%, 80%, 90%, and 100%. The data analysis used in this study is descriptive quantitative which shows the size of the growth area of the bacterial colonies of *P. acnes* expressed in mm (millimeters) around the paper disc.

Results: From the results of the data that has been obtained based on the measurement of the diameter of the inhibition zone of the red betel leaf ethanol extract at concentrations of 70%, 80%, 90%, and 100%, which is 16 mm; 18mm; 18.5mm; and 21 mm. The intermediate category was produced by red betel leaf extract at a concentration of 70%, 80%, 90% and the sensitive category at a concentration of 100%.

Conclusion: The conclusion of this study was that the administration of red betel leaf extract with a concentration of 100% was able to inhibit the growth of *P. acnes* bacteria with an inhibition zone diameter of 21 mm or included in the sensitive category.

Key words : Antibacterial, *Propionibacterium acnes*, Red Betel (*Piper crocatum*), Kirby Bauer.

INTRODUCTION

Skin disease is a disease in the outermost part of the body, which can be caused by chemicals, sunlight, bacteria, viruses, fungi, weak immunity, and personal hygiene factors (Triana & Fitria, 2019). One of the diseases caused by bacterial infection is acne. Acne (*Acne vulgaris*) is a skin disease caused by blockage of dead skin cell follicles, excess oil production on the skin which causes clogged facial skin pores, triggering bacterial activity and inflammation caused by acne-causing bacteria including *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* in sebaceous follicles (Efrata *et al.*, 2018). Acne is not a dangerous skin disease, but it has a big impact on sufferers both physically and psychologically because it can cause anxiety and decrease self-confidence (Saragih *et al.*, 2016). Many of the acne sufferers who use drugs inappropriately and can cause various new problems such as allergic reactions, irritation, resistance and hypersensitivity. In addition, there are complications from increasingly inflamed acne such as comedonal acne, conglobatal acne, papulo-pustular acne and other severe acne (Try *et al.*, 2021).

To prevent the use of drugs inappropriately, it is necessary to develop research on the use of medicinal plants as an effort to treat acne. One of the medicinal plants that has the potential as a therapy for acne treatment is red betel leaf (Kumalasari *et al.*, 2020). In a study conducted by Syafriana and Rusyita (2017) stated in their research that red betel leaf (*Piper crocatum*) is one type of plant that contains chemical compounds such as alkaloids, flavonoids, tannins, and essential oils. The results of the study explained that the minimum inhibitory concentration value of the ethanolic extract of red betel leaf (*Piper crocatum*) was able to inhibit the growth of *P. acnes* bacteria at a concentration of 10%. In this study, using 70% ethanol extract of red betel leaf with disk diffusion method (Kirby Bauer) with various concentrations of 70%, 80%, 90%, and 100%.

METHOD

A. Research Design

The design of this study was an experiment that tested the inhibition of Red Betel leaf extract (*Piper crocatum*) against the growth of *Propionibacterium acnes* bacteria. Experimental design is a research in which the researcher intentionally provides treatment or intervention to the research subject. The treatment in this study was red betel leaf extract with concentrations of 70%, 80%, 90%, and 100%.

B. Time and Location of Research

This research was conducted in February-March 2022 at Stikes Mitra Keluarga Bekasi Timur.

C. Research Sample

The samples in this study were Red Betel Leaf (*Piper crocatum*) and pure culture of *Propionibacterium acnes* ATCC 11827.

D. Research Variables

The variables in this study are independent variables. Independent variables are independent variables, unlike independent variables which are always paired with the dependent variable (Sugiyono, 2018). The independent variable in this study was the diameter of the inhibition zone on the media containing *P. acnes* bacteria after administration of red betel leaf extract with varying concentrations.

E. Tools and Materials

The tools used are glass and non-glass tools, analytical balance (IKA, Ohaus MB 120), incubator (Memmerth IN-30), autoclave (AGGRAM), Laminar air flow (UK 1200 MM), rotary evaporator (IKA-RC 2), oven (IKA 125), and P100 micropipette (Acura digital 825).

The materials used were red betel leaf (*Piper crocatum*), 70% ethanol, distilled water, NA (*Nutrient agar*) and MHA (*Mueller hinton agar*) medium, *Propionibacterium acnes* ATCC 11827 bacteria culture.

F. Making Red Betel Leaf Extract

Wash the red betel leaves and then air dry them until they are completely dry. Once dry, puree with a blender. Weigh the fine powder as much as 100 grams and given 500 ml of 70% ethanol and stored in a place that is protected from light for 3 days while stirring occasionally. Then filtered, the residue was re-maceration with added 250 ml of 70% ethanol and stored for 2 days. Then evaporation is carried out using a *rotary evaporator*.

G. Qualitative Phytochemical Screening

1. Alkaloid Test

Weigh 1 gram of red betel leaf simplicia powder, add 5 ml of chloroform and 10 ml of 10% ammonia. Strain into a test tube and add 10 drops of 2N H₂SO₄ to the filtrate. Shake until two layers are formed, then transferred to 3 test tubes as much as 1 ml. To each tube, a few drops of *Mayer*, *Wagner* and *Dragendorff* reagents were added. Positive results of alkaloids are indicated by the formation of a precipitate with *Mayer's* reagent forming a white precipitate, with *Wagner's* reagent forming a brown precipitate and *Dragendorff's* reagent forming an orange precipitate (Trian, 2020).

2. Tannin Test

Weigh as much as 20 mg, then add 96% ethanol. Then 2-3 drops of 1% FeCl₃ solution were added. Positive results are indicated by the formation of a bluish black or greenish color (Eka, 2017; Trian, 2020).

3. Flavonoid Test

Weigh as much as 200 mg, then add 5 ml of 96% ethanol and heated for 5 minutes. Then add a few drops of concentrated HCl and 0.2 g of Mg powder. Positive results are indicated by the appearance of a dark red color for three minutes (Trian, 2020).

4. Saponin Test

Weigh as much as 2 grams then put into a test tube, add distilled water and boil for 2-3

minutes, and then cooled, then shaken vigorously. Positive results are indicated by the formation of stable foam (Triana, 2020).

5. Essential Oil Test

The 0.5 gram thick extract was diluted with 1 ml of 96% ethanol solvent and then heated on a hot plate using a watch glass until the residue was obtained. Positive results are indicated by the characteristic odor produced by the residue (Rukmini *et al.*, 2020).

H. Resistance Test

The diameter of the inhibition test was carried out using the Kirby Bauer method, in which a sterile cotton swab that had been dipped in a suspension containing *P. acnes* bacteria was rubbed on the MHA medium evenly. Then attached to each paper disk that has been soaked in the extract according to the concentration for $\pm$ 30 minutes and sterile distilled water. Paper disks were placed on the surface of the MHA media and incubated for 24 hours at 37°C. The test material is categorized as positive if the laboratory test results on Red Betel leaf extract can inhibit the growth of *P. acnes* which is characterized by the formation of an inhibitory zone formed around the paper disk (Basarang, 2019).

RESULTS

A. Plant Determination

Determination of a plant aims to find out the truth of the identity of the plant, whether the plant is really the desired plant. Thus errors in the collection of materials to be studied can be avoided. The determination was carried out at the Bogor Research Center-Indonesian Institute of Sciences (LIPI), Cibinong, West Java. The results of the determination can be seen in the following table:

Number	Kol Number	Type	Family
1	Red Betle Leaf	<i>Piper crocatum</i> Ruiz and Pav.	Piperaceae

B. Plant Organoleptic

Organoleptic tests are carried out as an initial introduction to the plants that will be used for testing. This is done by looking at the physical appearance of plants by observing which includes smell, color, shape, and taste. The results of organoleptic tests can be seen in the following table:

Organoleptic Test	Result
Smell	Strong aromatic
Colour	Purplish red
Form	Flat like a heart with a tapered tip
Flavour	Bitter and spicy

C. Plant Yield

The yield of this research extract was obtained through the extract process using the maceration method. Yield is the ratio of the amount of extract produced from plant extraction, the resulting yield indicates the value of the extract produced is increasing, the yield can be expressed from the comparison of the weight of the extract with the weight of the simplicia powder. The yield of plants can be seen in the following table:

Sample	Powder Weight (g)	Extract Weight (g)	Extract Yield Value (%)
Red Betle Leaf	100 g	13,5 g	13,5%

D. Phytochemical Screening

Phytochemical screening is a preliminary stage in a phytochemical research with the aim of providing an overview of the class of compounds contained in the plant to be studied. The

phytochemical screening method that will be carried out is by looking at the color test reaction using a color reagent. The results of phytochemical screening can be seen in the following table:

Secondary Metabolic Compound	Extract Test Results	Reagen	Information
Flavonoid	+	Wagner	Chocolate precipitate
Alkaloid	+	FeCl ₃ 1%	Blue or greenish black color
Tannin	+	Concentrated HCl + Mg	Dark red color
Saponin	+	Aquadest	Forms stable foam
Essential Oil	+	Ethanol 96%	Presence of residue characteristic odor

E. Inhibitory Zone Diameter Test

The antibacterial bioactivity test was carried out using samples of red betel leaf with three replications of treatment. The antibacterial bioactivity test of the red betel leaf ethanol extract used four concentration variations, namely 70%, 80%, 90%, and 100%. The results of the diameter of the inhibition zone can be seen in the following table:

Treatment	Average Inhibition Zone Diameter (mm)	Information
70%	16 mm	Intermediate
80%	18 mm	Intermediate
90%	18,5 mm	Intermediate
100%	21 mm	Sensitive
Control (+)	26 mm	Sensitive
Control (-)	0	Resistant

Information:

Positive control = Clindamycin

Negative control = Aquadest

DISCUSSION

In this study, the sample used was red betel plant. The plant parts used in this study were leaves that had been collected in February 2022. The red betel plants that have been collected are then determined at the Research Center for Biology-Indonesian Institute of Sciences, Cibinong, West Java. Determination of plants is done to ensure the correctness of the plants to be used, by matching the morphological characteristics of red betel leaf and green betel leaf to the literature. Based on the results of the determination that has been made, it can be seen that the red betel leaf plant is included in the type of *Piper crocatum* Ruiz and Pav. with the *Piperaceae* family.

Furthermore, after determining the plants, organoleptic tests were carried out on red betel leaf plants. The organoleptic test aims to see the physical appearance of red betel leaf and green betel leaf which includes smell, color, shape, and taste. Based on the results of organoleptic tests, it is known that red betel leaf has a purplish red color, while for smell, shape, and taste, it has a strong aromatic odor, flat leaf

shape resembling a heart with a tapered leaf tip, and has a bitter and spicy taste. These results are the same as research conducted by Nani and Tri (2016) that red betel leaves have red leaves with a purple underside, have an elliptical shape with a tapered top, and have a bitter taste.

In this research, the red betel leaf which has been determined and organoleptic test is then extracted using 70% ethanol solvent by maceration method. According to Gede et al. (2019) Ethanol solvent is a polar solvent so it is often used to identify bioactive compounds. Ethanol can dissolve compounds in plants because ethanol is able to degrade cell walls so that bioactive compounds are easier to get out of plant cells. Then from the results of the maceration extraction, the results of the viscous extract were obtained, where the viscous extract was calculated to obtain the yield value of the extract. Extract yield is the ratio of the weight of the resulting extract to the weight of the sample simplicia. High extract yields indicate that the amount of juice that can be extracted or removed from the plant tissue is quite optimal. The yield of red betel leaf extract obtained in this study is higher when compared to the yield of red betel leaf extract obtained in Septiani's research (2017) using 70% ethanol solvent, which is only 10.10% with the maceration method.

The next research data obtained are the results of phytochemical screening. Phytochemical screening is a test carried out to determine the content of the secondary metabolite compounds contained in plants. Based on the results obtained in this phytochemical screening, red betel leaf positive contains alkaloids, flavonoids, tannins, saponins and essential oils. The results of this phytochemical screening are the same as those of the study conducted by Vianey et al. (2022), which in his research compared the antioxidant activity of red betel leaf using a maceration extraction process with 70% ethanol solvent, both of which were positive for alkaloids, flavonoids, tannins, and saponins, while the essential oil content was in accordance with Ayu's research (2018) where in his research it was stated that the positive red betel leaf contains essential oil and qualitative analysis was carried out.

Based on the results table, it is known that the ethanolic extract of red betel leaf has antibacterial activity against the growth of *P. acnes* which is indicated by the formation of an inhibition zone around the disc. From the results of the data that has been obtained based on the measurement of the diameter of the inhibition zone of the red betel leaf ethanol extract at concentrations of 70%, 80%, 90%, and 100%, which is 16 mm; 18mm; 18.5mm; and 21 mm. From the measurement results, it can be seen that the red betel leaf ethanol extract test with a concentration of 70%, 80%, 90% showed intermediate results, while the red betel leaf ethanol extract with a concentration of 100% showed sensitive results. Based on the results, red betel leaf extract was proven to inhibit the growth of *P. acnes* bacteria. The results of this study are in accordance with the research conducted by Wahyuningsih & Sumaryono (2021) which also proved that the ethanolic extract of red betel leaf can inhibit the growth of *P. acnes* bacteria.

As for the positive control, the antibiotic clindamycin was used, with an average inhibition zone of 26 mm in the sensitive category. Clindamycin has a greater lipophilic effect because it has the element of chlorine it has, its penetration into bacterial cells is better than lincomycin. It is known that clindamycin is effective against gram-positive anaerobic bacteria, one of which is *P. acnes*. It has an antibacterial effect mechanism by binding to the 50S ribosomal subunit of bacteria and inhibiting bacterial protein synthesis due to its activity against *S. aureus*, streptococci, and anaerobes (Akramullah, 2021). The results of the positive control of clindamycin in this study were higher than those conducted by Mayefis et al. (2020) where the positive control results of clindamycin on *P. acnes* showed an inhibition zone diameter of 9.03 mm, while the negative control used aquadest, where the results did not show an inhibition zone. This indicates that the control used has no effect on the antibacterial test.

Determination of the antibacterial activity of red betel leaf extract was carried out by the agar diffusion method. The principle of this method is to use solid media and paper discs, then the inhibition of bacterial growth is determined by measuring the diameter of the zone of inhibition of bacterial growth. The inhibition zone for bacterial growth is a clear area around the paper disc (Susi, 2018).

This diffusion bioactivity test was started by rubbing the bacterial suspension on the surface of the *Mueller Hinton agar* media, then attaching a paper disk containing red betel extract with varying concentrations, a positive control paper disk for the antibiotic clindamycin, and a paper disk containing a negative control of distilled water. Then incubated at 37°C for 24 hours. After 24 hours, the inhibition zone was measured using the Kirby-bauer method, namely measuring the inhibition zone around the paper disk vertically and horizontally using a caliper or ruler with units of millimeters (mm). Then record the diameter and compare

the measurement results according to the 2015 CLSI (*Clinical Laboratory Standards Institute*) table to determine the bacteria in the category of resistant, intermediate, or sensitive. A bacterium is said to be sensitive to antibiotics if the bacteria can be inhibited properly which is characterized by the formation of a clear zone around the disc during the test, the intermediate category if the bacteria can be inhibited but with weaker inhibition, and the resistant category if the bacteria can be inhibited but show inhibition. very weak or no inhibition is formed at all (Susi, 2018).

The limitation of this research is the possibility of confounding variables such as contamination from plant raw materials and bioactivity tests. Because plant raw materials are natural products, plants can be degraded by various microorganisms, both bacteria and fungi. Contaminants of plant raw materials can come from soil, post-harvest processing, transportation, and storage. The effect that can arise due to the presence of microorganisms in plant raw materials is a decrease in active compounds, therefore prevention can be done by drying plants properly which can reduce microorganism contamination in raw materials, where the condition for growing microorganisms in a material is the presence of water (Ningsih, 2014).

Furthermore, the main causes of contamination include unhygienic procedures carried out and airborne microorganisms during the test process. Prevention that can be done to reduce the occurrence of contaminants is to store test materials to be used according to a predetermined temperature, maintain body and environmental hygiene, and carry out aseptic procedures (John *et al.*, 2019).

CONCLUSION

The average diameter of the inhibition zone of red betel leaf at concentrations of 70%, 80, 90% indicates the intermediate category, and at a concentration of 100% indicates the sensitive category. So it can be concluded that the sensitive category of red betel leaf extract was obtained at the highest concentration of 100%.

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BIOACTIVITY TEST AGAINST ANTIBACTERIAL COMPOUND LEAF EXTRACT OF SUGAR APPLE LEAVES (*Annona Squamosa*) AGAINST *Staphylococcus aureus*

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Abstract

Introduction: Infectious diseases are one of the major health problems globally, most often caused by bacteria. Treatment for bacterial infections is done with antibiotics, but their use can cause resistance. Sugar apple (*Annona squamosa*) leaves contain metabolites such as alkaloids, flavonoids, and saponins that have antibacterial potential. This study aims to determine the sensitivity response of *Staphylococcus aureus* bacteria to Sugar apple leaf extract with concentrations of 20%, 40%, 60%, 80%, and 100%. **Methods:** This study used an experimental design with a disk diffusion technique (Kirby Bauer). Sugar apple leaves are collected from the yard of a resident of Harapan Jaya, Bekasi, West Java. Sugar apple leaf extract was obtained by maceration technique using 70% ethanol. Extract concentrations are 20%, 40%, 60%, 80%, and 100%. control (+) chloramphenicol and control (-) distilled water. **Result:** Antibacterial test using disk diffusion method (Kirby Bauer). The average results obtained are concentrations of 20%: 1.5mm, 40%: 2mm, 60%: 2.5mm, 80%: 2.75mm, and 100%: 4.5mm. The higher the concentration of the extract given, the greater the inhibition zone formed. **Conclusion:** The bioactivity of the ethanol extract of Sugar apple (*Annona squamosa*) leaf as an antibacterial can inhibit the growth of *Staphylococcus aureus* bacteria.

Keywords: Antibacterial, Kirby Bauer, Maceration, *Staphylococcus aureus*, Sugar apple (*Annona squamosa*).

INTRODUCTION

Infectious diseases are one of the major health problems globally caused by pathogenic microorganisms, which are capable of causing illness and death (Yadav *et al.*, 2020). Infectious diseases are often caused by bacteria. One of them is *Staphylococcus aureus* which belongs to the group of gram-positive pathogenic bacteria that can cause various diseases such as abscesses, endocarditis, skin infections, pneumonia, meningitis, and sepsis. The severity of bacterial infections in humans varies, including respiratory tract infections, eye infections, urinary tract infections, and the Central Nervous System (CNS) (Widiastuti *et al.*, 2019). Treatment therapy for infectious diseases caused by bacteria is done by giving antibiotics. However, antibiotics that are used inappropriately can trigger antibiotic resistance (Ballo *et al.*, 2021). With this, research is needed in addition to using chemicals, one of which is the development of natural ingredients that contain antibacterial compounds to avoid the occurrence of resistance to antibiotics. A study conducted by Isramilda *et al* (2020) stated that decoction Sugar apple (*Annona squamosa*) leaves with 0.9% NaCl using the good method was able to inhibit *Staphylococcus aureus* with a diameter of 6.35 mm with a concentration of 100%. While research by Dewangga dan Nirwana (2016) stated that the infusion of Sugar apple (*Annona squamosa*) leaves was compared to 70% ethanol extract of Sugar apple (*Annona squamosa*) leaves with concentrations of 50%, 25%, and 12.5%. The inhibition obtained was much better and no maximum concentration was found that compensated for its positive control on the growth of *Staphylococcus aureus*. In the study, Tansil *et al* (2016) stated that the 96% ethanol extract of Sugar apple leaves with concentrations of 50%, 25%, and 12.5% in *Escherichia coli* was less inhibitory when compared to *Staphylococcus aureus*. Based on previous research, no research has been conducted on the bioactivity test of the antibacterial compound of Sugar apple leaf (*Annona squamosa*) against *Staphylococcus aureus* at doses of 100%, 80%, 60%, 40%, and 20%. Therefore, researchers are interested in researching bioactivity tests of antibacterial compounds using 70% ethanol extract of Sugar apple leaves against *Staphylococcus aureus* bacteria by disc diffusion method (Kirby Bauer).

METHOD

A. Research design

This research design uses experimental research which aims to determine the emergence of the consequences of the treatment given by the researcher intentionally (Payadnya dan Trisna, 2018). The treatment in this study was Sugar apple (*Annona squamosa*) leaf extract with concentrations of 100%, 80%, 60%, 40%, and 20%.

B. Time and Location of Research

This research was carried out at the Phytochemical and Microbiology Laboratory of STIKes Mitra Keluarga, East Bekasi in February - March 2022.

C. Research Sample

The samples in this study were pure cultures of *Staphylococcus aureus* ATCC25923 and Sugar apple (*Annona squamosa*) leaves.

D. Research Variables

The variables in this study include the independent variable namely the concentration of Sugar apple (*Annona squamosa*) leaf extract of 100%, 80%, 60%, 40%, and 20% (w/v). The dependent variable is diameter zone of inhibition formed from the growth of *Staphylococcus aureus* bacteria and the control variable used chloramphenicol as control (+) and sterile distilled water as control (-)

E. Tools and materials

The tools used are glass and non-glass tools, analytical balance (IKA, Ohaus MB 120), incubator (Memmerth IN-30), autoclave (AGGRAM), Laminar air flow (UK 1200 MM), rotary evaporator (IKA-RC 2), oven (IKA 125), and P100 micropipette (Acura digital 825). The materials used were Sugar apple (*Annona squamosa*) leaves, 70% ethanol, distilled water, NA (*Nutrient agar*) and MHA (*Mueller hinton agar*) medium, *Staphylococcus aureus* bacteria culture ATCC25923.

F. Making Sugar apple Leaf Extract

Wash the Sugar apple leaves and dry them with the wind until they are completely dry. After that, mashed with a blender. Weigh the fine powder as much as 100 grams and give 500 ml of 70% ethanol and stored it in a place protected from light for 3 days while stirring occasionally. Then filtered, the residue was macerated again by adding 250 ml of 70% ethanol and stored for 2 days. Then the concentration was carried out using a rotary evaporator to produce a thick extract of Sugar apple leaves with a concentration of 100%.

G. Qualitative Phytochemical Screening

1. Alkaloid Test

1 ml of Sugar apple leaf extract was taken into two test tubes, then 5 drops of Mayer and Dragendorff reagents were added to the test tube. Positive results when a precipitate forms on the sample. A white precipitate is formed indicating (+) Mayer, and a yellow-red (+) Dragendorff. the precipitate is formed (Aliwu *et al.*, 2020).

2. Flavonoid Test

As much as 1 ml of Sugar apple leaf extract then add 1 ml of ethanol to a test tube and heat on a bath. Then three drops of concentrated HCl and MgCl₂ were added, and the changes were seen. A positive result was obtained with a reddish-brown color change Putri *et al.*, 2020).

3. Saponin Test

2 ml of Sugar apple leaf extract is put into a test tube then add distilled water and shaken for 30 seconds, observe the changes that occur. A positive result is indicated by the formation of a stable foam for 10 minutes with a height of 1 to 3 cm (Rante *et al.*, 2020).

4. Terpenoid Test

Take 5 ml of extracted Sugar apple leaves into a test tube. added with 2 ml of chloroform and 3 ml of concentrated sulfuric acid and 2 ml of chloroform. A reddish-brown color change indicates a positive result (Astuti *et al.*, 2021).

H. Sensitivity Test

The inhibition test was carried out using the Kirby Bauer method. Each disc paper was soaked for

± 30 minutes in a solution of Sugar apple leaf extract with various concentrations (20%, 40%, 60%, 80%, and 100%), distilled water as a negative control and chloramphenicol as a positive control, then the disc paper was placed on the surface of the MHA media which had been swabbed with bacterial suspension evenly with a sterile cotton swab and incubated at 37°C for 24 hours. Sugar apple leaf extract is said to be positive if the given treatment forms an inhibition zone around the paper disc (Syarifah *et al.*, 2018).

RESULTS

Based on research that has been carried out at the Phytochemistry and Microbiology Laboratory of STIKes Mitra Keluarga, East Bekasi from February 18 – March 30, 2022. The results of the study are as follows:

A. The yield of Sugar apple Leaf Extract

The result of extracting the leaves of Sugar apple (*Annona squamosa*) using the maceration method. Obtained with the % yield value as shown in the following table:

No.	Leaf Weight (grams)	Powder Weight (grams)	Extract Weight (grams)	yield (%)
1.	500	100	11,642	11,642

B. Phytochemical Screening of Sugar apple Leaf Extract

The results of the phytochemical screening test of Sugar apple leaf extract (*Annona squamosa*) obtained include the Alkaloid, Flavonoid, Saponin, and Terpenoid tests as shown in the following table:

No	Identification	Observation result	Information
1.	Alkaloids (Dragendorff's reagent)	The solution is dark brown and there is a yellow-reddish precipitate	+
2.	Alkaloids (Mayer's reagent)	The solution is dark brown and there is a white precipitate	+
3.	Flavonoids	Reddish brown solution	+
4.	Saponins	The solution is brown and stable foam is formed	+
5.	Terpenoids	Brown solution	-

Results :

+ : Positive contains secondary metabolites

- : Negative contains secondary metabolites

C. Sugar apple Leaf Extract Bioactivity Test

The results of the observation of the bioactivity test of Sugar apple leaf extract (*Annona squamosa*) on the growth of *Staphylococcus aureus* bacteria with various concentrations, namely 100%, 80%, 60%, 40%, 20%, control (+) and control (-) which were replicated 3 times. . The results

obtained are shown in the following table:

Treatment	Inhibition Zone Diameter (mm)			Average (mm)	Information
	Replication I	Replication II	Replication III		
20%	1.5	1	2	1.5	resistance
40%	2	1.5	2.5	2	resistance
60%	2.5	2	3	2.5	resistance
80%	2.75	2.5	3	2.75	resistance
100%	5	4.5	4	4.5	resistance
Control (+)	26	28	27	27	sensitive
Control (-)	0	0	0	0	resistance

Results :

Control (+) : Chloramphenicol

Control (-) : Aquadest

DISCUSSION

In this study, the sample used was the leaf part of the Sugar apple (*Annona squamosa*) plant that grows in the gardens of the residents of Harapan Jaya village, Bekasi, West Java. This sample was collected in early February 2022 and determined at the Center for Biological Research – Indonesian Institute of Sciences (LIPI), Cibinong, West Java, to ensure the correctness of the plants to be used, by matching the morphological characteristics of Sugar apple leaves with the literature. After that, the identification of Sugar apple leaf simplicia which includes smell, color, taste, and shape is carried out. In this study, simplicia was dried and extracted by the maceration method and concentrated with a rotary evaporator to obtain a thick extract. Maceration was carried out on Sugar apple leaf powder using 70% ethanol because it was able to attract more compounds in the simplicia. The maceration process was carried out for three days with maceration for two days. Then the % yield value from wet sorting of Sugar apple leaves was 149%. Then on dry sorting of Sugar apple leaves, the % yield value was 43.738%. Meanwhile, in the thick extract of Sugar apple leaves, the % yield value obtained was 11.642%. According to FHI in 2017 the requirement for a good % yield is < 7.2%. So based on the results of the calculation of the % yield value that has been obtained is appropriate because it meets the requirements of a good % yield. Then the % yield value from wet sorting of Sugar apple leaves was 149%. Then on dry sorting of Sugar apple leaves, the % yield value was 43.738%. Meanwhile, in the thick extract of Sugar apple leaves, the % yield value obtained was 11.642%. According to FHI in 2017 the requirement for a good % yield is < 7.2%. So based on the results of the calculation of the % yield value that has been obtained is appropriate because it meets the requirements of a good % yield. Then the % yield value from wet sorting of Sugar apple leaves was 149%. Then on dry sorting of Sugar apple leaves, the % yield value was 43.738%. Meanwhile, in the thick extract of Sugar apple leaves, the % yield value obtained was 11.642%. According to FHI in 2017 the requirement for a good % yield is < 7.2%. So based on the results of the calculation of the % yield value that has been obtained is appropriate because it meets the requirements of a good % yield.

Then in this study phytochemical screening of the extracts that have been obtained to determine the presence or absence of secondary metabolites is observed from the precipitate formed and the color changes that occur. The results of phytochemical screening tests on 70% ethanol extract of Sugar apple (*Annona squamosa*) leaves can be seen in Table 5.2 showed that Sugar apple leaf extract was positive for terpenoids, flavonoids, alkaloids, and saponins. The results terpenoid test obtained negative results because when observed it did not show a reddish-brown color change in the solution. Positive results of Alkaloids and Flavonoids are indicated by the formation of colored precipitates while saponins are

indicated by the presence of stable foam formed above the solution.

In the bioactivity test of Sugar apple leaf extract, concentration variations were made, namely 100%, 80%, 60%, 40%, and 20%. Based on the results of the measurement data obtained in Table 5.3 It is known that the leaf extract of Sugar apple (*Annona squamosa*) has antibacterial activity against the growth of *Staphylococcus aureus* which is indicated by the formation of inhibition of the growth of *Staphylococcus aureus* bacteria in variations in the concentration of the Sugar apple leaf extract. The data obtained from the measurement of the diameter of the inhibition zone of Sugar apple leaf extract with a concentration of 20% obtained an inhibition zone with an average of 1.5 mm, a concentration of 40% obtained an average of 2 mm, a concentration of 60% obtained an average of 2.5 mm, 80% is 2.75mm and 100% concentration is 4.5mm. The data was obtained from the positive control with an average of 27mm while the inhibition zone was not obtained in the negative control. The results of this study are following research conducted by Erlina *et al* (2018) about the antibacterial effect and phytochemical activity of sugar apple leaf extract which states that the high concentration of sugar apple leaf, the greater the antibacterial activity so that bacterial growth can be maximally inhibited.

Activity in inhibiting *Staphylococcus aureus* in the treatment of ethanol extract of Sugar apple leaf (*Annona squamosa*) is due to the presence of active compounds in the extract of Sugar apple leaf. Based on research (Dewangga & Nirwana, 2016). stated that the ethanol extract of Sugar apple leaves contains saponins, alkaloids, flavonoids, and terpenoids that function as antibacterial. In Sugar apple leaf extract, there are active compounds whose respective mechanisms are in inhibiting bacterial growth. According to Jangnga *et al* (2018) Flavonoid compounds work to inhibit bacterial growth by causing damage to the permeability of lysosomes, microsomes, and bacterial cell walls. These flavonoid compounds can inhibit the movement of bacteria actively and spontaneously. Alkaloids are capable of causing changes in the composition of amino acids and cells, resulting in damage and causing bacterial cell death (Gurrapu & Mamidala, 2017). Saponins as antibacterials work through hydrogen bonds by forming complex compounds on the cell membrane so that they can destroy the permeability properties of the bacterial cell wall and cause cell death (Dewangga & Nirwana, 2016). The active compounds in the ethanolic extract of Sugar apple leaves that were previously mentioned above against the growth of *Staphylococcus aureus* bacteria in this study could be inhibited.

The results of the data from these measurements can be seen that the test given the treatment of Sugar apple leaf ethanol extract at various concentrations showed results that were resistant to the growth of *Staphylococcus aureus*. While the results obtained from the treatment of chloramphenicol antibiotics showed sensitive results. So that the inhibition zone obtained in the positive control using the antibiotic chloramphenicol was greater than the treatment with various concentrations of Sugar apple leaf extract. This is because antibiotics are derived from a substance obtained by chemical synthesis or microorganisms so that they can inhibit and kill microorganisms at low concentrations (Cahyani, 2021). In this study, chloramphenicol was used as a positive control. Chloramphenicol is a broad-spectrum antibiotic and Has activity capable of inhibiting protein synthesis in bacterial cells (Mahdiva *et al.*, 2021). The interpretation of the results of bacterial growth against antibacterial or antibiotic substances in this study was categorized based on the formation of a bacterial inhibition zone around the paper disc which was then compared with the 2018 CLSI table. The standard for assessing the diameter of the antibiotic inhibition zone based on the CLSI (*Clinical Laboratory Standards Institute*) contained a level of resistance. bacteria to antibiotics are grouped into three categories, namely resistant, intermediate, and sensitive.

CONCLUSION

The diameter of the inhibition zone for the growth of *Staphylococcus aureus* bacteria which was replicated 3 times at concentrations of 100%, 80%, 60%, 40%, and 20% was formed with an average of 4.5mm, 2.75mm, 2.5mm, 2mm and 1, 5mm. So it falls into the category of resistance.

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BIOACTIVITY TEST OF ANTIBACTERIAL COMPOUNDS OF EXTRACTS LEMON PEEL (*Citrus limon*) AGAINST BACTERIA *Staphylococcus aureus*

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Abstrak

Introduction: Infectious diseases are caused by living microorganisms ranging from bacteria, viruses and fungi. Infectious diseases that often occur are usually caused by bacteria. Lemon (*Citrus limon*) is one of the plants that has the potential to be a natural antibacterial because it contains bioactive compounds such as flavonoids, terpenoids, tannins, and citric acid which can inhibit antibacterial growth.

Method : This research is a quantitative experimental study using the maceration extraction method using 70% ethanol as solvent and the Disk Diffusion method (Kirby Bauer) with various concentrations of 20%, 40%, 60%, 80% to 100%.

Result : The results of the data that has been obtained based on the sensitivity response of *Staphylococcus aureus* bacteria to Lemon (*Citrus limon*) flavedo peel extract at stratified concentrations ranging from 20%, 40%, 60%, 80% to 100%, with an inhibition zone diameter of 1, respectively. 5mm, 1.8mm, 2.3mm, 2.8mm, 3.5mm.

Conclusion: The conclusion of this study is that the administration of red betel leaf extract with a concentration of 100% resulted in the largest diameter of the inhibition zone, the data obtained were analyzed by the One Way Anova test, resulting in a P value <0.05.

Keywords : Antibacterial, *Citrus limon*, Kirby Bauer, Maceration, One Way Anova

INTRODUCTION

Infectious diseases are caused by living microorganisms ranging from bacteria, viruses and fungi. Infectious diseases that often occur are usually caused by bacteria (Cahyani, 2021). One of the bacteria that causes infectious diseases is *Staphylococcus aureus*. One of the problems caused by *Staphylococcus aureus* bacteria is several types of infectious diseases such as skin diseases such as impetigo, cellulitis, paronychia, osteomyelitis (bone infection), pneumonia (respiratory tract infection), toxic shock syndrome, food poisoning with symptoms of nausea and vomiting, and diarrhea (Wikananda *et al.*, 2019). The growth of bacteria that cause infection needs to be inhibited in order to prevent bad health effects if not treated. Natural materials derived from plants can be used as an alternative treatment of infectious diseases, especially those caused by bacteria. One of the plants that has the potential as an antibacterial one of which is lemon (*Citrus limon*) because there are many bioactive compounds that act as antibacterials (Indriani *et al.*, 2015). Lemon (*Citrus limon*) can be used as a natural antibacterial because there are bioactive compounds such as terpenoids, flavonoids, tannins, and also citric acid that can inhibit bacterial growth (Saragih *et al.*, 2019). Astuti (2021) stated that the antibacterial sensitivity test of lemon peel extract (*Citrus limon*) on *Escherichia coli* and *Salmonella typhi* bacteria, the method used was Kirby Bauer disc.

Variations in stratified concentrations ranging from 10%, 30%, 50%, 70% and 90% showed greater inhibition of *Escherichia coli* bacteria than *Salmonella typhi* bacteria. Concentration of 10% in *Escherichia coli* bacteria showed an inhibition zone diameter of 12.17 mm at the largest concentration, namely 90% showed an inhibition zone diameter of 22.10 mm and was categorized as sensitive while *Salmonella typhi* bacteria did not show any inhibition zone at all. The differences between previous studies and those to be studied are in the solvent, the sample used, the extraction method, and the type of bacteria used, so that researchers are interested in conducting research on the bioactivity of antibacterial compounds with the aim of knowing the ability of the flavedo peel extract of Lemon (*Citrus limon*) against *Staphylococcus aureus* by using maceration extraction method with 70% technical ethanol solvent.

METHOD

A. Research Design

The design of this study is a quantitative experiment by testing the inhibitory power of the ethanolic extract of flavedo lemon peel using the maceration extraction method using 70% ethanol as a solvent and the Disk Diffusion method (Kirby Bauer) with various concentrations of 20%, 40%, 60%, 80%. up to 100%.

B. Time and Location Research

This research was conducted in February-March 2022 at the Phytochemistry and Microbiology Laboratory of STIKes Mitra Keluarga, East Bekasi.

C. Research Sample

The samples in this study were flavedo peel of lemon (*Citrus limon*) and pure culture of *Staphylococcus aureus* ATCC 6538.

D. Research Variables

The variables in this study were divided into three, namely independent, dependent, and control variables. The independent variable used was the concentration of lemon peel extract (*Citrus limon*) used, namely 100%, 80%, 60%, 40%, and 20% (w/v). The dependent variable was the growth of *Staphylococcus aureus* bacteria in the antibacterial compound test by measuring the diameter of the inhibition zone. The control variables were further divided into 2, namely positive using clindamycin and negative using sterile distilled water.

E. Tools and Materials

The tools used are glass and non-glass tools, analytical balance (IKA, Ohaus MB 120), incubator (Memmerth IN-30), autoclave (AGGRAM), Laminar air flow (UK 1200 MM), rotary evaporator (IKA-RC 2), oven (IKA 125), and P100 micropipette (Acura digital 825).

The materials used were lemon flavedo peel (*Citrus limon*), 70% ethanol, distilled water, NA (Nutrient agar) and MHA (Mueller hinton agar) medium, *Staphylococcus aureus* ATCC 6538 bacteria culture.

F. Preparation of Lemon Lime Flavedo peel extract

Wash and clean the peeled lemon flavedo skin and then air dry it without direct sunlight, puree with a blender. 100 grams of sample from simplicia powder of lemon flavedo peel was weighed and then put into an erlenmeyer. The simplicia powder that was already in the erlenmeyer was added with 500 ml of 70% ethanol and soaked and then stirred for 10 minutes with a stirring rod. The mouth of the Erlenmeyer was wrapped and then added with aluminum foil, allowed to stand for 3 days and stirred occasionally every day for 10 minutes, stored in a dark place and protected from sunlight. Filtering was carried out after 3 days of maceration using a glass funnel and coated with filter paper around the funnel to separate the macerate from the filtrate. The filtrate I was stored, while the extract was remaceration by adding 250 ml of 70% ethanol to the extract into the Erlenmeyer and closed again using aluminum foil, stored again for a day and stirred occasionally. Filter again using a funnel lined with filter paper to obtain the filtrate from the remaceration. The results of the filtrate I and filtrate II were mixed and then concentrated with a rotary evaporator using a temperature of 40°C, a pressure of 200mBar, and a rotational speed of 60rpm (Verdiana *et al.*, 2018). The resulting thick extract was concentrated again with a water bath at a temperature setting of 50°C after that it was left at room temperature until the remaining solvent evaporated, the extract was weighed and then stored in a container and the yield calculation was carried out.

G. Phytochemical screening

1. Alkaloid Test

The simplicia powder of flavedo lemon peel was weighed as much as 1g, added 5 ml of chloroform, 10 ml of 10% ammonia. In this test, the simplicia powder that has been added with chloroform and ammonia is filtered and filtrated in a test tube, after which 10 drops of 2N H₂SO₄ are added. The

mixture was shaken to form two layers, then transferred into 3 test tubes as much as 1 ml. Add a few drops of Mayer, Wagner, and Dragendorf reagents to each tube.

2. Tanin Test

20 mg of the sample was weighed and then concentrated HCL was added until it was submerged. A solution of 1% FeCl is added as much as 2-3 drops. A positive result indicates the formation of a greenish black color

3. Fenolik Test

20mg sample was weighed and then dissolved in 96% ethanol. FeCl₃ 1% is added as much as 2-3 drops. A positive result containing phenol indicates a darker color change.

4. Flavonoid Test

200 mg of the sample was weighed and then extracted with 5 ml of 96% ethanol. heated using a test tube for five minutes. The addition of Mg as much as 200 mg. Positive results can be seen from the appearance of a dark red color within three minutes.

5. Saponin Test

2 grams of the sample is weighed and then put in a test tube, after that it is mixed with aquadest until the sample is submerged, heated until there is steam rising for 2 to 3 minutes. Then let it cool, and shake vigorously. Positive results can be seen from the presence of foam.

6. Terpenoid Test

The terpenoid test was carried out by dissolving chloroform into the extract solution, as much as 2ml of concentrated sulfuric acid and 0.5 ml of anhydrous acetic acid were added through the tube wall. Positive results are indicated by the formation of a brownish ring (Bhernama, 2020),

H. Resistance Test

The diameter of the inhibition test was carried out using the Kirby Bauer method, in which a sterile cotton swab that had been dipped in a suspension containing *Staphylococcus aureus* bacteria was rubbed on the MHA medium evenly. Then attached to each paper disk that has been soaked in the extract according to the concentration for $\pm$ 30 minutes and sterile distilled water. Paper disks were placed on the surface of the MHA media and incubated for 24 hours at 37°C. The test material is categorized as positive if the laboratory test results on flavedo lemon peel extract can inhibit the growth of *Staphylococcus aureus* which is characterized by the formation of an inhibitory zone formed around the paper disk (Basarang, 2019).

RESULT

A. Plant Determination

Determination of plants is carried out to determine the authenticity of plants and plant parts used in the study are correct and in accordance with those referred to in the literature review. The results of the determination confirmed that the sample of the type of lemon in this study was *Citrus x limon* (L.) Osbeck, with the family *Rutaceae*. The determination was carried out at the Bogor Research Center – Indonesian Institute of Sciences (LIPI) Cibinong, West Java.

No.	No. Kol	Type	ethnic group
1	Jeruk Lemon	<i>Citrus x limon</i> (L.) Osbeck	<i>Rutaceae</i>

B. Organoleptic Extract Flavedo Lemon Peel

Organoleptic testing of the extract was carried out by observing simply and subjectively. The organoleptic used in this study was a thick extract of lemon peel (*Citrus limon*).

Organoleptic Test	Result
Texture	Thick extract
Color	Yellowish-brown
Smell	Aromatic

C. Plant Yield

The extract yield in this study was obtained through a maceration extraction process. Yield can be expressed from the comparison of the weight of the extract with the weight of the simplicia powder. The yield of plants can be seen in the following table:

Sample	Powder Weight (g)	Extract Weight (g)	Extract Yield Value (%)
1000g	100 g	20,9 g	20,9%

D. Phytochemical screening

Phytochemical screening is a preliminary stage in a phytochemical research conducted to prove the content of antibacterial compounds. Lemon contains antibacterial compounds including citric acid, flavonoids, terpenoids and tannins. The results of phytochemical screening can be seen in the following table:

Compound Metabolites Secondary	Extract Test Results	Reagent	Information
Tanin	+	HCL Pekat + FeCl 1%	Dark green color
Fenolik	+	Etanol 96% + FeCl ₃ 1%	Black
Flavonoid	+	Etanol 96% + Mg	Dark red color
Saponin	+	Aquadest	Forms stable foam
Terpenoid	+	Kloroform + H ₂ SO ₄ + Asam asetat anhidrat	Form a brown ring
Alkaloid	-	Mayer, Wagner, Dragendoff	No white precipitate is formed

E. Inhibitory Zone Diameter Test

The bioactivity test of antibacterial compounds was carried out with 3 replications. The results of the bioactivity test of antibacterial compounds can be seen in the following table:

Treatment	Observation Zone Obstacle (mm)				Bacterial Activity
	Cawan I	Cawan II	Cawan III	Rerata (mm)	
20%	1mm	1,5mm	2mm	1,5mm	Resistan
40%	1,5mm	2mm	2mm	1,8mm	Resistan
60%	2,5mm	1,5mm	3mm	2,3mm	Resistan
80%	3mm	3mm	2,5mm	2,8mm	Resistan

100%	3mm	4mm	3,5mm	3,5mm	Resistan
Kontrol (+)	31mm	31mm	31mm	31mm	Sensitif
Kontrol (-)	0mm	0mm	0mm	0mm	Resistan

Keterangan : Kontrol (+) = Vankomisin
Kontrol (-) = Aquadest

F. ONE WAY ANOVA TEST RESULT

The One Way Anova test on the lemon flavedo peel extract treatment group resulted in a P value <0.05. This shows a significant difference in the average diameter of the *Staphylococcus aureus* growth inhibition zone between the negative, positive control treatment groups, the concentration of lemon lime flavedo peel extract 20%, 40%, 60%, 80%, and 100%.

	One Way Anova test	Sig.
N		21
Mean		6,143
Standard deviation		10,457
Diameter of the zone of inhibition of growth of <i>Staphylococcus aureus</i>		0,000

DISCUSSION

In this study, the sample used in this study was Lemon (*Citrus limon*) which grows in the Majalengka area, West Java. Researchers used local lemon plants purchased from fruit suppliers who supply the lemons to the Bantar Gebang market, Bekasi. According to a brief interview conducted between the researcher and the supplier, it is known that the cultivation of Lemons in the area has been started since 2000 with an open area of 4 hectares. There are more than 2000 trees planted, with the age of the plants can usually be harvested after 2-3 years. Plant determination was carried out at the Bogor Research Center – Indonesian Institute of Sciences (LIPI) Cibinong, West Java. The results of the determination can be seen in appendix 1.

Lase (2019) stated in his research that the type of lemon used was *Citrus limon* (L.) Burm.fil. from the family *Rutaceae* and plant determination was carried out in Herbarium Medanense (MEDA). The thick extract can be obtained after going through the evaporation process, and no longer contains liquid filters and the consistency remains liquid at room temperature. The water content of the thick extract is between 5-30% (Aksara *et al.*, 2017). Organoleptic test is carried out to see the physical appearance of the sample to be used. The extraction process using the maceration method was carried out for 4 days in an Erlenmeyer flask which was stored in a cabinet protected from the sun, with occasional stirring. After that, remaceration was carried out for 1 day with the replacement of the same solvent. The results of the maceration and remaceration processes were combined and obtained results of 538 ml, and concentrated using a series of vacuum rotary evaporators with a pressure of 200 mBar or equivalent to ± 20 Bar, with a playback speed of 60 rpm at a temperature of 40 which took 2 days (Verdiana *et al.*, 2018). The separation process between the solvent and the sample using a rotary evaporator has produced an extract which will then be thickened again using a water bath, in order to obtain the desired thick extract. The thickening process uses a water bath for 2 days with a temperature set of 50. The results of the extraction of lemon peel (*Citrus limon*) by researchers in the wet yield were 11%, the dry yield was 22.7%, the thick extract yield was 20.9%.

Lemon contains antibacterial compounds including citric acid, flavonoids, terpenoids and tannins. The antibacterial power of lemon peel is caused by the four compounds that are mutually sustainable such as terpenoids that can react with porins (transmembrane proteins) on the outer membrane of the bacterial cell

wall, forming strong polymer bonds and damaging the porin and permeability of the bacterial cell wall so that the bacterial cell lacks nutrients. Rahmitasari *et al.*, 2020). The phytochemical screening test on tannins also showed positive results by showing a blackish green color. Tannins have antibacterial activity that can interfere with protein transport in the inner layer of cells and inactivate enzymes (Saragih *et al.*, 2019). Positive flavonoids show a dark red color for 3 minutes, the respiratory system slows down because flavonoids can inhibit energy metabolism so that they can become antibacterial. Citric acid which can make the pH of bacterial cells decrease so that bacterial growth is inhibited (Nizhar, 2015) in (Saragih *et al.*, 2019). Hidayah *et al.*, (2017) stated that the positive content of phenolic is indicated by the formation of a darker color, the way phenolic works starts from the peptidoglycan component that is disturbed in the cell wall so that the synthesis of the cell wall is disturbed and is not formed completely. Positive phenolics are indicated by the formation of a greenish black color, where the way saponins work as antibacterials starts from denatured proteins so that the ability of bacterial membranes can be damaged, this damage to cell membranes greatly interferes with the survival of bacteria (Sudarmi *et al.*, 2017). Alkaloids showed negative results, which means that there are no alkaloid compounds in lemons as antibacterial on the flavedo peel of lemons.

The antibacterial bioactivity test of the ethanolic extract of the flavedo peel of Lemon (*Citrus limon*) which was carried out by the researchers overall was in the resistant category. Resistance is the resistance of a microorganism to a certain antimicrobial (Luthfiyyah and Monika, 2018). The results were resistant because the antibacterial inhibition carried out by the researchers was very low and classified as weak because the data intervals between the researchers and previous studies were in different categories even though the samples used were the same. Researchers got the results of the diameter of the inhibition zone from five stratified concentrations ranging from 20 - 100% of 1.5 - 3.5 mm. Based on the measurements in this study, it was known that the test of ethanol extract of flavedo lemon peel on *Staphylococcus aureus* with stratified concentration variations were all categorized as resistant because they showed results of 5 mm in the five variations in concentration, which means that the antibacterial compound of lemon flavedo peel extract can inhibit the growth of *Staphylococcus aureus* bacteria but with weak strength. The positive control showed an inhibition zone diameter of 31mm and was categorized as sensitive, while the negative control showed no inhibition zone at all and was included in the resistance category, the results of the inhibition test with concentration variation and control group treatment.

Testing the antibacterial activity of the peel extract of the flavedo part and with the albedo of Lemons when compared with lemon juice certainly produces a greater inhibitory power in the lemon juice, this is because there are more metabolite compounds that act as antibacterial contained in the flesh of the fruit than in the fruit. skin part. Ali *et al.*, (2017) stated that lemon peel extract was stronger in inhibiting gram-positive bacteria in the form of *Staphylococcus aureus* by showing a larger diameter of the inhibition zone than *Escherichia coli*, which belongs to the gram-negative bacteria group. Lemon peel extract showed that *Staphylococcus aureus* bacteria, which is a gram-positive type, are more susceptible to the active substances in the extract because the outer membrane is formed by a layer of peptidoglycan which is easily penetrated by the active compound compared to *Escherichia coli* which is a gram-negative bacteria due to differences in cell wall structure. Another cause is the plasticity ability of *Escherichia coli* cell walls which can secrete transpeptidase needed to survive against changes in pH and make cell walls with more complex lipopolysaccharide structures. This is supported by research by Astuti (2021) that in testing with two types of gram-negative bacteria can show different results. *Escherichia coli* showed the diameter of the inhibition zone, while *Salmonella typhi* did not show any inhibition zone at all. This is presumably because the pathogenicity of *Salmonella typhi* is higher than *Escherichia coli*. The limitations of the researcher in conducting this research are the series of vacuum rotary evaporators that have not been completely validated, the evaporation temperature is not stable, the difference in the solvent used in the study is technical 70% ethanol, and also the variety of plants used.

CONCLUSION

The sensitivity response of *Staphylococcus aureus* to the flavedo peel extract of Lemon (*Citrus limon*) at stratified concentrations ranging from 20%, 40%, 60%, 80% to 100% with inhibition zone diameters of 1.5mm, 1.8mm, 2, respectively. ,3mm, 2.8mm, 3.5mm can inhibit bacterial growth by showing resistant results or showing weak inhibition. The data analysis test using One Way Anova on Lemon Orange flavedo peel extract resulted in a P value <0.05 indicating a significant difference in the average diameter of the *Staphylococcus aureus* growth inhibition zone against the treatment group.

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STUDY OF ETNOPHARMACOLOGICAL UTILIZATION BY THE COMMUNITY OF TARUMAJAYA REGENCY, NORTH BEKASI

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Abstract

Background: Research on ethnopharmacology of medicinal plants in Tarumajaya Regency, North Bekasi aims to determine the types of plants that are efficacious as traditional medicines, plant parts that can be used as ingredients in traditional medicine processing, and how to use them in traditional medicine.

Method: The research design used was an exploratory survey and observational method, through interviews with 140 respondents using a questionnaire that included the name of the disease, the local name of the plant, the part of the plant used, and the method of manufacture. Samples were selected based on purposive sampling and purposive consecutive sampling.

Results: The results showed that the medicinal plants used by the people of Tarumajaya Regency, North Bekasi were 21 types of medicinal plants from 21 families, consisting of turmeric (*zingiberaceae*), sweet saga (*polysonaceae*), aloe vera (*liliaceae*), bitter (*acanthaceae*), star fruit wuluh (*oxalidaceae*), somjawa (*talinaaceae*), cocor duck (*crassulaceae*), noni (*rubaceae*), cat's whiskers (*lamiaceae*), green betel (*piperaceae*), gondola (*basellaceae*), castor leaf (*euphorbiaceae*), ciplukan (*solanaceae*), papaya (*caricaceae*), brotowali (*menispermaceae*), suji leaf (*asparagaceae*), ginger (*zingiberaceae*), guava (*myrtaceae*), lime (*rutaceae*), flower telang (*leguminosae*), fig (*moraceae*).

Conclusion: The most widely used plants were the *Zingiberaceae* family, with a very good level of public knowledge about the use of drugs. The most widely used medicinal plants come from gardens, namely the leaves, with a processing process by boiling with water and used for drinking.

Keywords: Ethnopharmacology, Medicinal Plants, Tarumajaya, North Bekasi

INTRODUCTION

The general public's trust in medicinal plants has decreased due to the increase in the pharmaceutical industry with chemicals (Conde *et al.*, 2014). People are starting to lose knowledge about medicinal plants around them, especially those that are endemic. According to Sulfiyana *et al.* (2019), it is stated, along with the times, people who are slowly living in urban areas are starting to abandon the habit of using plants as medicine to cure diseases.

The lack of awareness of Indonesian people towards the knowledge of medicinal plants in the environment where they live will have an impact on the loss of public knowledge in recognizing the names of medicinal plants and their uses in the treatment of diseases. In addition, the loss of information about medicinal plants causes the next generation to abandon the habit of cultivating and utilizing medicinal plants so that the potential of the herbal medicine business becomes scarce (Hidayat, 2016).

According to the 2013 World Health Organization (WHO) report on *Enhancing the Role of Traditional Medicine in Health Systems: A Strategy for the African Region*, several countries that use plants as traditional medicine include Belgium (45%) and England (90%). Users of this traditional medicine on the African continent reach 90% in Burundi and Ethiopia and 70% in Benin (Muganga *et al.*, 2013).

According to WHO (2010) regarding *Guidelines for Registration of Traditional Medicines in the WHO Africa Region* 2010, almost 20 years traditional medicines are widely used in various parts of the world and some user countries include Nigeria, Ghana, Zambia and Mali, which reach 60%. In addition, those who use traditional medicine for health make up about 80% of the population in many countries.

Another research on ethnopharmacology was conducted by Mulyani *et al.* (2019) using observation methods and exploratory surveys showed that, out of 22 families of medicinal plants used by the community in Warukin and Tanta Hulu villages, South Kalimantan, there were 28 types of medicinal plants

that were useful for treating disease or maintaining health.

It can be seen from several studies that have been carried out, there are differences in the location of the research, the number of plants obtained, and the families of plants that are often used by the surrounding community, so researchers are interested in conducting this research in Pahlawansetia Village and Samudrajaya Village.

METHODS

Research Design

The research design used in this research was the method of observation and exploratory survey.

Research Time and Place

The implementation of this research was carried out in Tarumajaya Regency, North Bekasi. In addition, this research was carried out in several houses of residents who grew traditional plants for further observation purposes. The collection of research data begun in February 2022-April 2022.

Population and Sample

The population in this study were people who had been registered in Tarumajaya Regency, North Bekasi. Samples were selected based on purposive sampling and purposive consecutive sampling techniques. Determination of the number of samples using the formula below:

$$n : \sqrt{N} + 1$$

Information:

n = Number of respondents

determined N = Number of FC
(Family Card)

The specified number of samples was.

a. Pahlawansetia

Village. $n =$

$$\sqrt{4866} + 1$$

$$n = \sqrt{4867}$$

$$n = 69 \sim 80$$

b. Samudrajaya

Village.

$$n = \sqrt{2510} + 1$$

$$n = \sqrt{2511}$$

$$n = 50 \sim 60$$

The total respondents who were involved in this research were 119 people, which were divided into 69 people representing Pahlawansetia Village and 50 people representing Samudayajaya Village. According to Nursalam and Medika (2011), this study used inclusion criteria. The inclusion criteria that met the requirements and become the research sample are:

1. Age above 30 years.
2. Married men and women.
3. Married men and women who were willing to be research respondents.
4. Married men and women who could read and write.

According to Nursalam and Medika (2011), the exclusion criteria in this study were respondents where the research subject could not represent the sample because it did not meet the requirements as a research sample, for example the condition of married women and men who were sick and experiencing disturbances of consciousness such as epilepsy.

Research Variables

The variables used in this study were public knowledge, the value of knowledge of medicinal plants, sources of acquisition of medicinal plants, plant organs that are often used, and the percentage of how to use medicinal plants.

Proceeding 2nd International Allied Health Student Conference

Research Materials and Tools

Tools preparation

The tools that needed to be prepared to continue the research were writing instruments, 12 megapixel cellphone camera, scissors, plant identification application (PlantNet), *Kitab Tanaman Obat Nusantara* book written by Alternative *et al.* (2019), *Praktikum Farmakologi* book written by Silalahi *et al.* (2019), *Karakteristik Morfologi Tumbuhan* book written by Liunokas *et al.* (2021), and *Penuntun Farmakologi* book written by Eliyanoor and Benbasyar (2016).

Ingredient

The material used was the respondent sheet

(questionnaire).Lampiran 7 Dokumentasi lembar kuesioner.

NO	QUESTIONS	ASSESSMENT		
		Yes	No	No Idea
1	Did you know that there are types of medicinal plants that can be used for this village?			
2	Was your first knowledge of medicinal plants hereditary?			
3	Did you know about medicinal plants first from media information?			
4	Do you know what medicinal plants are used for traditional events?			
5	Do you think there are still many medicinal plants in this village?			
6	Do you think herbs can cure Covid-19?			
7	Do you think herbs can boost immunity?			

NO	QUESTIONS	ASSESSMENT		
		Yes	No	No Idea
1	Have you ever used medicinal plants for the treatment and maintenance of health?			
2	Do you use family medicinal plants to treat disease and maintain health?			
3	Do you make your own traditional concoctions?			
4	In your opinion, is traditional medicine effective and efficacious?			
5	Do you cultivate plants that can be used to treat disease?			

Data Analysis

Data analysis was also carried out on informant data such as age, gender, education and occupation. Furthermore, the collected medicinal plant data was calculated using the formula (Mulyani *et al.*, 2020).

$$\frac{\sum \text{certain plant organs}}{\sum \text{total plant}} \times 100\%$$

Percentage of sources of acquisition of medicinal plants using the formula:

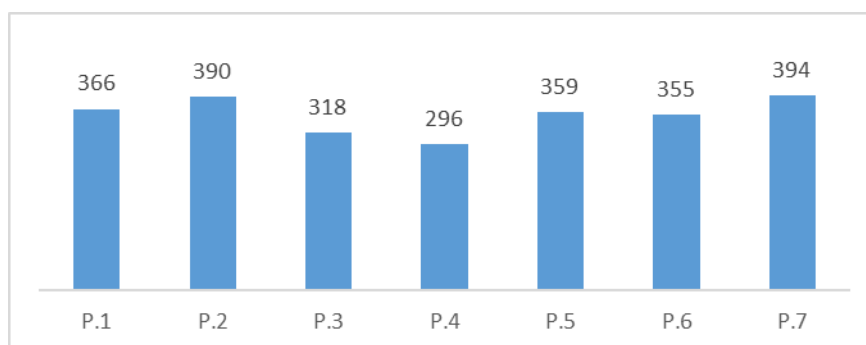
$$\frac{\sum \text{source of acquisition of the type obtained by the respondent}}{\sum \text{The total source of all processing mentioned by respondent}} \times 100\%$$

Percentage of how to use medicinal plants using the formula:

$$\frac{\sum \text{certain ways of use}}{\sum \text{total plant}} \times 100\%$$

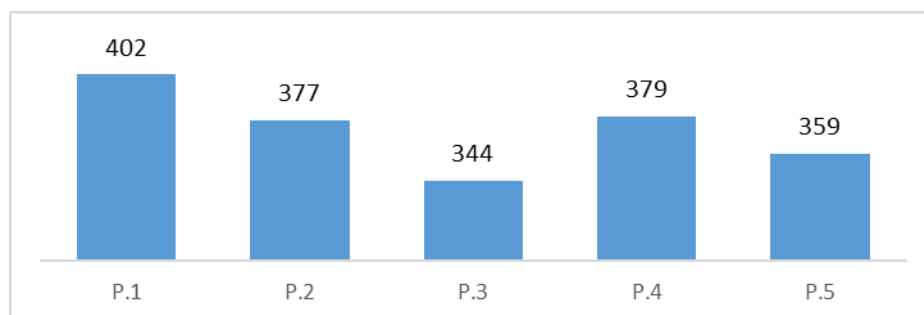
RESULTS

The level of knowledge and benefits of medicinal plants by the community
Diagram 5.1 Score scores on public knowledge.



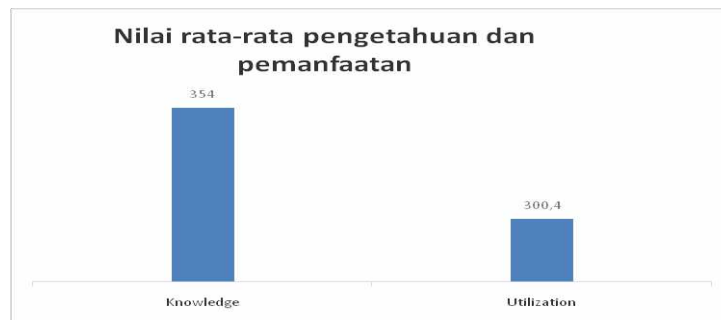
Based on diagram 5.1, the results shown were very good public knowledge of questions 1, 2, 3, 5, 6, 7. For question number 4, people's knowledge was in a good range. It can be interpreted that people know about medicinal plants in their environment.

Diagram 5.1 The scores of the questionnaire for the Use of Medicinal Plants.



Based on diagram 5.2, the results showed that people's knowledge of questions 1-5 was very good. Public knowledge was within good limits. In other words, people still know how medicinal plants are used for treatment.

Diagram 5.2 Average value of knowledge and utilization of medicinal plants.



Based on diagram 5.3, the results of the study indicate that the level of public knowledge about the use of medicinal plants is in the very good range. It can be interpreted that people know very well how a plant can be processed into materials that are beneficial to health, for example for traditional medicine. It is a note that empirically the use of plants in the community still exists and has not become extinct. It can still be developed and documented.

DISCUSSION

Based on the results of the study, the level of public knowledge about knowledge and utilization of medicinal plants is very good. It can be seen in diagram 5.1 that the score level of community knowledge about medicinal plants is very good, especially on questions 1,2,3,5,6,7 which show very good results. For question 4, the range is good. Based on diagram 5.2, the score level for the use of medicinal plants in questions 1-5 is very good so that the knowledge of community utilization is in a good range. It can be seen in diagram 5.3 that the average value of knowledge and utilization of medicinal plants is in the very good range, with a knowledge value of 354 and utilization of 300.4. It can be seen that the community is very aware of how a plant can be processed into ingredients that are beneficial for health and for traditional medicine. It is a note that empirically the use of plants in the community still exists and has not become extinct. It is still being developed and documented.

CONCLUSION

From the results of data analysis, the value of knowledge and use of medicinal plants by respondents in the two villages of Pahlawansetia and Samudrajaya is very good. The score for knowledge of medicinal plants is 354 and the utilization of medicinal plants is 300.4.

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EVALUATION OF THE SENSITIVITY RESPONSE OF *Staphylococcus aureus* ATCC 6538 TO ANTIBIOTICS INHIBITING THE SYNTHESIS OF BACTERIAL CELL WALLS

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Abstract

Introduction: The cause of nosocomial infections is bacteria. One of the bacteria that causes nosocomial infections is *Staphylococcus aureus*. The use of antibiotics with the right dose is the main choice in treating bacterial infections. But in practice antibiotics are often used inappropriately, causing bacterial resistance. Resistance is a condition where antibiotics fail to kill bacteria. To reduce this problem, it is necessary to evaluate the sensitivity to antibiotics. The purpose of this study was to determine the sensitivity response of *Staphylococcus aureus* ATCC 6538 to antibiotics in the class of inhibitors of bacterial cell wall synthesis with concentrations of 15, 20, 25, and 30 g.

Methods: This research is a laboratory experimental study using the Kirby Bauer method with Ampicillin as a sample of antibiotics. The data analysis used in this research is descriptive which shows the size of the inhibition zone diameter and turbidity in the MIC method.

Results: The results of this study showed that the antibiotic Ampicillin with a concentration of 30 g was able to inhibit the growth of *S. aureus* with an average diameter of the inhibition zone of 29 mm or included in the sensitive category.

Conclusion: The conclusion of this study is that the antibiotic Ampicillin is still effective in inhibiting the growth of *S. aureus*. With a sensitive category at a concentration of 30 g

Keywords: Antibiotics, Nosocomial infections, Kirby Bauer, Resistance, *Staphylococcus aureus*.

INTRODUCTION

According to Sihombing (2020) Nosocomial infections are diseases that are generally acquired from hospitals. This disease can trigger an increase in morbidity and mortality worldwide. Khan *et al.* (2017) states that the percentage of nosocomial infections in developed countries is 7% while developing countries are 10%. According to research results Sihombing (2020) states that the percentage of hospitalized patients is at risk of nosocomial infection by 20%.

Research result Klevens *et al.* (2007) reported that every year as many as 1.7 million patients who are undergoing treatment in hospitals are exposed to nosocomial infections. WHO (2005) in Sardi (2021) added that as many as 5% - 15% of patients in developed countries who were hospitalized had nosocomial infections. Abubakar (2017) stated that the most dominant nosocomial infection was bacteria. Erlin *et al.* (2020) stated that one of the bacteria that causes nosocomial infections is *Staphylococcus aureus*. According to Kuslovic (2020) in his research explained that the bacteria *S. aureus* is a type of bacteria with a pathogen.

Artati *et al.* (2016) stated that the use of antibiotics with the right dose is still the main choice in treating nosocomial infectious diseases caused by *S. aureus*. Roni *et al.* (2019) added, although the use of antibiotics needs to pay attention to the right procedure, in practice antibiotics are often used inappropriately, causing bacterial resistance with varying impacts. Kuswandi (2019) explained that bacteria that are resistant to antibiotics can increase the impact of mortality on the risk of infectious diseases. Meanwhile, according to WHO in Tjay *et al.* (2015), Every year antibiotic resistance in European Union countries reaches 25,000 deaths. Given the problems and impacts caused by bacterial resistance to antibiotics, it is necessary to do research on *surveillance*

returned to evaluate the resistance of nosocomial bacteria, such as *S. aureus*, to antibiotics. The importance of conducting research on surveillance of bacterial resistance to antibiotics is reported in the research results Iliya *et al* (2020) stated in his research that the isolates taken from patients at the Klambu District health facility were tested for sensitivity using the disc diffusion method using several antibiotics and got the results that the highest sensitivity was obtained from chloramphenicol antibiotics and the lowest was penicillin.

METHOD

Research design

This study was conducted using an experimental design, with the aim of testing the sensitivity of the antibiotic cell wall synthesis inhibitor class to the growth of *S. aureus* bacteria.

Population and Sample

The samples in this study were pure cultures of *S. aureus* ATCC 6538 collection of the Parasitology Laboratory of the University of Indonesia and cell wall inhibitor antibiotics, namely amoxicillin, co amoxiclav, penicillin, ampicillin, and cefadroxil.

Research Tools and Materials

The tools that will be used in this research are measuring cup (Pyrex, USA), erlenmeyer (Pyrex, USA), beaker glass (Pyrex, USA), test tube (Pyrex, USA), test tube rack, measuring pipette (Pyrex, USA), 50-100µl micropipette (Socorex, Switzerland), yellow tip, analytical balance (Acuplus, China), laminar air flow (Esco, Singapore), autoclave (Hirayama, HG-80, 76L, Japan), incubator (Memmerth IN- 30, Germany), Bunsen, petri dish (Pyrex, USA), vortex mixer VM 300 (Gemmy, Germany), ose needle, paper disk, sterile cotton swab, tweezers, label paper, spray bottle, tube rack, cotton stopper, wrapping, aluminum. The ingredients to be used are pure isolate of *S. aureus*, antibiotics amoxicillin (Bernofarm), co amoxiclav (Indofarma), penicillin (Phapros), ampicillin (Errita Pharma), and cefadroxil (Dexamedica) with doses of 45, 50, 55, 60µg, Mueller Hinton Agar (MHA), Nutrien Agar (NA), Nutrien Broth (NB), NaCl 0.9%, sterile Aquadest, and Mc Farland 0.5.

Tool Sterilization

All tools that will be used in research with microorganisms must be sterilized before use. The tools and media will be sterilized by the wet heat method, namely autoclaving at 121 °C for 15 minutes. Before sterilization, tools and materials must be wrapped first. Tools made of iron that are not waterproof (wet) such as ose needles and tweezers are then sterilized using the fixation method or incandescent with a Bunsen flame. (Hadijah, 2021).

Production of Mueller Hinton Agar (MHA) Media

Weigh 38 grams of MHA media then put it into an Erlenmeyer and mix it with 1 liter of distilled water then heated on a hot plate until dissolved and sterilized using an autoclave for 15 minutes at a temperature of 121°C. The MHA media was put into a petri dish using a pipette as much as 25 ml of a petri dish (Utomo *et al.*, 2018).

Preparation of Nutrient Agar (NA) Media

Weigh 1.68 grams of NA medium then put it into the omogenyr and dissolve it in 60 ml of distilled water. Homogenize using a hotplate magnetic stirrer. The media was sterilized using an autoclave at 121°C for 15 minutes, then poured 15 ml into each sterile petri dish and at room temperature for ± 30 minutes (Puspasari *et al.*, 2020).

Preparation of 0.5 . Mac Farland Solution

Mac Farland's solution is a turbidity comparison solution which is equivalent to 1.5x10⁸ CFU/ml bacteria (Amanda *et al.*, 2019). Mac Farland 0.5 was made using 2 ingredients, namely 1% Barium Chloride and 1% Sulfuric Acid by inserting it into a test tube of 0.5 ml of 1% Barium Chloride then adding 9.95 ml of 1% Sulfuric Acid. Mix until homogeneous, then cover with cotton (Apriani dan Fathir, 2021).

Bacterial Rejuvenation

Bacterial rejuvenation was carried out using 5 ml of NA media which had been compacted in a test tube in the form of an inclined media with a slope of 30°. *S. aureus* bacterial colonies were taken as much as one ose and then streaked on NA media and incubated in an incubator at 37°C for 24 hours. (Lestari *et al.*, 2020).

Bacterial Suspension Manufacturing

Bacteria that have been rejuvenated are taken with a sterile ose needle, suspended in 5 ml of 0.9% NaCl in a test tube until it reaches the same turbidity as the standard Mc Farland turbidity of 0.5 (Base *et al.*, 2019).

Determination of Test Solution

1) Preparation of Antibiotic Solution Concentration 15 g / 50 l.

Antibiotics as much as 165 mg dissolved in 50 ml of distilled water (Solution A). Take 1 ml of solution A, add aquadest to 10 ml. Drop on a paper disk as much as 50 l.

2) Preparation of Antibiotic Solution Concentration 20 g / 50 l.

Antibiotics as much as 220 mg dissolved in 50 ml of distilled water (Solution A). Take 1 ml of solution A, add aquadest to 10 ml. Drop on a paper disk as much as 50 l.

3) Preparation of Antibiotic Solution Concentration 25 g / 50 l.

Antibiotics as much as 275 mg dissolved in 50 ml of aquadest (Solution A). Take 1 ml of solution A, add with aquadest to 10 ml. Drop on a paper disk as much as 50 l.

4) Preparation of Antibiotic Solution Concentration 30 g / 50 l.

330 mg of antibiotic dissolved in 50 ml of aquadest (Solution A). Take 1 ml of solution A, add aquadest to 10 ml. Drop on a paper disk as much as 50 l.

Testing the Inhibitory Power of Antibiotics Using the Kirby Bauer Method

The bacterial suspension that has been made is scratched onto the solidified MHA media using the 4 quadrant scratch plate method. Blank disk measuring 6 mm was dripped with 50 l of antibiotic solution at each concentration of 15, 20, 25, and 30 g. Paper disks containing antibiotics were placed in a petri dish containing MHA media and bacterial culture using tweezers, then incubated at 37°C for 24 hours. Then the diameter of the inhibition zone around the paper disk was measured using a caliper or ruler (Hadijah, 2021).

RESULTS

The sensitivity response test of *Staphylococcus aureus* bacteria to antibiotics amoxicillin, co amoxiclav, penicillin, ampicillin, and cefadroxil by disc diffusion method using concentrations of 15, 20, 25, 30 g, and negative control with three replications, shows the results as shown in table 5.1.

Table 1. Result of Measurement of Inhibitory Zone Diameter for *Staphylococcus aureus* growth after being given antibiotics

Average Inhibition Zone Diameter (mm)	
Treatment	Amp
15 µg	26,7
20 µg	27,2
25 µg	28,2
30 µg	29
Category	Sensitive

description : Ampisilin (Amp)

Based on the data above, it can be seen that the diameter of the inhibitory zone after being given antibiotics with various concentration levels showed a sensitive category to the antibiotic Ampicillin with a concentration of 30 g, the average diameter of the inhibition zone was 29 mm.

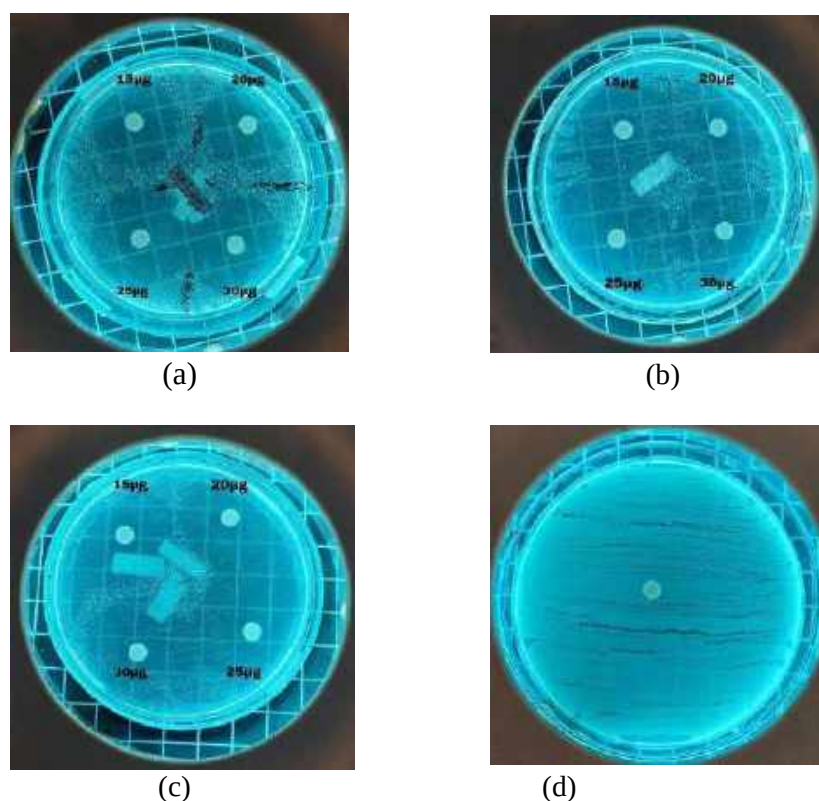


Figure 1. Replication 1 (a), Replication 2 (b), Replication 3 (c) and Negative control (d)

DISCUSSION

This study aims to determine the sensitivity response of *S. aureus* bacteria to antibiotics in the class of inhibitors of bacterial cell wall synthesis. The antibiotic used in this study was Ampicillin. According to Fatimah *et al.* (2022) Ampicillin is an antibiotic that is very effective in inhibiting the growth of *Staphylococcus aureus* and other gram-positive bacteria. This is reinforced by the statement Jamilatun (2019) in his research showed that the sensitivity test of several antibiotics against several isolates of *S. aureus* bacteria gave results, namely that among all isolates were sensitive to Ampicillin with a concentration of 10 g. This indicates that with a small concentration of Ampicillin still able to inhibit the growth of *S. aureus*. This statement is supported by Fatimah *et al.* (2022) that in his research, the results of the antibiotic Ampicillin had better sensitivity and were still more effective in inhibiting *S. aureus* bacteria.

After experimenting with the antibiotic Ampicillin in table 1, it is known that the results obtained from the measurement of the diameter of the inhibitory zone of the antibiotic Ampicillin at a concentration of 15 g obtained an average inhibition zone of 26.7 mm, a concentration of 20 g was 27.2 mm, a concentration of 25 g was equal to 28.2 mm, and the concentration of 30 g was 29 mm. From the measurement data that has been obtained, it is known that the antibiotic Ampicillin on *S. aureus* bacteria all showed sensitive results using graded concentrations.

The interpretation of the results of the category of bacterial growth response to the antibiotic Ampicillin in this study, especially for the disc diffusion method, was based on the formation of a clear zone or inhibition zone around the paper disc compared to *National Community for Clinical Laboratory Standard* (NCCLS) in Fatimah *et al.* (2022) which are grouped into three categories, namely *Staphylococcus aureus* bacteria are said to be resistant if the clear zone formed is <15 mm, intermediate is 16-17 mm, and sensitive is 18 mm.

The results of the sensitivity test to the antibiotic Ampicillin showed that *S. aureus* was sensitive to the

antibiotic ampicillin. According to Sumampouw (2018) that ampicillin is very effective against *S. aureus* because both antibiotics belong to the semisynthetic beta-lactam chemical class which is effective in inhibiting gram-positive by inhibiting cell wall synthesis and murein production.

Several studies on antibiotic sensitivity tests gave different results. Based on this, it proves that the pattern of bacterial sensitivity differs from one area to another. Different habitats or sources of *S. aureus* bacteria allow different sensitivity characters to each antibiotic. This is in accordance with and reinforced by Bhavya's (2014) statement which says that bacterial resistance to antibiotics varies widely in an area because antibiotics have different sensitivities depending on the environment and the bacterial isolates themselves.

Staphylococcus aureus is one of the gram positive bacteria. The cell wall of gram-positive bacteria is composed of very thick peptidoglycan to maintain the integrity of the cell. If there is damage to the cell wall during its formation, bacterial cell lysis will occur so that the bacteria lose the ability to form colonies and will die. Inhibiting the formation of cell walls can occur due to the administration of antibiotics (Ajizah *et al.*, 2007). In research Kurniawan *et al.* (2019) added that positive bacteria have cells that are pretty simple stuff so that's what makes it easier for antibiotics to get into cells and find targets to work on.

Antibiotics of the class of inhibitors of bacterial cell wall synthesis have a mechanism of action by preventing cross-linking of peptidoglycan during the final stage of cell wall synthesis, by inhibiting bound proteins. When the antibiotic experiences resistance, what happens is that the bacteria produce beta-lactamase enzymes in gram-negative or gram-positive bacteria which then secrete the beta-lactamase enzyme to exit the cell in large enough quantities so that the drug that wants to penetrate the cell wall becomes inactive (Sagita *et al.*, 2020).

In this study, the method used is disc diffusion. This method is done by applying a bacterial suspension that has previously been made. The bacterial suspension was made by dissolving the bacteria into NaCl 0.9 and then equalizing the turbidity with Mac Farland 0.5. The process of balancing the bacterial suspension with Mac Farland 0.5 with the aim of estimating the number of bacteria in the liquid suspension by comparing the turbidity. Mac Farland standard 0.5 is the most commonly used standard in clinical microbiology laboratories with an estimated bacterial count of 1.5×10^8 CFU/ml (Aviany dan Pujiyanto, 2020).

CONCLUSION

Sensitivity testing using the Kirby Bauer method on the antibiotic Ampicillin, with concentrations of 15 g, 20 g, 25 g, and 30 g showed a sensitive category. Ampicillin antibiotics obtained the largest average diameter results, namely at concentrations of 15 g, 20 g, 25 g, and 30 g, the average inhibition zones were 26.7 mm, 27.2 mm, 28.2 mm, and 29 mm. Based on the results obtained, it can be concluded that the antibiotic Ampicillin is still effective in inhibiting the growth of *Staphylococcus aureus* and the use of higher concentrations will result in a larger diameter of the inhibition zone.

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EVALUATION OF USE OF ANTIHYPERTENSION DRUG CLASS CALCIUM CHANNEL BLOCKER (CCB) IN OUTPATIENT HYPERTENSION PATIENTS IN A PRIVATE HOSPITAL IN WEST BEKASI IN 2020

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Abstract

Introduction: Hypertension is a disease with a high prevalence in Indonesia. Antihypertensive drugs widely used in Indonesia are Calcium Channel Blocker (CCB). This study aims to evaluate antihypertensive therapy for the CCB class of outpatient hypertension patients at a West Bekasi private hospital in 2020.

Method: This study uses a descriptive observational method with a retrospective approach. Data were obtained from medical records of outpatient hypertension patients with a total of 100 patients with inclusion criteria being patients with a diagnosis of hypertension, without comorbidities, men and women aged 45-59 years, receiving drugs Calcium Channel Blocker class.

Results: The most widely used antihypertensive drug was the single antihypertensive drug, Amlodipine (31%). The most commonly used combination of CCB class antihypertensive drugs with Angiotensin Receptor Blockers (ARBs). The results of the evaluation of the use of CCB class antihypertensive drugs in one of the West Bekasi private hospitals in 2020 showed the results, namely the right indication (100%), the right drug (91%), and the right dose (99%).

Conclusion: The use of the drug under study was following the provisions of rational drug use.

Keywords: Calcium Channel Blocker (CCB), Evaluation of Drug Use, Hypertension.

INTRODUCTION

Hypertension is a condition of a person with a systolic blood pressure of ≤ 140 mmHg and diastolic blood pressure of ≤ 90 mmHg, which is obtained after repeated examinations a maximum of three times with a distance of one minute. If the initial blood pressure is $< 130/85$ mmHg, there is no need to repeat it (Unger et al., 2020). The prevalence of hypertension has increased by 8.31% from 2013 to 2018 in Indonesia (Kemenkes RI, 2019). In West Java, the prevalence of hypertension increased by 10.2% from 2013 to 2018 (Dinkes Jabar, 2019). In Bekasi City, the highest hypertension cases were primary hypertension cases in outpatients with an age range of 45 years to >75 , as many as 10,489 (7.97%) (Dinkes Kota Bekasi, 2014). The results of previous research by Musnelina and Khotimah in 2017 at the Depok City Hospital showed that the types of antihypertensive therapy used were single and combination therapy. Combination treatment was used more with a percentage of 56.9% compared to single therapy of 43.08% (Musnelina dan Khotimah, 2017).

Based on the data in previous studies, the researcher wishes to examine the evaluation of the use of antihypertensive drugs class Calcium Channel Blocker (CCB) because it is known that the most widely used therapy is class drugs Calcium Channel Blocker dihydropyridine such as amlodipine and nifedipine as pharmacological therapy (Musnelina dan Khotimah, 2017). In this study, three indicators were used, namely the right indication, the right drug, and the right dose. These three points need to be evaluated because the prevalence of hypertension is still high in Indonesia, and the treatment given to patients is still not rational on the exact value of the drug and the right frequency. Evaluation of the accuracy of the

indication, the right drug, and the right dose is expected to help reduce the prevalence of hypertension in Indonesia.

METHOD

This study uses a descriptive observational method using a retrospective approach. The observational study design is where the researcher does not treat the variables and only observes the situation. Descriptive research aims to obtain an overview of a condition that occurs in a specific population (Masturoh dan Anggita, 2018). The study was conducted at a West Bekasi private hospital from February to March 2022. The study's participants were all outpatient hypertension patients at a West Bekasi private hospital from January to December 2020. The number of samples in this study was 100 patients. The sample taken is a population that meets the inclusion and exclusion criteria. In this study, patients met the inclusion criteria: if patients with a diagnosis of hypertension, without comorbidities, men and women aged 45-59 years, and receiving class drugs Calcium Channel Blocker. And patients cannot be included in the study if conditions are obtained, such as: in the patient data, the diagnosis is unclear, the dosage is not precise, or the data are difficult to read. Sampling by technique non-probability consecutive. On consecutive technique sampling, all subjects who came sequentially and met the sample selection criteria were included in the study until the sample size was met. Drop out for clinical research is 10% (Sastroasmoro dan Ismael, 2011). Calculation of the number of samples based on the Slovin's formula is as follows:

Slovin's formula:

$$n = \frac{N}{1 + N (e)^2}$$

Information:

n = sample unit (number of respondents needed)

N = population (sum of population size of hypertensive patients in one of the West Bekasi Private Hospitals year 2020)

e = error value used

The total number of samples included was 100 patients.

Data collection is done by looking at the data on hypertension patients in the medical records from January - December 2020 then, the medical record number is recorded. Medical record data with numbers that have been recorded, collected and selected based on inclusion criteria. Furthermore, the data from the patient's medical record is copied into the data accumulation sheet in the form of patient identity (initial name, age, gender), date of the hospital visit, patient's complaints and diagnoses, disease history, and treatment history, blood pressure data, treatment profile, recapitulation data then data analysis. Processing is done by looking at the use of CCB drugs contained in patient data and then described by calculating the percentage using the formula for all data according to the criteria divided by the total number of case data then multiplied by 100%. Then the data is obtained in the percentage of drug use class Calcium Channel Blocker, then the calculated data is displayed in the form of a table containing the percentage. The rationale for the use of antihypertensive drugs was evaluated using the criteria of the Indonesian Ministry of Health in 2011, namely the right indication, the right drug, and the right dose then adjusted according to the hypertension classification table according to the International Society of Hypertension (Unger et al., 2020), table of Oral Antihypertensive Drugs in the 2021 Hypertension Management Consensus electronic book: Updates PERHI Consensus 2019 (Lukito et al., 2021) and Algorithm Hypertension Therapy in JNC 8: Evidence-based Guidelines Management of Adult Hypertensive Patients (Muhadi, 2016). The criteria used will be adjusted to the conditions in the medical record. The results will be presented in a table containing the percentage of the rationality of the drug.

RESULTS

Patient Characteristics Data

According to the gender characteristics of hypertension patients, there were more women, 58 patients (58%), while 42 patients (42%) were male, which can be seen in table 1. According to the age characteristics of hypertension patients, there were 25-49 years old (25%), 50-54 years old with the highest number (38%), and 55-59 years old (37%), which can be seen in table 2.

Table 1. Characteristics by Gender

Gender	Amount	Percent (%)
Woman	58	58
Men	42	42
Total	100	100

Table 2. Characteristics by Age

Age (years)	Amount	Percent (%)
45 – 49	25	25
50 – 54	38	38
55 – 59	37	37
Total	100	100

Profile of the Use of CCB Antihypertensive Drugs

The pattern of using antihypertensive drugs in a private hospital in West Bekasi, shown in table 3 that single and combination CCB drugs are used. The single CCB drug used is Amlodipine. The most widely used combination of two antihypertensive drugs is CCB and ARB. Single drugs are more widely used than combination drugs.

Table 3. Patterns of Use of CCB Antihypertensive Drugs in a West Bekasi Private Hospital Year 2020

Pattern Giving	Drug Class	Medicine name	Amount	Percent (%)
Single (55%)	CCB	Amlodipine 5mg	31	31
	CCB	Amlodipine 10mg	24	24
Combination (45%)	CCB + ARB	Amlodipine 5mg + Valsartan 80mg	4	4
	CCB + ARB	Amlodipine 5mg + Valsartan 160mg	8	8
	CCB + ARB	Amlodipine 10mg + Valsartan 160mg	1	1
	CCB + ARB	Amlodipine 5mg + Candesartan 8mg	5	5
	CCB + ARB	Amlodipine 5mg + Candesartan 16mg	3	3
	CCB + ARB	Amlodipine 10mg + Candesartan 8mg	1	1
	CCB + ARB	Amlodipine 10mg + Candesartan 16mg	9	9
	CCB + ARB	Amlodipine 5mg + Telmisartan 80mg	2	2
	CCB + Beta Blockers	Amlodipine 5mg + Bisoprolol 5mg	6	6
	CCB + Beta Blockers	Amlodipine 10mg + Bisoprolol 5mg	1	1
	CCB + Thiazide	Amlodipine 10mg + HCT 25mg	1	1
	CCB + ACEI	Amlodipine 5mg + Perindopril arginine 5mg	2	2
	CCB + ARB + Beta Blockers	Amlodipine 5mg + Telmisartan 80mg + Bisoprolol 5mg	1	1
	CCB + ARB + Beta Blockers	Amlodipine 5mg + Candesartan 16mg + Bisoprolol 5mg	1	1
Total			100	100

Evaluation of the Use of CCB Antihypertensive Drugs

Evaluation of the appropriateness of the use of CCB antihypertensives in this study was based on the International Society of Hypertension Global Hypertension Practice Guidelines year (Unger et al., 2020), JNC 8: Evidence-based Guidelines Handling of Adult Hypertensive Patients (Muhadi, 2016), Consensus on Management of Hypertension 2021: Update PERHI Consensus 2019 (Lukito et al., 2021). Evaluation of this accuracy includes the right indication, the right drug, and the right dose. The results obtained are the value of the right indication of 100%, the right drug of 91%, and the right dose of 99% which can be seen in table 4.

Table 4. Evaluation of the Use of CCB Antihypertensive Drugs in a Private Hospital West Bekasi 2020

Criteria	Appropriate		Not appropriate		Total	
	Amount	Percent (%)	Amount	Percent (%)	Amount	Percent (%)
Right indication	100	100	-	-	100	100
Right drug	91	91	9	9	100	100
Right dose	99	99	1	1	100	100

DISCUSSION

Patient Characteristics Data

Patient data obtained by gender and age were used to determine the distribution of outpatient essential hypertension patients at a West Bekasi Private Hospital in January – December 2020. Based on data from table 1, which discusses the distribution of essential hypertension patients by type gender, the results show that women are more affected by hypertension when compared to men. Judging from the percentage that women get 58% and men 42%. The results of this study are in line with research conducted by Falah in 2019, that there is a significant relationship between gender and the incidence of hypertension. Women have a 0.4 times higher chance of experiencing hypertension than men (Falah, 2019). This can be strengthened by several research results, such as Azhari's 2017 study which states that women have a 2.7 times chance of developing hypertension (Azhari, 2017). Wahyuni and Eksanoto's research in 2013 said that women would experience an increased risk of hypertension after menopause over 45 years because of a decrease in estrogen which serves to increase blood levels (Wahyuni dan Eksanoto, 2013). This can be strengthened by several research results, such as Azhari's 2017 study which states that women have a 2.7 times chance of developing hypertension (Azhari, 2017). Wahyuni and Eksanoto's research in 2013 stated that women will experience an increased risk of hypertension after menopause over 45 years because of a decrease in estrogen which serves to increase blood levels. High-Density Lipoprotein (HDL) plays a role in maintaining blood vessels. HDL levels in postmenopausal women will decrease due to reduced estrogen hormone if followed by a bad lifestyle. The impact that will be caused when HDL is low and the amount of Low-Density Lipoprotein (LDL) is high, namely the occurrence of atherosclerosis so that blood pressure will be high (Wahyuni dan Eksanoto, 2013). Atherosclerosis is a common condition that develops when a sticky substance called plaque builds up in the arteries. Atherosclerosis develops slowly. This can be caused by cholesterol, fat, blood cells, and other substances in the blood-forming plaque. When plaque builds up, it causes the arteries to narrow. This reduces the supply of oxygen-rich blood to the tissues of vital organs in the body (NHLBI, 2022).

The distribution of hypertensive patients based on age in table 2 can be seen that there are fewer hypertensive patients in the 45 - 49 year age range (25%) when compared to the age range 50 – 54 years (38%) and 55 – 59 years (37%). The results of this study are in line with the results of research from Kristiyowati in 2020, namely the age range of most hypertension patients, namely the age of < 50 years as many as 156 patients from 224 patients (69.64%) (Kristiyowati, 2020). The results of Nuraeni's research (2019) also support this study, it is said that individuals aged ≥ 45 years are 8.4 times more at risk of suffering from hypertension when compared to individuals aged ≤ 45 years (Nuraeni, 2019). After the age

of 45 years, the arterial walls will thicken due to the accumulation of collagen in the lining of the blood vessels. There will be a narrowing of the blood vessels, and the blood vessels will become stiff so that the systolic blood pressure will increase (Kristiyowati, 2020).

Profile of the Use of CCB Antihypertensive Drugs

Hypertensive patients can be given non-pharmacological therapy and pharmacological therapy. Non-pharmacological therapies that can be given include limiting salt consumption, changing meal times, reducing weight, regular exercise, not smoking, and reducing alcohol consumption (Kemenkes RI, 2013). This study did not analyze non-pharmacological therapy because the data were not included in the medical records and patients who came for outpatient treatment were for control or follow-up treatment. Pharmacological therapy given is single oral antihypertensive therapy, a combination of two drugs, or a combination of three drugs. The medications used by outpatients at one of the West Bekasi Private Hospitals are CCB, ARB, Thiazid, ACEI, and antihypertensive drugs. Beta-Blockers single or in combination. Data on the Pattern of Use of CCB Antihypertensive Drugs in a private hospital in West Bekasi in 2020 can be seen in table 3.

The use of antihypertensive drugs prescribed by doctors in table 3 shows that single therapy is more widely used than combination antihypertensive drugs. The single antihypertensive drug used was Amlodipine at a dose of 5 mg or 10 mg. Amlodipine has a broad scope for treating hypertension in terms of blood pressure. CCB antihypertensive drugs also play a role in preventing heart failure. When compared to Beta Blockers, CCB class of drugs showed greater effectiveness in slowing the development of plaque buildup in the neck blood vessels that carry blood to the brain (carotid atherosclerosis) and thickening of the left side of the heart muscle (LV hypertrophy) (Mancia et al., 2013).

In addition to the single drug used in one of the West Bekasi Private Hospitals, combination therapy is also used. The combination therapy used is two-drug combination therapy and three-drug combination therapy. The most widely used combination of CCB drugs with ARBs, namely Amlodipine with valsartan, Candesartan, or Telmisartan. These results are in line with the algorithm that is used as a reference, namely from the 2021 hypertension management consensus: PERHI Consensus Update that the combination of two antihypertensive drugs that are often used is ACEI or ARB with CCB or diuretic (Lukito et al., 2021). ESH also said that CCB and ARB are an effective drug combination to use. In addition, the results of several studies show that the use of two drugs from two classes of antihypertensive drugs can increase blood pressure reduction when compared to only one drug (Mancia et al., 2013). CCB drugs, either singly or in combination, are included in the first-line hypertension drugs for the general population or patients who do not have CKD co-morbidities (Muhadi, 2016). CCB antihypertensive drugs block calcium from entering the arteries, allowing coronary and peripheral arteries to widen (Kemenkes RI, 2013). Amlodipine does not reduce the intrinsic ability of the heart muscle to generate force and contract and does not worsen heart failure. These drugs have a longer duration of activity and can take once a day (BPOM RI, 2020). ARBs work by blocking the binding of angiotensin II to the receptor. This drug has the effect of widening blood vessels to facilitate the work of the heart (Kemenkes RI, 2013). Because the impact of these two drugs is the same, it can be said that if these two drugs are combined, an additive effect will occur so that the blood pressure decrease can occur more quickly. But there must be control over the use of this drug combination because the side effects caused if not controlled can cause hypotension which is dangerous for the body. The combination of CCB and ARB drugs most widely used by the research sample was Amlodipine 10mg + Candesartan 16mg with a percentage of 9%.

The following combination of two drugs combines CCB class antihypertensive drugs with Beta-Blockers. According to the drug therapy algorithm for hypertension that is used as a reference, the combination of Beta-Blockers with diuretics or other classes of drugs is recommended if there are specific indications such as angina, post-AMI, heart failure, and heart rate (Lukito et al., 2021). The combination of these two drugs should not be used if the patient does not have the specific indications mentioned above. Work mechanism Beta Blockers is to decrease heart rate and pumping power. Beta-Blockers can reduce mortality and morbidity in elderly patients, can reduce the risk of coronary heart disease, and can prevent

the recurrence of myocardial infarction and heart failure (Kemenkes RI, 2013). Class antihypertensive drugs Beta-Blockers are not included as an effective drug when combined with CCB class drugs because of the particular indications included for Beta Blockers (Lukito et al., 2021). The most widely used drug combination by the research sample was Amlodipine 5mg + Bisoprolol 5mg with a percentage of 6%.

In addition, the combination of the two drugs used was a CCB class antihypertensive drug with a thiazide one patient only used. The thiazide class of antihypertensive medication is an effective combination when combined (Mancia et al., 2013). Diuretics work to remove body fluids through urine so that they can reduce the number of body fluids and the burden on the heart. If the patient has hypokalemia, additional potassium or spironolactone supplements may be given. Other diuretics that can be used such as triamterene or hydrochlorothiazide (Kemenkes RI, 2013). CCB and thiazide class of antihypertensive drugs are recommendations in the first line of hypertension treatment. Combining these two drugs can help lower blood pressure in hypertensive patients. The drug used in this study was a combination of Amlodipine 10mg + HCT 25mg with a percentage of 1%.

The following two-drug combinations are CCB antihypertensive drugs with ACEI with a percentage of 2%. ACEI class antihypertensive drugs are included in drugs that are effective in combination with CCB drugs (Mancia et al., 2013). ACEI group drugs work by inhibiting ACE activity, inhibiting angiotensin I to angiotensin II at the receptor. This drug has the effect of widening blood vessels to facilitate the work of the heart. These ACEI class drugs can cause coughing, so if you want to replace them, they can be replaced with ARB class drugs because they have a similar mechanism of action (Kemenkes RI, 2013). The combination of CCB + ACEI antihypertensive drugs used by the patients in this study was Amlodipine 5mg + Perindopril Arginine 5mg.

The drug combination is safe and tolerable. If the patient has hypertension that cannot be controlled by a combination of two drugs, a third drug can be added. However, combining these three drugs is not recommended for the first-line treatment of hypertension (Adrian dan Tommy, 2019). A combination of three drugs is recommended if blood pressure is not controlled with a combination of two drugs, namely RAS Blocker (ACEIs or ARBs), CCBs, and diuretics. Combining medications with antihypertensive class Beta Blockers must have particular indications such as angina, post-AMI, heart failure, and heart rate control (Lukito et al., 2021). In this study, three drugs were combined from the antihypertensive drug class CCB + ARB + Beta Blockers. The ARB class drugs used were Telmisartan 80mg and Candesartan 16mg. ARBs work by blocking the binding of angiotensin II to the receptor. This drug widens blood vessels to facilitate the working heart (Kemenkes RI, 2013). The drug class Beta Blockers used is bisoprolol 5 mg. The mechanism of action of the drug Beta-Blockers is to decrease heart rate and pumping power. Drug Beta Blockers can reduce mortality and morbidity in elderly patients, can reduce the risk of coronary heart disease, and can prevent the recurrence of myocardial infarction and heart failure (Kemenkes RI, 2013).

Evaluation of the Use of CCB Antihypertensive Drugs

Rational use of drugs must be ensured to ensure that patients receive drugs as needed in the long term at affordable prices. The use of drugs can be called rational if they meet several parameters of the accuracy of the drugs that have been determined (KemenKes RI, 2011).

Right Indication

The results of the evaluation of the accuracy of antihypertensives in this study can be seen in table 4. Appropriate indications for the use of drugs are if the medications prescribed are by the diagnosis of hypertension based on blood pressure measurements. The use of antihypertensive drugs can be said to be the right indication seen from the administration of antihypertensive drugs based on medical reasons and needed by the patient (Lisni et al., 2020). Based on the analysis of all samples in the study, the indication accuracy value was 100%. This value was obtained because the administration of antihypertensive drugs was by the diagnosis given by the doctor, namely essential hypertension. Prescribing medications in hospitals follows the availability of medicines at the hospital. Therefore, prescribing drugs can be different from prescribing drugs in hospitals or other pharmacy installations. The results of this study are in line with

the results of Lisni's research (2020), namely the value of the accuracy of drug indications is 100% from 119 electronic prescription data conducted at one of the Bandung City Health Centers (Lisni et al., 2020).

Right Drug

The results of the evaluation of the accuracy of the drug in this study can be seen in table 4. The right drug in hypertension therapy is the suitability of drug selection from several types of antihypertensive drugs. Then, the medication is adjusted to the diagnosis and therapy guidelines (Lisni et al., 2020). The first line of treatment in patients aged <60 years who are not black with a target of reducing blood pressure to systolic blood pressure <140 mmHg and diastolic blood pressure <90 mmHg, namely thiazide-type diuretics or ACEI or ARB or CCB in single or combination drugs (Muhadi, 2016).

The results of this study showed the exact value of the drug was 91%. Evaluation of drug accuracy was carried out in this study by comparing the therapy given to patients with antihypertensive treatment on the algorithm from JNC 8. 91 patients had received treatment according to the first-line drug algorithm according to JNC 8, namely patients received a single antihypertensive drug, antihypertensive drug two-drug combinations, and three-drug combinations. Antihypertensive drugs other than the CCB group are ARBs, Thiazides, ACEIs, and Beta Blockers. Nine patients did not meet the exact criteria for the drug because the patient who received a combination of class antihypertensive drugs, Beta Blockers (bisoprolol). Class antihypertensive drugs Beta-Blockers are used in patients with special indications such as angina, post-AMI, heart failure, and heart rate control (Lukito et al., 2021). According to Sholicha (2015), Beta Blockers are effective in treating angina, either used alone or in combination with CCB antihypertensive drugs (Sholicha, 2015). Dezs's research (2017) also says that Beta-Blockers are recommended for the first-line treatment of heart failure, coronary artery disease, and heart rhythm disorders, as well as hypertension with complications such as heart failure, angina pectoris, or a history of infarct disease (Dézsi dan Szentes, 2017). The sample in this study had a diagnosis of uncomplicated hypertension, so the antihypertensive drug class Beta Blockers are unsuitable for use.

The results of this study are in line with the research of Ekaningtyas, Wiyono, and Mpila (2021), namely the value of drug accuracy is 87.22% of the 133 total samples. In this study, the inaccuracy of 12.78% of the drug was due to the administration of monotherapy for patients with complications of Diabetes Mellitus and chronic kidney failure which should be given combination therapy (Ekaningtyas et al., 2021). Correspondingly, research is on administering drugs to patients with and without complications that have not been appropriate. Patients without complications were given combination therapy, while patients with complications were given monotherapy.

Right Dosage

The results of the evaluation of the accuracy of antihypertensive doses in the study can be seen in table 4. The right dose is suitable for administering antihypertensive drug doses with the therapeutic dose range seen from the use of doses per day and the patient's condition (Kristiyowati, 2020). The results of this study showed that patients who met the criteria for the right dose were 99 patients with a percentage of 99%, and patients who did not meet the requirements for the right dose were 1%. Patients who do not meet the right dose criteria are patients with medical record number 106xxxx. The guidelines used to evaluate the dose accuracy in the study were the Oral Antihypertensive Drugs Guidelines in the Consensus for the Management of Hypertension (Lukito et al., 2021).

The dose of Amlodipine based on the Oral Antihypertensive Drugs guidelines in the Consensus for the Management of Hypertension is 2.5 - 10 mg taken once a day (Lukito et al., 2021), while patients with medical record number 106xxxx received Amlodipine at a dose of 10 mg twice a day, one tablet so that the amount was declared incorrect because the amount given was too much. Administration of drugs that are too fast or too long causes unstable steady-state work, causing the drug effect to be less effective (Pandiangnan et al., 2017). Excessive doses can increase the risk of side effects and the emergence of toxic impacts so that the right drug is given to achieve therapeutic success in patients (Andriani dan Yulianti, 2012). According to Nugraheni (2021), using Amlodipine can cause side effects such as edema, nausea,

vomiting, abdominal pain, dry mouth, and constipation. The most specific side effect is edema due to using high doses of Amlodipine. Peripheral edema is caused by fluid accumulation in the blood due to hemodilution that occurs with the long-term use of high doses of Amlodipine (Nugraheni dan Hidayat, 2021).

The results of this study are in line with the results of Lisni's (2020) research, namely the dose accuracy value of 99.16% of the total 119 samples. The dose inaccuracy was 0.84% because in one patient the dose was lower than the minimum dose (Lisni et al., 2020). Inappropriate dosing is still found, although in a low percentage.

CONCLUSION

Based on research on patient characteristics, most hypertensive patients were female (58%) and aged 50-54 (38%). Based on the results of the study showed that the most widely used drug was the single antihypertensive drug, Amlodipine (31%). The most commonly used combination of CCB class antihypertensive drugs with ARB, namely Amlodipine 10 mg + Candesartan 16 mg (9%). The evaluation of the accuracy of antihypertensives showed that the right indication was 100%, the right drug was 91%, and the right dose was 99%.

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FORMULATION AND EVALUATION OF NANOEMULSION OF BASIL LEAF EXTRACT (*OCIMUM BASILICUM* L.) WITH TWEEN 80 CONCENTRATION VARIATIONS

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Abstract

Introduction: A nanoemulsion is a transparent emulsion system consisting of a mixture of oil and water and surfactant molecules to stabilize it. The nanoemulsion particle size ranged from 10-200 nm. The content of alkaloids, flavonoids, tannins, saponins and eugenol in basil leaves makes them useful as antibacterials. The purpose of this study was to evaluate the physical stability of the basil leaf extract nanoemulsion formulation using the surfactant tween 80 with concentrations of 36%, 37% and 38%.

Method: This study used an experimental design to determine the physical stability of nanoemulsion preparations by making three different concentrations of tween 80 (36%, 37% and 38%) and evaluating organoleptic tests, pH, viscosity, transmittance, particle determination (particle size and polydispersity index) and stability (centrifugation and freeze thawing).

Results: The results of the research on nanoemulsion preparations of basil leaf extract F1, F2 and F3 had average particle sizes of 14,43 nm, 14,73 nm and 15 nm with polydispersity indexes of 0,38, 0,42 and 0,37. The three formulas are brown, clear, have a distinctive smell of basil leaves, and have a transmittance value of above 90%. The results of the centrifugation test on all formulas did not occur in phase separation, and in the freeze thawing test, the results obtained did not change the color, odor, or phase separation. The preparation remained stable during the storage period of 14 days.

Conclusion: Basil leaf extract nanoemulsion with a concentration of 36% in F1 is the best nanoemulsion with clear characteristics of light brown color, characteristic odor of basil leaves, has a pH value of 6.24, viscosity 1333,33-1416,67 cP, transmittance 96,7%, particle size of 14,43 nm and a polydispersity index of 0,38. After centrifugation and freeze-thaw tests, it doesn't change color, smell, or separate into phases.

Keywords: Basil leaves, Nanoemulsion, Tween 80

INTRODUCTION

Emulsions generally degrade as energy levels increase and with time. Reducing the particle size is one of the methods used to solve the problem of emulsions. Nanoemulsions can be used to reduce size (Daud et al., 2017). A transparent emulsion system, consisting of a mixture of oil and water and surfactant molecules to stabilize it (Adi et al., 2019). The particle size of the nanoemulsion ranges from 10–200 nm (Ayuningtias et al., 2017). Nanoemulsions can be used to deliver drugs to the skin. Large surface area, low surface tension, and increased penetration can be achieved by utilizing the interfacial tension of the o/w type emulsion (Nurpermatasari and Ernoviya, 2020). Creaming, flocculation, coalescence and precipitation can be prevented by the presence of nanoemulsions, so that nanoemulsions are more stable than ordinary emulsions (Hanifah and Jufri, 2018). Nanoemulsion technology in Indonesia is currently being developed because it can increase skin permeability for drug penetration (Ayunin, 2017). Oil, water, surfactants and cosurfactants, which are the basic ingredients of nanoemulsions, can be formulated using other ingredients such as plants to enhance the effect of nanoemulsions. Basil is one of the plants that can be used as a mixture of nanoemulsion formulations containing secondary metabolites such as tannins, flavonoids, essential oils and saponins (Utami et al., 2021). Azkawati (2016) explained that basil contains alkaloids, tannins, flavonoids, saponins and eugenol compounds that can be used as antibacterials. Basil (*Ocimum basilicum* L.) leaves have the potential to have antibacterial effects on *Salmonella*, *Escherichia coli*, *Campylobacter jejuni* and *Clostridium aerogenes* (Rubab et al., 2017). Extracts from basil leaves have biological activity that can be used as a deodorant, antidepressant, antipyretic, antidiabetic,

antihyperglycemic, as well as antibacterial (Wahid et al., 2020). According to Kindangen et al. (2018), basil (*Ocimum basilicum* L.) leaves have antibacterial properties. The antibacterial results of the ethanolic extract of basil leaves contained in the anti-acne gel had an inhibition zone diameter of 9,7 mm, 14,4 mm and 19,1 mm, which had the ability to inhibit *Staphylococcus aureus* bacteria at concentrations of 0.5%, 1%, and 1,5%. Arianto and Cindy (2019) explained in their research that variations of tween 80 and sorbitol obtained stability during the nanoemulsion experiment within 12 weeks of storage at room temperature, low and high. Kristiani et al. (2019) conducted research on *Ocimum basilicum* L. nanoemulsion with essential oil where at a concentration of 2,5% *Salmonella typhi* bacteria could be inhibited and obtained nanoemulsion stability with surfactant, namely tween 80 and co-surfactant PEG 400. However, research on nanoemulsion with basil leaf extract (*Ocimum basilicum* L.) using various concentrations of surfactant tween 80 and 36%, 37% and 38% has never been done. Sorbitol was used in this study as a cosurfactant. Based on this, the researchers wanted to formulate and test the stability of the nanoemulsion extract of *Ocimum basilicum* L. by making three variations of the concentration of tween 80 (36%, 37% and 38%), which aim to determine the physical stability of the formulation in the presence of different concentrations of tween 80.

METHOD

A. Research Design

The design of this research is experimental and uses the descriptive analysis method.

B. Research Location and Time

This research was conducted at the Pharmaceutical Technology Laboratory of STIKes Mitra Keluarga in February–March 2022.

C. Population and Sample

The population and sample in this study were basil leaf extract (*Ocimum basilicum* L.) which were obtained from a supplier Herbal Palapa Muda Perkasa located in Kalimulya, Depok City.

D. Procedure

1. Plant Determination

This examination aims to determine whether the basil used is a species of *Ocimum basilicum* (L.). The basil plant samples were determined at the Herbarium Bogoriense, Botanical Division of the BRIN Cibinong Biological Research Center.

2. Extraction By Maceration Method

The maceration method was used to extract 1.500 grams of simplicia powder at room temperature. Maceration with 5 liters of 96 percent ethanol was completed in 3x24 hours. The extract was filtered through filter paper every 24 hours until the filtrate was obtained (Yamlean, 2017). At about 40°C, the filtrate was evaporated with a rotary evaporator to separate the solvent from the solute and get a thick basil leaf extract (Ahmadita, 2017; Ariani et al., 2020).

3. Basil Leaf Extract Phytochemical Screening

a. Alkaloid test

50 mg of the extract was dissolved with a few mL of HCL and then filtered. To the filtrate, 1-2 drops of Mayer, Wagner and Dragendorff reagents were added in different test tubes. The Mayer reagent will be characterized by the presence of a white or yellowish precipitate in the tube. The Wagner reagent will be marked by the presence of a brown or blackish red precipitate in the tube. The Dragendorff reagent will be marked by the presence of an orange precipitate (Kumalasari and Andiarna, 2020).

b. Flavonoid Test

A total of 40 mg of extract was added with 100 mL of hot water. Then boil for 5 minutes, then filtered. The filtrate was measured as much as 5 mL and then 0,05 mg of Mg powder and 1 mL of concentrated HCL were added. If it gives a brown or yellow color after vigorous

shaking, it indicates flavonoid positive (Wowor et al., 2022).

c. Tannin Test

A total of 0,1 grams of extract added 10 mL of distilled water. Then filtered, the filtrate added with reagent iron (III) chloride (FeCl_3) 1% 5 mL. If it causes a dark blue or green-black color, the identification results show a positive tannin (Kumalasari and Andiarna, 2020).

d. Phenolic Test

A total of 2 mL of the sample was added 3-4 drops of FeCl_3 . If a green, red, purple, blue or solid black color is formed, the identification indicates a positive phenol (Wowor et al., 2022).

4. Formulation of Basil Leaf Extract Nanoemulsion

The nanoemulsion was made using an aqueous phase consisting of propyl paraben and methyl paraben dissolved in distilled water, and then the aqueous phase was manually stirred with a stirring rod. The solution was then heated on a hot plate, cooled and filtered (Mixture a). Furthermore, the basil leaf extract was dissolved with some sorbitol and some other sorbitol was used to dissolve sunflower oil. The extract that had been dissolved with sorbitol was put into a glass beaker containing sunflower oil that had been dissolved in sorbitol (Mixture b). Put the mixture into a glass beaker containing tween 80, then stir using a mixer. Put mixture b into a glass beaker containing tween 80 and mixture a, then stir using a mixer at 450 rpm for 15 minutes. After thoroughly mixing, the preparation is placed in a sonicator for 30 minutes (Arianto and Cindy, 2019). Table 1 shows the formula for basil leaf extract nanoemulsion.

Table 1. Nanoemulsion Preparation Formula (Arianto and Cindy, 2019)

Material Composition (%)	Formula			Function
	I	II	III	
Basil Leaf Extract	0,5	0,5	0,5	Antibacterial
Sunflower Oil	5	5	5	Oil Phase
Tween 80	36	37	38	Surfactant
Sorbitol	22	22	22	Cosurfactant
Methyl Paraben	0,1	0,1	0,1	Preservative
Propyl Paraben	0,02	0,02	0,02	Preservative

5. Characteristics and Evaluation of Nanoemulsion Preparations

a. Organoleptic Test

Visual observations included color, odor and phase separation of nanoemulsion preparations at room temperature (25°C) for 14 days of storage (Arianto and Cindy, 2019).

b. pH Test

Measurement of the pH of the preparation was carried out using a pH meter. The pH of the nanoemulsion preparation must be in accordance with the pH of the skin, which is 4,5–6,5. A number of nanoemulsions are inserted into the pH meter. Observations were made at room temperature (25°C) for 14 days of storage (Arianto and Cindy, 2019).

c. Viscosity Test

Viscosity testing using a Brookfield LV-801 viscometer at room temperature (25°C) for 14 days. A total of 100 mL of the nanoemulsion preparation was measured, then put on spindle number 4. Place the preparation under the spindle that has been installed. The spindle is lowered to the limit of the spindle submerged in the preparation. The speed of the viscometer is set at the correct rpm. The measurement results will be shown on the screen of the Brookfield viscometer (Zulfa et al., 2019). The viscosity value of a good semisolid preparation is between 500 and 5000 cP (Kusuma *et al.*, 2017).

d. Transmittance Test

The transmittance percentage was measured at 650 nm with a UV-Vis spectrophotometer. It was carried out at the beginning of the observation after the preparation was made by

dissolving 100 mL of the basil leaf extract nanoemulsion in 5 mL of distilled water (Huda and Wahyuningsih, 2018). The nanoemulsion preparation looks clear and transparent, as shown by the fact that 90–100% of light can pass through it (Indalifiany et al., 2021).

e. Nanoemulsion Particle Determination Test

The determination of particles from nanoemulsion preparations was measured by a particle size analyzer (Horiba Scientific, Nanoparticle Analyzer SZ-100), which includes particle size and polydispersity index. Testing was conducted at the beginning of the observation after the preparation was made by means of 100 mL of nanoemulsion sample dissolved in 50 mL of distilled water (Maharini et al., 2018). The nanoemulsion particle size ranges from 10–200 nm (Ayuningtias et al., 2017). The polydispersity index value 0,5, including monodispersion, indicates uniform droplet size (Adi et al., 2019).

f. Physical Stability Test

1) Centrifugation Test

The nanoemulsion preparation was put into Eppendorf as much as 1,5 mL, then centrifuged for 30 minutes at a speed of 3000–4000 rpm for 30 minutes at the beginning of the observation after the preparation was made (Winarti et al., 2016). The centrifugation test makes sure that the nanoemulsion preparation is stable and that the phases don't separate (Arianto and Cindy, 2019).

2) Freeze Thawing Test

This test was carried out by storing the nanoemulsion preparation for 24 hours at a cold temperature ($4^{\circ}\pm 2^{\circ}\text{C}$), then moving the preparation to room temperature ($25^{\circ}\pm 2^{\circ}\text{C}$) for 24 hours (1 cycle). A total of 6 cycles were run for testing. The freeze thawing requirement is stable during storage; there is no change in color, odor or phase separation (Rismarika et al., 2020).

RESULTS

A. The Result Of Plant Determination

The determination test was carried out on March 4, 2022 at the Herbarium Bogoriense, Botany Division of the BRIN Cibinong Biology Research Center. The results of the determination test obtained are correct if the basil plant has the Latin name *Ocimum basilicum* (L.) with the *Lamiaceae* tribe.

B. Basil Leaf Extraction Results

The results of the basil leaf extract are blackish brown, have a distinctive aroma of basil leaves and have a thick texture.

C. Basil Leaf Phytochemical Screening Results

The results of phytochemical screening of basil leaf extract were positive for containing alkaloids with Mayer and Wagner reagents, flavonoids, tannins and phenolics. Meanwhile, with Dragendorff's reagent the results obtained were negative for alkaloids.

D. Results Characteristics and Evaluation of Nanoemulsion Preparations

1. Results of Organoleptic Observations of Nanoemulsion

Tables 2, 3 and 4 show the organoleptic results of the basil leaf extract nanoemulsion having different colors in the three formulas. All formulas have a distinctive odor of basil leaves and no separation occurs.

Table 2. Tween 80 Formulation I Organoleptic Test Results 36%

Replication	Days to-	Color	Odor	Phase Separation
Rep 1	0	Clear, Light brown	Typical basil leaves	Not separating
	7	Clear, Light brown	Typical basil leaves	Not separating
	14	Clear, Light brown	Typical basil leaves	Not separating
Rep 2	0	Clear, Light brown	Typical basil leaves	Not separating
	7	Clear, Light brown	Typical basil leaves	Not separating
	14	Clear, Light brown	Typical basil leaves	Not separating
Rep 3	0	Clear, Light brown	Typical basil leaves	Not separating
	7	Clear, Light brown	Typical basil leaves	Not separating
	14	Clear, Light brown	Typical basil leaves	Not separating

Table 3. Tween 80 Formulation II Organoleptic Test Results 37%

Replication	Days to-	Color	Odor	Phase Separation
Rep 1	0	Clear, slightly dark brown	Typical basil leaves	Not separating
	7	Clear, slightly dark brown	Typical basil leaves	Not separating
	14	Clear, slightly dark brown	Typical basil leaves	Not separating
Rep 2	0	Clear, slightly dark brown	Typical basil leaves	Not separating
	7	Clear, slightly dark brown	Typical basil leaves	Not separating
	14	Clear, slightly dark brown	Typical basil leaves	Not separating
Rep 3	0	Clear, slightly dark brown	Typical basil leaves	Not separating
	7	Clear, slightly dark brown	Typical basil leaves	Not separating
	14	Clear, slightly dark brown	Typical basil leaves	Not separating

Table 4. Tween 80 Formulation III Organoleptic Test Results 38%

Replication	Days to-	Color	Odor	Phase Separation
Rep 1	0	Clear, Dark brown	Typical basil leaves	Not separating
	7	Clear, Dark brown	Typical basil leaves	Not separating
	14	Clear, Dark brown	Typical basil leaves	Not separating
Rep 2	0	Clear, Dark brown	Typical basil leaves	Not separating
	7	Clear, Dark brown	Typical basil leaves	Not separating
	14	Clear, Dark brown	Typical basil leaves	Not separating
Rep 3	0	Clear, Dark brown	Typical basil leaves	Not separating
	7	Clear, Dark brown	Typical basil leaves	Not separating
	14	Clear, Dark brown	Typical basil leaves	Not separating

2. Nanoemulsion pH Test Results

Table 5 shows the results of the nanoemulsion pH test of the basil leaf extract, which was stable and met the skin pH range of 4,5–6,5.

Table 5. pH Test Results

Days to-	pH		
	F1	F2	F3
	Tween 80 36%	Tween 80 37%	Tween 80 38%
	Average $\pm$ SD	Average $\pm$ SD	Average $\pm$ SD
0	6,24 $\pm$ 0,02	6,36 $\pm$ 0,02	6,41 $\pm$ 0,02
7	6,24 $\pm$ 0,02	6,36 $\pm$ 0,02	6,41 $\pm$ 0,02
14	6,24 $\pm$ 0,02	6,36 $\pm$ 0,02	6,41 $\pm$ 0,02

3. Nanoemulsion Viscosity Test Results

Table 6 shows the results of the nanoemulsion test results of stable basil leaf extract and meets the viscosity requirement range of 500-5000 cP.

Table 6. Viscosity Test Results

Days to-	Viscosity (cP)		
	F1	F2	F3
	Tween 80 36%	Tween 80 37%	Tween 80 38%
	Average $\pm$ SD	Average $\pm$ SD	Average $\pm$ SD
0	1416,67 $\pm$ 520,42	2833,33 $\pm$ 144,34	3000 $\pm$ 250
7	1336,33 $\pm$ 386,79	2166,67 $\pm$ 144,34	2166,67 $\pm$ 144,34
14	1333,33 $\pm$ 381,88	1916,67 $\pm$ 144,34	2166,67 $\pm$ 144,34

4. Nanoemulsion Transmittance Test Result

Table 7 shows the transmittance test results of the basil leaf extract nanoemulsion that met the transmittance requirement range of 90-100%.

Table 7. Transmittance Test Results

Transmittance (%)		
F1	F2	F3
Tween 80 36%	Tween 80 37%	Tween 80 38%
Average $\pm$ SD	Average $\pm$ SD	Average $\pm$ SD
96,7 $\pm$ 0,005	97,9 $\pm$ 0,003	91,7 $\pm$ 0,02

5. Nanoemulsion Particle Determination Test Result

Table 8 shows the particle size test results for basil leaf extract nanoemulsions that meet the requirements for a particle size of 10–100 nm.

Table 8. Particle Size Test Results

Particle Size (nm)		
F1	F2	F3
Tween 80 36%	Tween 80 37%	Tween 80 38%
Average $\pm$ SD	Average $\pm$ SD	Average $\pm$ SD
14,43 $\pm$ 0,21	14,73 $\pm$ 0,06	15 $\pm$ 0,12

Table 9 shows the results of the nanoemulsion polydispersity index test of basil leaf extract meeting the range of requirements for the polydispersity index <0.5.

Table 9. Polidispersity Index Test Results

Polydispersity Index		
F1	F2	F3
Tween 80 36%	Tween 80 37%	Tween 80 38%
Average $\pm$ SD	Average $\pm$ SD	Average $\pm$ SD
0,38 $\pm$ 0,04	0,42 $\pm$ 0,06	0,37 $\pm$ 0,01

6. Physical Stability Test Results of Basil Leaf Nanoemulsion

The stability of basil leaf nanoemulsion was tested using centrifugation and freeze-thawing. The results of the centrifugation test of basil leaf extract nanoemulsion using surfactant tween 80 with concentrations of 36%, 37 and 38% with 3 replications met the requirements for centrifugation, namely no separation. Then the results of the freeze thawing test using surfactant tween 80 with concentrations of 36%, 37% and 38% with 3 replications for 6 cycles met the freeze thawing requirements, namely no change in color, odor and phase separation.

DISCUSSION

The manufacture of basil leaf extract is done using maceration method, using 96% ethanol as solvent. The extract obtained was blackish brown in color, had a distinctive aroma of basil leaves and had a thick texture. The results obtained in accordance with the research of Risnayanti and Dalimunthe (2022) are dark brown-black in color, have a distinctive aroma of basil leaves and have a thick extract texture. The extraction of 1.500 g of dried simplicia of basil leaves resulted in a thick extract of 105,4 g of basil leaves with an extract yield of 7,03%. The yield obtained is in accordance with the requirements of the Indonesian Herbal Pharmacopoeia (2017), which is not less than 5,6%.

The results of the phytochemical test of alkaloids with Mayer's reagent on samples of basil leaf extract (*Ocimum basilicum* L.) were positive for alkaloids, these results were in accordance with previous research conducted by Warsi and Sholichah (2017) when the sample was dripped with Mayer reagent, a white precipitate was formed. In the alkaloid test using Wagner's reagent on a sample of basil leaf extract (*Ocimum basilicum* L.) the results obtained were positive for containing alkaloids, these results were in accordance with previous research conducted by Kumalasari and Andiarna (2020) which formed a blackish red precipitate. Testing of alkaloids with Dragendorff's reagent on samples of basil leaf extract (*Ocimum basilicum* L.) showed negative results, namely the formation of an orange precipitate, where the results were the same as the research conducted by Harianja et al. (2020) negative result no orange precipitate formed. Identification of flavonoids in samples of basil leaf extract (*Ocimum basilicum* L.) positive for flavonoids, these results are in accordance with previous studies conducted by Harianja et al. (2020) formed an orange-red and brown solution. Identification of tannins in samples of basil leaf extract (*Ocimum basilicum* L.) positive for tannins, these results are in accordance with previous research conducted by Kumalasari and Andiarna (2020) which formed a blackish green color. The results of the phenol phytochemical test on the basil leaf extract sample (*Ocimum basilicum* L.) were positive for containing phenol, these results were in accordance with previous research conducted by Harianja et al. (2020) a greenish black solution was formed.

There are three formula modifications in this study, namely at a concentration of tween 80, consisting of F1 (tween 80, 36%), F2 (tween 80, 37%) and F3 (tween 80, 38%). Each formula was carried out three replications to see the physical stability of the nanoemulsion preparation. There are several test evaluations to see the physical stability of nanoemulsion preparations modified with tween 80 concentration, namely organoleptic tests, pH, viscosity, transmittance, particle determination and stability (centrifugation and freeze thawing tests). In this study, changing the amount of Tween 80 didn't change how stable the nanoemulsion was physically. Because of this, these three formulas were part of a stable and good formulation because they met the test evaluation requirements during storage.

In this study, organoleptically, there were slight differences in color observations, namely formula 1 tween 80 36% was clear light brown, formula 2 tween 80 37% was clear, slightly dark brown and formula 3 tween 80 38% was clear dark brown. The use of basil leaf extract and variations of tween 80 as a surfactant have an effect on the color of the nanoemulsion preparations, where the higher the tween 80 used, the darker the color of the resulting preparation. Meanwhile, the observation of odor and phase

separation gave the same results, which had a characteristic odor of basil leaves and no separation occurred.

The pH value of the preparation was determined by using the pH test on the three formulas. Because these topical preparations will be applied to the skin, it is important to consider how safe these preparations are when used (Zulfa, 2020). The pH of the topical preparation is between 4,5-6,5 according to the pH range of the skin. Skin irritation can occur if the pH of the preparation is too acidic and scaly skin can occur if the pH is too alkaline caused by damage to the acid mantle in the stratum corneum (Rahmaniyah, 2018). Based on the test results, the pH value of the nanoemulsion preparations ranged from 6,24-6,41 so that the three formulas were included in the safe skin pH range, which was between 4,5-6,5 for topical use. The pH measurement results show that the higher the concentration of tween 80 used, the higher the pH of the nanoemulsion preparation obtained, namely F1 (tween 80 36%) an average of 6,24, F2 (tween 80 37%) an average of 6,36 and F3 (tween 80 38%) an average of 6,41. This is possible because tween 80 has a pH of 6-8, so the higher the concentration of tween 80, the higher the pH value of the preparation (Handayani et al., 2018). After 14 days, the pH of the three formulas did not change. This shows that the preparation was stable.

Viscosity testing on nanoemulsion preparations was carried out to determine the viscosity. The release of the active ingredient from the preparation is influenced by the viscosity of the preparation (Hidayati, 2020). The viscosity value of semisolid preparations for topical use ranges from 500-5000 cP (Kusuma et al., 2017). The viscosity test on the basil leaf extract nanoemulsion was measured 3 times for 14 days, namely days 0, 7 and 14. On day 0 of the three formulas, the viscosity values ranged from 1416,67 - 3000 cP. On the 7th day of the three formulas the viscosity values ranged from 1336,33 – 2166,67 cP. On the 14th day of the three formulas, the viscosity values ranged from 1336,33 – 2166,67 cP. The three formulas fall into the required range between 500-5000 cP. The results of the viscosity measurement showed that the higher the concentration of tween 80 used, the higher the viscosity of the nanoemulsion preparation. This increase in viscosity is due to the effect of surfactant concentration in the preparation, which induces flocculation between the dispersed particles, increasing the viscosity of the nanoemulsion preparation (Hakim et al., 2018). The viscosity values of the three formulas showed a decrease during storage, but remained within the required range. Improper temperature conditions and storage procedures can cause a decrease in viscosity. This means that the surface tension of the preparation has gone down, which could make the preparation less stable (Mardikasari et al., 2016).

The clarity of the nanoemulsion formulations was assessed using the transmittance test. The percentage of transmittance to ensure the purity of the nanoemulsion preparations as a result of visual observation using distilled water as a comparison. The transmittance value shows the comparison between the light intensity that appears after interacting with the test substance and the light intensity that appears before engaging the test substance (Daud et al., 2017). The preparation has a clear and transparent appearance, as evidenced by the transmittance percentage of 90-100% (Indalifiany et al., 2021). The results of the measurement of the transmittance of the nanoemulsion preparations obtained are F1 (tween 80 36%) an average of 96,7%, F2 (tween 80 37%) an average of 97,9% and F3 (tween 80 38%) an average of 91,7%. The three formulas have transmittance values that are not much different and fall into the required range between 90-100%. The formation of nanoemulsions is indicated by a clear preparation, therefore the higher the clarity value, the better the nanoemulsion preparation (Daud et al., 2017).

The particle determination test was carried out at the beginning of the observation after the preparation was made, namely day 0 of the three formulas. The results of the nanoemulsion preparation particle size obtained are F1 (tween 80 36%) an average of 14,43 nm, F2 (tween 80 37%) an average of 14,73 nm and F3 (tween 80 38%) an average of 15 nm. The particle sizes of the three formulas were in accordance with the requirements ranging from 10-200 nm (Ayuningtias et al., 2017). The smaller the particle size the better for drug absorption and release (Winarti et al., 2016). The measurement results showed that the formulations of formulas 1, 2 and 3 had different sizes but were not significant. This is due to the difficulty of uniforming the two different systems, as well as other parameters such as duration and speed of stirring (Nurpermatasari and Ernoviya, 2020). The particle size of the nanoemulsion is also affected by the surfactant concentration. Surfactants act as emulsifiers, reducing the amount of free energy required to make nanoemulsions by lowering the interfacial tension in the oil-in-water system. The surfactants produce a film layer on the droplet surface in an o/w nanoemulsion system consisting of a

nonionic surfactant. Droplets will not enter the dispersion medium because of the film layer (Maharini et al., 2018). The nanoemulsion preparation has an average particle size of about 5-200 nm, so the nanoemulsion preparation has high kinetic stability (Mardikasari et al., 2016). Nanoemulsion preparations with a particle size of less than 90 nm have been shown to be more stable against sedimentation (Pratiwi et al., 2018).

The polydispersity index is a measurement of the molecular mass distribution of the sample (Handalis, 2018). The polydispersity index value shows information about the physical stability of the dispersion system and the uniformity of droplet size (Zulfa, 2020). All formulas that have a polydispersity index value <0,5 indicate that the resulting globules vary. The range of polydispersity index values that are well accepted is 0 (monodisperse particles) to 0,5 (large size distribution) (Adi et al., 2019). The low polyspersity index value indicates that the resulting dispersion system is more stable over time (Maharini et al., 2018). The results of the polyspersity index of nanoemulsion preparations obtained are F1 (tween 80 36%) an average of 0,38, F2 (tween 80 37%) an average of 0,42 and F3 (tween 80 38%) an average of 0,37. The polyspersity index value of the three formulas yielded a value of less than 0,5 which indicates that the level of particle size uniformity and will be stable in long-term storage (Maharini et al., 2018).

To determine the mechanical stability of the preparation, a centrifugation test was carried out. The centrifugation test on the basil leaf extract nanoemulsion was carried out at the beginning of the observation after the preparation was made, namely day 0 for each formula. Centrifugation testing can describe the stability of nanoemulsion preparations under the influence of the earth's gravity for a period of one year (Hakim et al., 2018). The purpose of the centrifugation test is to see whether the nanoemulsion preparation is stable by observing the phase separation after centrifugation. This test is needed to determine the impact of product transportation shocks on the physical appearance of a product (Daud et al., 2017). The results obtained from this test, the three formulas remained stable and there was no phase separation. Indicates that the preparation is stable for one year against the force of gravity.

A freeze thawing test was used to see whether the nanoemulsion preparation changes after storage and temperature differences. The results obtained in this test after 6 cycles were consistent. There was no difference between before and after the test. The three formulas remained stable and did not change organoleptically in terms of color, odor and phase separation. This is due to the presence of tween 80 in the preparation, which helps to keep the nanoemulsion preparation stable (Aulia, 2017).

CONCLUSION

From the research that has been done, it can be concluded:

1. Basil leaf extract (*Ocimum basilicum* L.) can be formulated into nanoemulsion preparations with variations in tween 80 concentrations of 36%, 37% and 38%. The three formulas are included in the requirements for nanoemulsion preparations, namely organoleptic, pH, viscosity, transmittance, particle determination and stability. And has good physical stability during the storage period of 14 days.
2. Basil leaf extract nanoemulsion (*Ocimum basilicum* L.) with a concentration of 36% in F1 is the best nanoemulsion with clear characteristics of light brown color, characteristic odor of basil leaves, has a pH value of 6,24, viscosity 1333,33 – 1416,67 cP, transmittance 96,7%, particle size of 14,43 nm and polydispersity index of 0,38. It remains stable without changing color, odor or phase separation after centrifugation and freeze thawing tests.

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MATOA LEAF EXTRACT PHYTOCHEMICAL SCREENING TEST (*Pometia pinnata* J.R.Forst. & G.Forst.) FROM BEKASI AT DIFFERENT MACERATION TEMPERATURES

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Abstract

Introduction: Matoa plant (*Pometia pinnata* J.R.Forst. & G.Forst.) is a plant that can grow in the tropics. The matoa plant is thought to contain a lot of secondary metabolites which are used as traditional medicine. One of the properties of matoa leaves is to have pharmacological effects such as antibacterial, diuretic and analgesic.

Method: This study used a descriptive experimental design to determine the results of macroscopic examination, the percentage of thick extract and the content of secondary metabolites of matoa leaves with variations in room temperature (25-30°C), 40°C and 50°C.

Results: The results of macroscopic examination of matoa leaves are green, oval leaf shape, shiny and smooth leaf surface. The yield of 70% ethanol extract of Matoa leaves with variations in maceration temperature obtained 29,6% extract at room temperature (25-30°C), 26,9% at 40°C and 36,4% at 50°C. Phytochemical screening results on 70% ethanol extract showed that Matoa leaves contain metabolites of alkaloids, flavonoids, triterpenoids, tannins and saponins.

Conclusion: The content of secondary metabolites of matoa leaf extract are alkaloids, flavonoids, triterpenoids, tannins, and saponins and the results of the thick extract of Matoa leaves with 70% ethanol solvent, the results of thick extract of matoa leaves with 70% ethanol solvent, the highest yield percentage in the extraction treatment at a temperature of 50°C resulted in an extract yield of 36,4%, while in the treatment extraction at room temperature (25- 30°C) of 29,6% and at 40°C of 26,9%.

Keywords: Matoa leaf, Macroscopic, Extract Yield, Phytochemical Screening.

INTRODUCTION

Knowledge of medicinal plants and their development is sourced from forests and yards for the cultivation of medicinal plants and to support environmental sustainability. Since ancient times, plants have been used as traditional medicine. The cost of drugs cannot be reached by everyone, so plants are one of the alternative ways of treatment for the community (Dewatisari *et al.*, 2018). Along with the advancement of science and technology today, plants need to be scientifically proven in treating various diseases. For this reason, it is necessary to conduct research to analyze the content of compounds in plants so that they can be used as treatment. Phytochemical screening is a way to analyze bioactive compounds contained in plants (Harahap and Nurbaiti, 2021). Nababan (2020) explained that a bioactive compound can be extracted by extraction. The factors that influence the extraction are the size of the material, the ratio of the amount of the material to the solvent, the extraction temperature, the extraction time, the type of solvent and the stirring speed (Nugroho, 2017). Extraction is used to extract a component of the active substance present in the plant. One of the plants that can be extracted by the maceration method is the Matoa plant (Badaring *et al.*, 2020). According to Romainum (2019) the parts of the Matoa plant that can be used include the stems that can be used as a wood industry, Matoa leaves are used to treat high blood pressure and the seeds and fruit of matoa can be consumed as food (Maryam *et al.*, 2020). Previous research conducted by Maryam *et al.* (2020), samples of the ethanolic extract of Matoa leaves from the city of Jayapura obtained phytochemical screening results containing tannins, flavonoids, alkaloids, saponins and steroids. While the research of Martiningsih *et al.*, (2016), obtained the results of a phytochemical screening study of ethanol extract from Matoa leaves found in the Banyuasri area, Singaraja contains flavonoids and tannins. The research of Kuspradini *et al.* (2016) yielded an extract using n-hexane solvent of 0,99%, ethyl acetate of 2,88% and ethanol 96% of 12,26%. The differences between the previous research and the research to be studied are: where to grow the sample and the extraction solvent. In

previous studies, samples were taken from Jayapura, Banyuasri and South Kalimantan and the solvents used were 96% ethanol, ethyl acetate, and n-hexane, while this study used samples of Matoa leaves that grew in the Bekasi area and used 70% ethanol as solvent. Based on the description above, it is necessary to conduct research on "Phytochemical Screening Test of Matoa Leaf Extract (*Pometia pinnata* J.R.Forst. & G.Forst.) from Bekasi at Different Maceration Temperatures". This is because the extraction temperature can affect the yield of extracts and differences in the place where plants grow can also affect the results of phytochemical screening. Therefore, research on the phytochemical screening test of Matoa leaf extract at different maceration temperatures needs to be carried out.

METHOD

A. Research Design

The design of this study used a descriptive experimental design. The treatment carried out in this study was a variation of maceration temperatures include room temperature (25-30°C), 40°C and 50°C.

B. Research Location and Time

The research was conducted at the Chemical Laboratory of Stikes Mitra Keluarga Bekasi Timur in February - March 2022.

C. Population and Sample

The population and sample in this study were 4 kg of fresh Matoa leaves obtained on Jl. Bantar Gebang No.29 RT.009/RW.003 Cijengkol Village, Setu District, Bekasi City.

D. Research Variable

The variables observed in this study were the content of secondary metabolites of Matoa leaves growing in Bekasi and the percentage yield of the extract with different maceration temperatures.

E. Procedure

1. Identification (Determination) of samples

Determination of this sample was carried out at the Herbarium Bogoriense, Botany Division of the Biological Research Center, BRIN Cibinong.

2. Extraction Maceration

The maceration method was carried out by soaking the simplicia using 70% ethanol as a solvent. The matoa leaf powder was weighed as much as 100 grams and put into an erlenmeyer. Then it was soaked with 500 ml 70% ethanol solvent which was covered with aluminum foil. The extraction process was carried out by maceration method using room temperature (25-30°C), 40°C, 50°C. At 40°C and 50°C, the samples were placed in an incubator. Maceration was carried out for 3 x 24 hours and occasionally stirred using a stirring rod. The filtrate and residue were filtered, then remaceration using residual samples for 2 days with as much new solvent as the initial solvent. Furthermore, the filtrate and remaceration results are combined. The filtrate was concentrated with a rotary evaporator at a temperature of 40°C with a pressure of 175 and rpm 50 in order to obtain a thick extract of matoa leaves (Wijaya *et al.*, 2018).

RESULTS

A. Results Morphological Examination of Matoa Leaves

Based on Table 1, the results of the morphological examination of Matoa leaves have a length of 31,5-35,5 cm, width of 8-10,5 cm, green color, oval shape, tapered tip, blunt leaf base, flat leaf edge, shiny and smooth leaf surface and pinnate leaf veins.

Table 1. Morphological Examination of Matoa Leaves

Parameter	Observation result	Papuan Plant Series Book (Garuda & Syafruddin, 2014)
Long	31,5 – 35,5 cm	30 – 40 cm
Wide	8 – 10,5 cm	8 – 15 cm

Color	Green	Green
Leaf Shape	Jorong	Jorong
Leaf Tip	Tapered	Tapered
Leaf Base	Blunt	Blunt
Leaf Edge	Flat	Flat
Leaf Surface Texture	Shiny and Slick	Shiny and Slick
Leaf Bone	Pinning	Pinning

B. Result Organoleptic Test of Matoa Leaf Powder

Based on Table 2, the organoleptic results of matoa leaf powder were in the form of a fine powder, lightgreen in color, with a weak characteristic odor and a bitter taste.

Table 2. Organoleptic Test of Matoa Leaf Powder

Organoleptic Test	Observation result
Shape / Texture	Fine Powder
Color	Light Green
Smell / Aroma	Weak Characteristic Smell
Flavor	Bitter

C. Result of Organoleptic Test of Matoa Leaf Extract with Various Temperature Variations

Based on Table 3, the organoleptic results of matoa leaf extract were thick extract, had a weak characteristic odor, had a bitter taste and blackish brown in color at temperatures (25-30°C) and 40°C. While using a temperature of 50°C to get a blackish green extract.

Table 3. Organoleptic Test of Matoa Leaf Thick Extract

Temperature Variation	Observation result		
	Temperature (25-30°C)	Temperature 40°C	Temperature 50°C
Shape	Thick Extract	Thick Extract	Thick Extract
Color	Dark-Chocolate	Dark-Chocolate	Green-Black
Smell	Weak Characteristic Smell	Weak Characteristic Smell	Weak Characteristic Smell
Flavor	Bitter	Bitter	Bitter

D. Yield of Matoa Leaf Extract

Based on Table 4, the yield of matoa leaf extract with a temperature (25-30°C) of 29,6%, a temperature of 40°C of 26,9% and 50°C of 36,4%.

Table 4. Extract Yield

Temperature Variation	Powder Weight	Extract weight	Yield (%)	Indonesian Herbal Pharmacopoeia (FHI)
(25-30°C)	100 g	29,6 g	29,6 %	Not less than 7.2%
40°C	100 g	26,9 g	26,9%	Not less than 7.2%
50°C	100 g	36,4 g	36,4%	Not less than 7.2%

E. Matoa Leaf Extract Phytochemical Screening Results

Based on Table 5 the results of phytochemical screening of matoa leaf extract with three different maceration temperature treatments. Positive results were found to contain alkaloids, flavonoids, triterpenoids, tannins, and saponins.

Table 5. Phytochemical Screening

Skrining Fitokimia	Pereaksi	(25-30°C)	40°C	50°C	Keterangan
Alkaloid	Mayer	(+)	(+)	(+)	Terbentuk endapan putih/kuning
	Dragendroff	(+)	(+)	(+)	Terbentuk endapan jingga atau merah bata
	Wagner	(+)	(+)	(+)	Terbentuk endapan merah kecoklatan

Flavonoid	Serbuk Mg + HCl pekat	(+)	(+)	(+)	Terbentuk larutan berwarna jingga-merah
Triterpenoid	Eter + Asam asetat pekat + Asam sulfat pekat	(+)	(+)	(+)	Terbentuk cincin berwarna merah keunguan
Tanin	FeCl ₃ 1%	(+)	(+)	(+)	Terbentuk larutan berwarna hijau kehitaman
Saponin	Air panas + HCl 2N	(+)	(+)	(+)	Terbentuk buih yang stabil

DISCUSSION

Organoleptic is a test carried out by observing the smell, taste and color of the simplicia (Ginting, 2017). Organoleptic testing on Matoa leaf samples showed fine powder, light green color, characteristic odor and bitter taste. Meanwhile, the organoleptic test on Matoa leaf extract showed thick extract, blackish brown-green color, weak characteristic odor and bitter taste.

Yield is the ratio of the weight of the resulting extract to the weight of the initial simplicia powder (Wijaya *et al.*, 2018). Extraction of 100 grams of Matoa leaf simplicia resulted in the yield of thick Matoa leaf extract at room temperature (25-30°C) of 29,6%, at 40°C of 26,9% and at 50°C of 36,4%. The yield of the thick extract of Matoa leaves obtained was in accordance with the requirements of the Indonesian Herbal Pharmacopoeia (2017), which was not less than 7,2%. Senduk *et al.* (2020) reported that the higher the content of substances attracted to raw materials, the higher the extract yield value. According to Dewatisari *et al.* (2018) high yield value shows the number of bioactive components contained therein. Maceration using a temperature of 50°C gives the yield the largest but the yield at 40°C was lower than the 25-30°C. In this study the results are not in accordance with the theory. This could be due to a procedural error in weighing the extract.

Phytochemical screening is the initial stage of research that aims to provide an overview of the class of compounds contained in the plant under study. In this study, phytochemical screening was carried out on samples of Matoa leaves growing in the Setu area, Bekasi using 70% ethanol as solvent. Sampling in the Setu area, Bekasi was carried out because no previous research had been carried out in the area and wanted to know the secondary metabolite compounds contained in Matoa leaves located in the Setu area, Bekasi.

Testing of alkaloids on samples of Matoa leaf extract used three reagents, namely Mayer reagent, Dragendorff reagent, and Wagner reagent. In testing the alkaloids before dropping HCl. This is because alkaline alkaloids are insoluble in air, but easily soluble in organic solvents. Alkaloids in the form of salts are easily soluble in polar solvents. The addition of HCl to alkaline alkaloids can help salt formation (Fajrin and Ida, 2019).

Alkaloid testing using Mayer's reagent on samples of Matoa leaf extract was positive for alkaloids, these results were in accordance with previous research conducted by Harahap and Nurbaiti, (2021). Assay for alkaloids with Mayer's reagent, nitrogen in the alkaloids reacts with metal ions K⁺ from potassium tetraiodomercurate (II) to form a precipitated potassium-alkaloid complex, resulting in a white or yellow precipitate (Harahap and Nurbaiti, 2021). In testing for alkaloids with Dragendorff's reagent on samples of Matoa leaf extract positive for alkaloids, these results are in accordance with the research of Hadi and Intan, (2019). Alkaloids test with Dragendorff's reagent, nitrogen can form coordinate covalent bonds with metal ions K⁺, thus forming an orange or brick red precipitate (Hadi and Intan, 2019). In testing for alkaloids with Wagner's reagent on samples of Matoa leaf extract positive for alkaloids, these results are in accordance with the research of Hadi and Intan, (2019). In the preparation of Wagner's reagent, iodine reacts with I⁻ ions of potassium iodide to produce I₃⁻ ions which are brown in color. In the Wagner test, the metal ion K⁺ forms a coordinate covalent bond with the nitrogen alkaloids forming a potassium-alkaloid complex bond which precipitates to form a brownish red precipitate (Hadi and Intan, 2019).

The flavonoid test on the matoa leaf extract was positive for flavonoids. In this study, the flavonoid test showed that at room temperature (25-30°C) an orange color was produced, at 40°C an orange red color was produced, at 50°C a brownish red color was produced. These results are in accordance with previous research conducted by Setiabudi and Tukiran (2017) the formation of a color

change from orange to red due to the formation of flavilium salts. The addition of concentrated HCl and magnesium powder to hydrolyze flavonoids into aglycones by hydrolyzing O-glycosyl. The complex compound was produced from the reduction of Mg with HCl with the formation of an orange-red solution (Dina, 2020). Bioactive components such as flavonoids are not resistant to high temperatures above 50°C (Yuliantari *et al.*, 2017).

Steroid/triterpenoid testing on Matoa leaf samples showed positive content of triterpenoids. The results obtained in this study are the formation of a red ring purplish. These results are in accordance with the research of Maryam *et al.* (2020) positive for steroids formed a green-blue ring, while positive for triterpenoids formed purplish red. Color change occurs through the formation of double bonds conjugated in the presence of oxidation of the steroid/triterpenoid compound group. The addition of anhydrous acetic acid to form an acetyl derivative. Meanwhile, the addition of concentrated sulfuric acid to hydrolyze water which reacts with acetyl derivatives forms a purplish red or green to blue ring (Khotimah, 2016).

The tannin test on the Matoa leaf samples was positive for tannins. The results obtained from the tannin test in this study were at room temperature (25-30°C), 40°C and 50°C a blackish green color was produced. These results are in accordance with previous research conducted by Widhoyo *et al.* (2019), which is a green – black color. The addition of 1% FeCl₃ tannins to form complex compounds with Fe⁺³ ions will form a blackish green color (Agustina *et al.*, 2017). At a temperature of 50°C, the catechol oxidase enzyme inactivation occurs and an enzymatic reaction occurs, thereby increasing the tannin content (Puspitasari, 2018).

The saponin test on the Matoa leaf samples was positive for saponins. These results are in accordance with previous research conducted by Prabowo and Noer. (2020) the addition of HCl will form a stable foam. The foam is not affected by acids, so the foam remains stable and does not wear off. Saponins can also lower the surface tension of water, so that foam forms on the surface of the water when shaken (Rossalinda *et al.*, 2021). Existence Glycosides are able to form foam in water which is hydrolyzed to glucose and other compounds so that a stable foam is formed (Nugrahani *et al.*, 2016).

CONCLUSION

The conclusions in this study are:

1. The content of secondary metabolites in matoa leaves growing in Bekasi are alkaloids, flavonoids, triterpenoids, tannins, and saponins.
2. Results of thick extract of Matoa Leaves with 70% ethanol solvent highest yield percentage in the extraction treatment at 50°C yielded an extract yield of 36,4%, while the extraction treatment at room temperature (25-30°C) yielded an extract yield of 29,6% and at 40°C the extract yield of 26,9%.

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FORMULATION AND EVALUATION OF NANOEMULSION FROM PAPAYA LEAF EXTRACT (*Carica Papaya* L.) WITH VARIATION CONCENTRATION OF TWEEN 80

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Abstract

Introduction: Nanoemulsion with small particle size can resolve less effective absorption of drugs. This preparation can be combined with Papaya leaf which can be efficacious as antibacterial because it contains alkaloid, flavonoid, saponin and tannin. The purpose of this study is to determine physical stability of nanoemulsion from Papaya leaf extract.

Method: Formulation of nanoemulsion based on variation concentrations of tween 80, F1 (36%), F2 (37%) and F3 (38%) with evaluation such as organoleptic test, pH test, viscosity test, transmission test, particle size test, Polydispersity Index (PI) test and physical stability test by centrifugation and freeze thawing which use cold temperature (4°C) and room temperature (25°C).

Results: All formulas were stable and met the requirements for evaluation of nanoemulsion with pH value 6,27-6,42; viscosity 1083-3250 Cp; transmission 90,83-97,03%; particle size 14,43-15,43 nm; PI 0,365-0,440.

Conclusion: All formulas had good physical stability and the best formula was F1 tween 80 36% because it had the smallest particle size and Polydispersity Index (PI) value, they were 14,43 nm and 0,365.

Keywords: Nanoemulsion, Papaya Leaf Extract, Physical Stability Test, Tween 80

INTRODUCTION

The development of drug delivery systems in the pharmaceutical sector has been carried out for several years. However, new problems began to emerge such as the ineffective absorption of drugs in the body. It is due to the low solubility of the drug and the size of the molecule is too large so that it is difficult to penetrate the absorption barrier (Ramadon and Mun'im, 2016). Drugs with a poor absorption rate will be wasted in the body. The impact is a low bioavailability (blood level of the drug) and can lead to failure of the drug's therapeutic effect (Permatasari et al., 2016). These problems cause the need for research on formulations that help drug delivery systems become more effective, which is by increasing drug absorption. The development of this nanotechnology system formulation can be used as one way to overcome these problems. One example of a nanotechnology system is nanoemulsion where the active substance is made in nano size (1-100 nm) (Larasati and Jusnita, 2020). Nanoemulsion consist of water, oil, surfactants and cosurfactants. Tween 80 surfactant with a concentration of 38% can be stable in nanoemulsion (Arianto and Cindy 2019). In addition to containing these components, nanoemulsion can also be combined with natural ingredients, one of which is Papaya leaf (*Carica papaya* L.) which has antibacterial properties because it contains alkaloids, flavonoids, saponins and tannins (Cahyanta et al., 2020). At concentration of 0,3%, Papaya leaf extract can be efficacious as an inhibitor of bacterial growth (Callixte et al., 2020). Based on several studies that have been carried out and see the advantages of nanoemulsion, the researcher is interested in conducting research on the formulation and evaluation of nanoemulsion from 96% ethanol extract of Papaya leaf (*Carica papaya* L.) with variations of tween 80 base with concentrations of 36%, 37% and 38%.

METHOD

A. Research Design

This research used an experimental design with descriptive analysis method.

B. Research Location and Time

This research was conducted at the Pharmaceutical Technology Laboratory of STIKes Mitra Keluarga Bekasi in February-March 2022.

C. Population and Sample

The population and sample in this study were Papaya leaf (*Carica papaya* L.) which were obtained from a supplier of extracts Palapa Muda Perkasa, Depok.

D. Research variable

The variable in this study was independent variable because the variable was not compared to other variables (Heri and Sriartha, 2019). The independent variables in this study were organoleptic, pH, viscosity, transmittance, particle size, Polydispersity Index (PI) and physical stability.

E. Research Tools

The tools used were rotary evaporator, analytical balance (Ohaus SPX222 Scout), brookfield viscometer (LV 801), digital pH meter (ATC), UV-Vis spectrophotometer, mixer (IKA RW 20), hot plate (IKA C-MAG), centrifugator, sonicator (Power Sonic405), particle size analyzer (Horiba SZ-100), refrigerator (Showcase), beaker glass (Iwaki Pyrex®), measuring cup (Iwaki Pyrex®), porcelain crucible (Iwaki Pyrex®), watch glass (Iwaki Pyrex®), glass bottle, spatula, dropper pipette, stirring rod.

F. Research Material

The ingredients used were 96% ethanol extract of Papaya leaf, sunflower oil (Kimia Jaya), tween 80 (Kimia Jaya), sorbitol (Samudra Kimia), methyl paraben (Kimia Jaya), propyl paraben (Kimia Jaya), 96% ethanol (Merck) and aquadest (Kimia Jaya).

G. Procedure

1. Papaya Leaf Preparation

The sample selection was done by purposive sampling method, which was a sampling method where the researcher determined the criteria for the sample to be used (Etikan and Bala, 2017). The criteria for a good sample were dark green and fresh Papaya leaf aged 2-5 months after planting (Roni et al., 2019). Papaya leaf samples were washed, wet sorting, chopping, drying, dry sorting and pollination (Febriansah, 2017).

2. Papaya Leaf Determination

Determination of plant served to ensure the truth and know the identity of the plant (Puspitasari and Proyogo, 2017). Plant determination was carried out at the Bogorinse Herbarium, LIPI Bogor.

3. Papaya Leaf Extraction

Papaya leaf was extracted by maceration method. 1000 g of Papaya leaf simplicia powder was macerated by soaking it in 5 L of 96% ethanol. The powder was soaked for 3 days at room temperature (25-30°C). Further, the extract was filtered. The filtrate obtained from the maceration process then concentrated with rotary evaporator at temperature 50°C to remove unwanted solvents in order to obtain 100 g thick extract of Papaya leaf (Pradiningsih and Mahida, 2019).

4. Papaya Leaf Phytochemical Screening

a. Alkaloid Test

50 mg of extract was dissolved in 1 mL of 2 M HCl then filtered. The filtrate was added 1 drop of Mayer, Wagner and Dragendorff reagents in different test tubes. If the extract was

positive for alkaloids, a white precipitate would be formed in the Mayer reagent, a brown precipitate in the Wagner reagent and an orange precipitate in the Dragendorff reagent.

b. Flavonoid Test

40 mg of extract added 100 mL of aquadest, boiled for 15 minutes then filtered. After that, 0,05 mg of Mg powder and 1 mL of HCl were added to 5 mL of the filtrate and the mixture was shaken vigorously. If the extract was positive for flavonoids, an orange color would be formed.

c. Saponin Test

5 mL of extract was added with aquadest and shaken vigorously. Let it stand for 10 minutes, added 1 drop of 2 N HCl. If a stable foam was formed for 10 minutes, then the extract was positive for saponins.

d. Tanin Test

0,1 g of extract was added to 10 mL of aquadest and filtered. 5 mL of 1% FeCl₃ was added to the filtrate. If a dark blue or black color was formed, then the extract was positive for tannins.

5. Formulation of Papaya Leaf Extract Nanoemulsion

Nanoemulsion was made by dissolving sunflower oil with some sorbitol. The remaining sorbitol was used to dissolve the Papaya leaf extract. This solution was mixed with a solution of sunflower oil and sorbitol and then homogenized (mixture 1). The aqueous phase was made by dissolving methyl paraben and propyl paraben with aquadest and then heated using a hot plate. The solution was cooled and added tween 80 with different concentrations 36%, 37% and 38% in each formula (mixture 2). This mixture was homogenized manually with a stirring rod. Mixture 1 and mixture 2 were homogenized with mixer at speed 450 rpm for 15 minutes. After that, the nanoemulsion was sonicated for 2 hours to remove the formed foam (Arianto and Cindy, 2019). Papaya leaf extract nanoemulsion formula can be seen in table 1.

Table 1. Papaya Leaf Extract Nanoemulsion Formula

Material Composition	Nanoemulsion Formula (%w/v)			Function
	F1	F2	F3	
Papaya leaf extract	0,5	0,5	0,5	Antibacterial
Sunflower oil	5	5	5	Oil phase
Tween 80	36	37	38	Surfactant
Sorbitol	22	22	22	Cosurfactant
Methyl paraben	0,1	0,1	0,1	Preservative
Propyl paraben	0,02	0,02	0,02	Preservative
Aquadest ad	100	100	100	Solvent

6. Evaluation of Papaya Leaf Extract Nanoemulsion

a. Organoleptic Test

Organoleptic test was carried out by observing the color, aroma and phase separation of the nanoemulsion stored at room temperature (25±2°C) for 14 days (Arianto and Cindy, 2019).

b. pH Test

This test was carried out using a pH meter by inserting the pH meter into the nanoemulsion which was stored at room temperature (25±2°C) for 14 days (Arianto and Cindy, 2019). The pH requirements of the preparation were adjusted to a safe pH for the skin, which was 4-10 (BSN, 2017).

c. Viscosity Test

Viscosity test was carried out using a brookfield viscometer. The nanoemulsion viscosity was measured with spindle number 4 at a speed of 12 rpm. This test was carried out on nanoemulsion stored at room temperature (25±2°C) for 14 days (Larasati and Jusnita, 2020). The viscosity of the nanoemulsion was adjusted to the viscosity of the topical preparation, which was 500-5000 Cp (Robert, 2020).

d. Transmittance Test

Transmittance test was carried out by dissolving 2 mL of nanoemulsion in 100 mL of aquadest, tested using a UV-Vis spectrophotometer at wavelength 650 nm (Pratiwi et al., 2017). This test was carried out on the first day of the nanoemulsion formulation. The transmission test requirement of nanoemulsion was 80-100% (FDA, 2020).

e. Particle Size Test

This test was carried out using Particle Size Analyzer (PSA) to determine the particle size of nanoemulsion (Prasetya et al., 2019). This test was carried out on the first day of the nanoemulsion formulation. 2 mL of nanoemulsion was dissolved in 100 mL of distilled water and the samples were tested at the Integrated Laboratory Research Center (ILRC), University of Indonesia. The nanoemulsion particle size requirement was 1-100 nm (FDA, 2020).

f. Polydispersity Index (PI) Test

This test was carried out using Particle Size Analyzer (PSA) to determine the particle size distribution of nanoemulsion. Sample preparation for this test was the same as for particle size testing. The Polydispersity Index (PI) requirement was <0,5 (Marzuki et al., 2019).

g. Physical Stability Test

1) Centrifugation Test

This test was carried out to observe the stability of nanoemulsion using a centrifuge with a rotation speed 3000 rpm for 30 minutes (Iskandar et al., 2021).

2) Freeze Thawing Test

This test was carried out by observing the stability of nanoemulsion at low temperature ($4\pm 2^{\circ}\text{C}$) for 24 hours and then transferred to room temperature ($25\pm 2^{\circ}\text{C}$) for 24 hours (1 cycle). This test was carried out for 6 cycles (Forestryana et al., 2020).

RESULTS

A. Determination Results of Papaya Leaf

Plant determination was carried out at the Bogorinse Herbarium, LIPI Bogor. Based on the results of the determination, it can be seen that the Papaya leaf used as a sample was a type of *Carica papaya* (L.) with the family Caricaceae.

B. Phytochemical Screening Results of Papaya Leaf Extract

Based on the results of phytochemical screening, it can be seen that the alkaloid test, saponin test and tannin test showed positive results, while the flavonoid test showed negative results.

C. Organoleptic Test Results of Papaya Leaf Extract Nanoemulsion

The best formula was formula 3 with the lightest color. The results of the Papaya leaf nanoemulsion organoleptic test can be seen in table 2.

Table 2. Papaya Leaf Extract Nanoemulsion Organoleptic Test

Formula	Color	Aroma	Phase Separation
F1	Clear, dark greenish yellow	Papaya leaf	No Separation
F2	Clear, slightly dark greenish yellow	Papaya leaf	No Separation
F3	Clear, bright greenish yellow	Papaya leaf	No Separation

Information :

F1 : Papaya extract leaf nanoemulsion with tween 80 36%

F2 : Papaya extract leaf nanoemulsion with tween 80 37%

F3 : Papaya extract leaf nanoemulsion with tween 80 38%

D. pH Test Results of Papaya Leaf Extract Nanoemulsion

All formulas were stable and complied with the skin's pH requirements. The results of the Papaya leaf nanoemulsion pH test can be seen in table 3.

Table 3. Papaya Leaf Extract Nanoemulsion pH Test

Day	pH		
	F1	F2	F3
	Average $\pm$ SD	Average $\pm$ SD	Average $\pm$ SD
0	6,27 $\pm$ 0,02	6,35 $\pm$ 0,02	6,42 $\pm$ 0,02
7	6,27 $\pm$ 0,02	6,35 $\pm$ 0,02	6,42 $\pm$ 0,02
14	6,27 $\pm$ 0,02	6,35 $\pm$ 0,02	6,42 $\pm$ 0,02

Information :

F1 : Papaya extract leaf nanoemulsion with tween 80 36%

F2 : Papaya extract leaf nanoemulsion with tween 80 37%

F3 : Papaya extract leaf nanoemulsion with tween 80 38%

E. Viscosity Test Results of Papaya Leaf Extract Nanoemulsion

The results of viscosity test showed that all formulas were in accordance with the viscosity requirements of topical preparations. The results of the Papaya leaf nanoemulsion viscosity test can be seen in table 4.

Table 4. Papaya Leaf Extract Nanoemulsion Viscosity Test

Day	Viscosity (Cp)		
	F1	F2	F3
	Average $\pm$ SD	Average $\pm$ SD	Average $\pm$ SD
0	1167 $\pm$ 144,34	2917 $\pm$ 144,34	3250 $\pm$ 250,00
7	1083 $\pm$ 144,34	2750 $\pm$ 250,00	3000 $\pm$ 250,00
14	1083 $\pm$ 144,34	2667 $\pm$ 144,34	2883 $\pm$ 144,34

Information :

F1 : Papaya extract leaf nanoemulsion with tween 80 36%

F2 : Papaya extract leaf nanoemulsion with tween 80 37%

F3 : Papaya extract leaf nanoemulsion with tween 80 38%

F. Transmission Test Results of Papaya Leaf Extract Nanoemulsion

Formula with the best transmittance percentage was formula 3. The results of the Papaya leaf nanoemulsion transmittance test can be seen in table 5.

Table 5. Papaya Leaf Extract Nanoemulsion Transmittance Test

Formula	Transmittance (%)
	Average $\pm$ SD
F1	90,83 $\pm$ 0,01
F2	93,23 $\pm$ 0,00
F3	97,03 $\pm$ 0,01

Information :

F1 : Papaya extract leaf nanoemulsion with tween 80 36%

F2 : Papaya extract leaf nanoemulsion with tween 80 37%

F3 : Papaya extract leaf nanoemulsion with tween 80 38%

G. Particle Size Test Results of Papaya Leaf Extract Nanoemulsion

Based on the particle size test, the best formula was formula 1. The results of the Papaya leaf nanoemulsion particle size test can be seen in table 6.

Table 6. Papaya Leaf Extract Nanoemulsion Particle Size Test

Formula	Particle Size (nm)
	Average $\pm$ SD
F1	14,43 $\pm$ 0,21
F2	15,20 $\pm$ 0,35
F3	15,43 $\pm$ 0,25

Information :

F1 : Papaya extract leaf nanoemulsion with tween 80 36%

F2 : Papaya extract leaf nanoemulsion with tween 80 37%

F3 : Papaya extract leaf nanoemulsion with tween 80 38%

H. Polydispersity Index (PI) Test Results of Papaya Leaf Extract Nanoemulsion

In the PI test the best formula is formula 1. The results of the Papaya leaf nanoemulsion PI test can be seen in table 7.

Table1. Polydispersity Index (PI) Test of Papaya Leaf Extract Nanoemulsion

Formula	Polydispersity Index (PI)
	Average $\pm$ SD
F1	0,365 $\pm$ 0,033
F2	0,411 $\pm$ 0,025
F3	0,440 $\pm$ 0,097

Information :

F1 : Papaya extract leaf nanoemulsion with tween 80 36%

F2 : Papaya extract leaf nanoemulsion with tween 80 37%

F3 : Papaya extract leaf nanoemulsion with tween 80 38%

I. Physical Stability Test Results of Papaya Leaf Nanoemulsion

Papaya leaf nanoemulsion stability test was carried out by centrifugation test and freeze thawing test. Based on the results of the study, it was known that all stable formulas did not occur in the phase separation either in the centrifugation test or in the freeze thawing test.

DISCUSSION

Formulation research and evaluation of Papaya leaf extract (*Carica papaya* L.) nanoemulsion with variations in concentration of tween 80 aims to obtain the best formula in terms of physical stability of the formulated nanoemulsion. This research was carried out in several stages, which were Papaya leaf sample preparation, Papaya leaf extraction, phytochemical screening of Papaya leaf extract, formulation and evaluation of nanoemulsion from Papaya leaf extract. The evaluations were organoleptic test, pH test, viscosity test, transmittance test, particle size test, Polydispersity Index (PI) test and physical stability test by centrifugation and freeze thawing.

Papaya leaf extraction was carried out with maceration method with 96% ethanol as solvent. The maceration method was chosen because the method is easy, the equipment used was simple and this method was a cold extraction which was suitable for the active substances contained in Papaya leaf which were not resistant to high heating (Julianto, 2019). The 96% ethanol solvent was chosen because it had the same polarity as the active substances contained in Papaya leaf, which was polar. In addition, 96% ethanol was neutral, safe and easy to obtain (Riwanti et al., 2020). The active substances in Papaya leaf to be extracted are alkaloids, flavonoids, saponins and tannins.

In 1000 g of Papaya leaf simplicia powder, 105,4 g of thick dark green extract was obtained, with a distinctive aroma of Papaya leaf with an extract yield of 10,54%. The extract yield was in accordance with the general requirements for extract yields in Farmakope Herbal Indonesia (2017), which is not less than

7,3%. In the research of Octavianni (2016), the yield of the extract was not much different, which was 10,07% with the characteristics of extract were thick dark green color and distinctive aroma of Papaya leaf. This showed that the extract results in this study are in accordance with previous studies.

Phytochemical screening is the initial stage to provide an overview of the class of compounds contained in the plant under study. In this study, phytochemical screening was carried out on the active compounds contained in Papaya leaf, which were alkaloids, flavonoids, saponins and tannins (Cahyanta et al., 2020). In the alkaloid test, the extract was added with HCl and three reagents, which were Mayer, Wagner and Dragendorff. The addition of HCl functioned to dissolve alkaloids from the extract, while the reagents of Mayer, Wagner and Dragendorff functioned to identify alkaloid compounds (Nugrahani et al., 2016).

Alkaloid test with Mayer reagent showed a positive result in the form of a white precipitate. These results are in accordance with the research of Harahap and Situmorang (2021) which also gave a positive result in the presence of a white precipitate. A white precipitate can be formed because the nitrogen ions in the alkaloids bind metal ions K^+ from $K_2[HgI_4]$, this results formation of a potassium-alkaloid complex in the form of a white precipitate (Baharudin, 2017).

In the alkaloid test with Wagner reagent gave positive results indicated by the presence of a brown precipitate. These results are in accordance with the research of Harahap and Situmorang (2021) which showed a positive result with a brown precipitate. The formation of a brown precipitate is caused by the reaction between nitrogen ions in the alkaloids with metal ions K^+ from KI_3 to form a potassium-alkaloid complex in the form of a brown precipitate (Baharudin, 2017).

The alkaloid test with Dragendorff reagent showed a positive result in the form of an orange precipitate. These results are in accordance with the research of Harahap and Situmorang (2021) which also gave a positive result in the presence of an orange precipitate. An orange precipitate can be formed because the nitrogen ions in the alkaloids bind metal ions K^+ from $K[BiI_4]$ This results in the formation of a potassium-alkaloid complex in the form of an orange precipitate (Baharudin, 2017).

Flavonoid testing was carried out by adding aquadest Mg and HCl powder to the extract. Aquadest functions to dissolve flavonoids while Mg and HCl powders function as reagents to identify flavonoid compounds. The results of the flavonoid test in this study were negative because no red color was formed. This result is different from the research of Ramadhani et al (2020) which showed a positive result in the form of a red solution. The formation of a red solution is caused by a reduction reaction caused by Mg and HCl so that a red or orange solution is formed (Nugrahani et al., 2016).

In the saponin test, the extract was added with aquadest and HCl. Aquadest functions to dissolve saponins from the extract and forms foam, while HCl serves to test the stability of the formed foam. The saponin test gave a positive result in the form of a stable foam formation. These results are in accordance with the research of Harahap and Situmorang (2021) which also formed a stable foam. Foam can be formed because the glycosides in the saponin compound are hydrolyzed into glucose and other compounds, the foam will remain stable even though it is reacted with HCl (Nugrahani et al., 2016).

The tannin test was carried out by adding aquadest and $FeCl_3$ on the extract. Aquadest functions to dissolve tannins from extracts and $FeCl_3$ serves to identify the phenol group in tannins. The tannin test gave a positive result in the form of a color change to black. These results are in accordance with the research of Ramadhani et al (2020) indicating a positive result in dark blue or black. This blackish color can be formed due to a complex reaction between the phenol group in tannins and metal ions Fe^{3+} (Baharudin, 2017).

Organoleptic test is a test carried out by observing the physical sample using the senses. Nanoemulsion is said stable if there is no physical change during storage. In table 2, it is known that all formulas have a distinctive Papaya leaf aroma and no phase separation occurs. However, there are color differences in each formula where the higher concentration of tween 80, the green color will fade. This result is different from the research of Arianto and Cindy (2019) which in this study, the nanoemulsion had a clear yellow color because there was no addition of extract.

The occurrence of color differences in each formula can be influenced by the 80% tween concentration. The higher concentration of tween 80%, the color of nanoemulsion will be brighter because tween 80 which is clear yellow will cover the dark green color of Papaya leaf extract. This is in accordance with the literature, where according to Sheskey et al (2017) tween 80 has the characteristics of a clear yellow color and according to Octavianni (2016) which stated that Papaya leaf extract was dark green. The

results of organoleptic test can be seen in table 2.

The pH test is carried out to determine sample acidity degree using pH meter. In this study, the nanoemulsion had a pH value that was not much different from the research of Arianto and Cindy (2019). In that study, the average pH was 6,5. The results of the pH test in this study were in accordance with the standard skin pH requirements 4-10 (BSN, 2017). If the pH is too acidic, it will irritate the skin while a pH that is too alkaline will make the skin dry which can cause scaly skin (Shabrina et al., 2020). Differences pH values in each formula can occur due to differences concentration of tween 80. The higher concentration of tween 80, the pH value will increase because tween 80 has an alkaline pH which is 6-8 (Handayani et al., 2018). The results of pH test can be seen in table 3.

Viscosity test is a test to determine the viscosity of a sample. The viscosity value of nanoemulsion in the study met the requirements because it was in accordance with the viscosity range of topical preparations 500-5000 Cp (Robert, 2020). This result is different from the research of Arianto and Cindy (2019) with an average viscosity of 225 Cp. This happened because in that study there was no addition of extract so the viscosity value was lower.

During 14 days storage at room temperature ($25\pm 2^{\circ}\text{C}$) all formulas decreased in viscosity value, this can occur due to the influence of temperature during storage. On the other hand, the viscosity value increases with increasing concentration of tween 80. This is in accordance with the research conducted by Zulfa et al (2019) which states that higher concentration of tween 80 had greater ability to reduce the size of the globule diameter so that the globule arrangement becomes denser and uniform. In addition, tween 80 has a thick consistency so it can increase the viscosity of nanoemulsion. The results of viscosity test can be seen in table 4.

The transmittance test is a test to determine the level of clarity of a sample. In this study, all formulas were in accordance with the requirements for transmittance of nanoemulsion because they were in the 80-100% range (FDA, 2020). If the nanoemulsion preparation has a transmittance of more than 80%, it is estimated that the particles formed have reached the nanometer size. The higher transmittance percentage, nanoemulsion will be clearer (Zulfa et al., 2019). The results showed that higher concentration of tween 80 had higher percentage of transmittance. This is because the clear yellow color at tween 80 will cover the dark green color of Papaya leaf extract. Color density can affect the clarity of a nanoemulsion (Jaya et al., 2017). The results in this study are not much different from the research of Aprilya et al (2021) with an average transmittance of 95,41%. The transmittance test results can be seen in table 5.

The particle size test is the main parameter in nanoemulsion because it states the particle size related to the absorption of the active substance into body. If the particle size gets smaller, it will increase the absorption of the active substance into body (Safrida et al., 2020). The particle size result in this study is different from the research of Arianto and Cindy (2019) which has an average particle size of 124,47 nm. Although different, the particle size in this study met the requirements for the particle size of nanoemulsion, which is 1-100 nm (FDA, 2020). Based on the results of the study, it can be seen that higher concentration of tween 80 had larger particle size. This is presumably due to the interaction between Papaya leaf extract and tween 80 which need further research. The addition of Papaya leaf extract can affect particle size of nanoemulsion. The results of the particle size test can be seen in table 6.

Polydispersity Index (PI) test is a test to determine the uniformity of particle size distribution in the sample. In this study, all formulas met the requirements Polydispersity Index (PI) of nanoemulsion, which is $<0,5$. If the PI value is smaller, the particle size of the nanoemulsion will be more uniform (Marzuki et al., 2019). These results are not much different from the research of Rusdi (2017) with an average Polydispersity Index (PI) value of 0,370. It can be seen that higher concentration of tween 80 had higher value of the Polydispersity Index (PI). In addition, the results also showed that the uniform distribution of all formulas was monodisperse, it means that the nanoemulsion had a uniform particle size shape (Syukri et al., 2020). The results of the Polydispersity Index (PI) test can be seen in table 7.

Centrifugation test is a test to determine the stability of nanoemulsion preparations. Nanoemulsion is said stable if there is no phase separation after centrifugation. If nanoemulsion is unstable after being centrifuged, it is feared that the nanoemulsion will not be able to withstand the shock effect during the product delivery process (Shabrina et al., 2020). The results of the centrifugation test on all formulas showed no phase separation after centrifugation. These results are in accordance with research conducted by Arianto and Cindy (2019).

Freeze thawing test is a test to determine the stability of nanoemulsion based on temperature changes. Nanoemulsion should remain stable after the freeze thawing test. If it does not meet the requirements of freeze thawing test, it is feared that the nanoemulsion preparation will become unstable due to the influence of temperature during storage (Nurdianti et al., 2018). The result of freeze thawing test on all formulas did not occur phase separation after storage for 6 cycles. These results are in accordance with the research of Arianto and Cindy (2019).

CONCLUSION

Nanoemulsion of 96% ethanol extract Papaya leaf with variation concentrations of tween 80 36%, 37% and 38% had good physical stability. The best formula of extract Papaya leaf nanoemulsion was formula 1 with concentration of tween 80 36% because it had the smallest particle size and PI values, they were 14,43 nm and 0,365.

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DETERMINATION OF VITAMIN C LEVELS IN FRESH FRUIT AND SWEET CRYSTAL Guava (*Psidium guajava* L.) USING UV-VIS SPECTROPHOTOMETRY METHOD

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Abstract

Vitamin C is a vitamin that is easily soluble in water, but is easily damaged by oxidation reactions by oxygen from the water. Vitamin C cannot be obtained alone, so to meet the needs of vitamin C can come from fruits, such as crystal guava fruit. Guava is a fruit that contains twice as much vitamin C as other fruits that contain vitamin C, so it is necessary to do research on vitamin C levels in crystal guava with UV/Vis spectrophotometry method. The purpose of this study was to determine the levels and differences in levels of vitamin C in fruit and candied guava, using UV-Vis spectrophotometry method. The results showed that the average vitamin C content in fresh fruit samples was 72.8 mg/100 g and candied fruit 236.08 mg/100 g. The results of the paired sample t-test or paired t-test obtained a 2-way (t-tailed) significance value of 0.001 or there was a significant difference in the average vitamin C levels between fresh fruit and candied crystal guava fruit. Thus, it can be concluded that crystal guava is a fruit that can be used as a food ingredient to meet the intake of vitamin C for humans.

Keywords : Crystal Guava, UV-Vis Spectrophotometry, Vitamin C

INTRODUCTION

To protect the body from various diseases, it is very important to have a healthy diet in everyday life. Adequacy of nutrients can be achieved by consuming healthy foods that are rich in vitamins, minerals, and fiber needed by the body (Yulianti and Kurniawati, 2017). One of them is ascorbic acid or vitamin C which is needed by the human body (Pratiwi et al., 2020).

Vitamin C has the meaning as an organic substance useful for maintaining metabolic functions (Hasanah, 2018). In addition, vitamin C is also a nutrient that acts actively as an antioxidant, effective in dealing with free radicals that can damage tissues or cells (Romalasari et al., 2017). The human body cannot synthesize vitamin C independently, so vitamin C is needed from outside the body. One way to meet the needs of vitamin C can come from fruits (Pratiwi et al., 2020).

Almost all fruits contain vitamin C, but most people only know the content of vitamin C in some fruits such as oranges and pineapples (Yulianto et al., 2022). In fact, apart from these two fruits, there are fruits that contain more vitamin C than oranges and pineapples, namely guava (Mayadewi and Sukewijaya, 2019). Guava (*Psidium guajava* L.) is one of the fruit varieties that has great potential in fulfilling nutrition for the community, because guava fruit contains twice as much vitamin C as fruits containing vitamin C, which is about 87 mg in 100 g guava. One example of guava fruit varieties is crystal guava fruit (Mayadewi and Sukewijaya, 2019).

Crystal guava is one of the guava varieties that has a great opportunity to be developed in Indonesia (Astrini et al., 2018). The guava is said to be crystal, because the flesh of the fruit is white rather clear with a large fruit size and has a slightly curved shape, not perfectly round resembling a crystal (Romalasari et al., 2017). There are many ways to consume crystal guava fruit, namely by consuming the fruit directly or processing it as candied crystal guava fruit (Hartanto et al., 2019).

Candied fruit is one of the processed foods favored by the community, because it has a sweet taste that is continuous with the distinctive taste of the fruit. The method that can be done in making candied fruit is the preservation method, by going through the soaking process using sugar water. Soaking can increase the sugar content in the fruit and reduce the water content. This can slow down microbial growth and make the fruit last longer (Hartanto et al., 2019).

According to research conducted by Padang and Maliku (2017), the results of the determination of

vitamin C levels in red guava fruit using the Na-2,6 Dichlorophenol Indophenol (DCIP) titration method were 42.9 mg/100 grams. This shows that red guava fruit contains vitamin C, so it is necessary to determine the content of vitamin C in other types of guava fruit such as crystal guava. Research conducted by Guntarti and Hutami (2019), obtained the results of Vitamin C Testing in crystal guava with variation of origin with the HPLC method, for vitamin C levels of crystal guava in the Bogor area of 41.39 mg/100g, Malang area of 67.46 mg/100 g, and the Gunung Kidul area of 86.08 mg/100 g. Differences in vitamin C levels occur due to differences in the height of the planting place on crystal guava fruit.

The difference between the previous research and the research to be studied is the sample and the research method that will be used. In previous studies using red guava fruit samples, the research method used was the titration method, while in this study using crystal guava fruit samples, the research method used was UV/Vis spectrophotometry. Based on this description, researchers are interested in conducting research on the determination of vitamin C levels in fresh and candied guava (*Psidium guajava* L.) by using UV/Vis spectrophotometry method.

METHOD

Research design

This research is included in quantitative research with the research design used is experimental.

Research Location and Time

This research was conducted at the Pharmaceutical Chemistry Laboratory of STIKes Mitra Keluarga, Bekasi City, West Java Province from March to April 2022.

Population and Sample

The population in this study was guava fruit crystal variety (*Psidium guajava* L.). The sample in this study was the fresh fruit of crystal guava obtained at Jalan Serelek No. 11 Cikarawang Karang Pulang Village, Dramaga Bogor Regency and candied crystal guava will be obtained by processing it yourself, using the same crystal guava fruit as fresh fruit samples.

Tools and materials

The tools used include a UV-Vis spectrophotometer (Thermo Scientific Genesys 10S UV-Vis v4.006) and glass cuvettes, glass beakers (Iwaki Pyrex), analytical balances (Ohaus), volumetric flasks (Iwaki Pyrex), micropipettes (Socorex) and tip, blender (Philips), measuring cup (Iwaki Pyrex), funnel (Iwaki Pyrex), volume pipette (Iwaki Pyrex), test tube (Iwaki Pyrex), measuring pipette (Iwaki Pyrex), dropper pipette, ball pipette, spatula, rod stirrer, filter paper, knife, cutting board, test tube rack, parchment paper, brown glass bottle, and vial.

The materials used include standard vitamin C or ascorbic acid (BBI Life Sciences), potassium permanganate (KMnO₄) 1%, aquadest and samples of fresh fruit and candied guava crystals.

Plant Determination Test

The determination test on crystal guava plants was carried out at the Herbarium Bogoriense LIPI, Bogor to avoid errors during sampling and collection and ensure that the fruit used was the crystal guava fruit in question (Puspitasari et al., 2019).

Making Candied Guava Crystal Ingredient

The ingredients used include 500 g of crystal guava fruit, 200 g of granulated sugar, ½ teaspoon of whitening, 1 teaspoon of salt, 700 ml of water, and 1 lime.

Ways of making

Fruit that has been peeled, seeds removed, and cut to the desired size, soaked in whitening water for 2 hours. Crystal guava fruit that has been soaked, then drained and washed until no whitening sticks. A total of 700 mL of water is boiled with 200 grams of sugar and 1 tsp of salt until boiling and cooled. After cooling, the crystal guava fruit is added, added with squeezed lime juice, and allowed to stand for 24 hours (Asih,

2017).

Preparation of Sample Test Solution Fresh Fruit Crystal Guava

Ripe guava fruit is cleaned, peeled, seeded, cut into small pieces, and crushed with a blender. The crystal guava fruit that has been blended is weighed as much as 2.5 grams and placed into a 50 mL volumetric flask and add distilled water to the mark and homogenized, then the sample solution is filtered using filter paper. The result of the filtered solution was taken as much as 35 mL, placed in a 50 mL volumetric flask, and added with distilled water up to the mark. The dilution was carried out again in a way, 35.71 mL of sample solution was taken, placed into a 50 mL volumetric flask, and distilled water was added to the mark (Chandra et al., 2019).

Candied Guava Crystal

Candied guava crystals are drained, cut into small pieces, and crushed using a blender. Candied guava crystals that have been blended are weighed as much as 2.5 grams and placed into a 50 mL volumetric flask and add distilled water to the mark and homogenized, then the solution is filtered using filter paper. The result of the filtered solution was taken as much as 35 mL, placed in a 50 mL volumetric flask, and added with distilled water up to the mark. The dilution was carried out again in a way, 35.71 mL of sample solution was taken, placed into a 50 mL volumetric flask, and distilled water was added to the mark (Chandra et al., 2019).

Qualitative Analysis Preparation of 1% KMnO₄ Solution

1% KMnO₄ solution was made by weighing 1 g of KMnO₄, then put into a 100 mL volumetric flask, and dissolved with distilled water to the mark. The solution was transferred to a 250 mL beaker and boiled using a hotplate at 70°C for 15 minutes. After the solution cools, the solution is filtered using filter paper and put into a brown glass bottle (Maulida et al., 2020).

Vitamin C Color Qualitative Test

Vitamin C color test was carried out by comparing the positive control with the negative control. The negative control was carried out by means of 1 mL of 1% KMnO₄ mixed with 1 mL of distilled water, while the positive control was carried out by means of 1 mL of standard solution of 100 ppm vitamin C reacted with 1 mL of 1% KMnO₄ and 1 mL of distilled water. For samples, 2 mL of sample filtrate was reacted with 1 mL of 1% KMnO₄ and 1 mL of distilled water. The color change to brown indicates the presence of ascorbic acid (Sari et al., 2021).

Quantitative Analysis

Preparation of Vitamin C Standard Solution 100 ppm

The standard solution of vitamin C 100 ppm was made by weighing 10 mg of standard standard vitamin C (ascorbic acid), then put into a 100 mL volumetric flask, and dissolved with distilled water to the mark to obtain a standard solution of vitamin C 100 ppm (Chandra et al., 2019).

Preparation of Vitamin C Standard Solution 50 ppm

A standard solution of 50 ppm vitamin C was made by pipetting as much as 25 mL of a standard solution of 100 ppm vitamin C, then placed into a 50 mL volumetric flask to obtain a concentration of 50 ppm. After that, distilled water was added to the mark, so that a standard solution of 50 ppm ascorbic acid was obtained (Chandra et al., 2019).

Determination of Maximum Wavelength of Vitamin C Solution

Standard solution of 50 ppm vitamin C was taken as much as 4 mL using a measuring pipette, then placed into a 10 mL volumetric flask to obtain a concentration of 20 ppm. Aquades were added to the mark and homogenized. After that, the maximum wavelength was checked, with a wavelength range of 200-400 nm and for the blanks to be used was distilled water (Chandra et al., 2019).

Preparation of Standard Curve for Vitamin C Solution

The standard solution was pipetted with a concentration of 50 ppm each as much as 2; 2.4; 2.8; 3.2; 3.6; and 4 mL (10; 12; 14; 16; 18; and 20 ppm), placed in a 10 mL volumetric flask, and mixed with distilled water up to the mark and dissolved. After that, the absorption is checked with the maximum wavelength that has been set and repeated three times (Chandra et al., 2019).

Determination of Accuracy

The accuracy test was carried out using the standard addition method. The sample solution was taken according to the concentration in the assay. Then the concentration of the standard solution of vitamin C was added as much as 80%-120%, so that the average standard solution taken was 2.91 mL (14.559 ppm) for a concentration of 80%; 3.64 mL (18.199 ppm) for 100% concentration; and 4.37 mL (21.838 ppm) for a concentration of 120%. The absorbance was measured at a predetermined maximum wavelength and the experiment was repeated three times. Acceptable accuracy values in the range of 80-120% (Yunita et al., 2019).

Determination of Precision

2 standard solutions of 50 ppm were taken; 2.4; 2.8 mL and each solution was placed into a 10 mL volumetric flask, then added with distilled water to the mark limit and obtained a concentration of 10; 12; 14 ppm. Absorbance is measured at a predetermined maximum wavelength. This solution was made three times (Yunita et al., 2019).

Determination of Vitamin C Levels

The diluted sample solution was checked for absorption at a predetermined maximum wavelength of vitamin C and carried out three times for each sample (Chandra et al., 2019).

Processing and data analysis

Vitamin C levels were calculated using a linear regression equation obtained from the vitamin C standard curve data. The data were processed and analyzed using the Microsoft Excel 2013 program. The data will also be statistically tested using the IBM SPSS program using the paired sample t-test or paired t-test. The data was first tested by homogeneity test and normality test, then continued with paired sample t test to see whether or not there was a significant difference in average vitamin C levels between fresh fruit and candied fruit in crystal guava.

RESULTS

Sampling

Crystal guava samples were taken from a plantation located on Jalan Serelek No. 11 Cikarawang Carang Village returns, Dramaga Bogor Regency. The criteria for crystal guava used by the researchers, namely the part of the fruit that was taken from the flesh, 4 months old, and ripe using the UV/Vis spectrophotometric method.

Vitamin C Color Test

Qualitative analysis of vitamin C content in fresh and candied guava fruit was carried out by testing the color of vitamin C using 1% KMnO₄ reagent. It was found that all samples of both fresh and candied fruit showed a color change from purple to brown. All samples showing positive results were compared with the (+) and (-) controls. The color test of vitamin C with 1% KMnO₄ reagent can be seen in table 1.

Table 1. Vitamin C Color Test With 1% KMnO₄ Reagent

Sample	Color	Results
Control -	Purple	-
Control +	Chocolate	+
Fresh Fruit Replication 1	Chocolate	+
Fresh Fruit Replication 2	Chocolate	+
Fresh Fruit Replication 3	Chocolate	+

Candied Fruit Replication 1	Chocolate	+
Candied Fruit Replication 2	Chocolate	+
Candied Fruit Replication 3	Chocolate	+

Information :

(+) : Positive contains vitamin C / reaction occurs

(-) : Negative contains vitamin C / no reaction occurs

Determination of the Maximum Wavelength of Vitamin C

Determination of the maximum wavelength for vitamin C in the range of 200-400 nm, the maximum absorbance value of vitamin C was 0.491 with a wavelength of 290 nm. The determination of the maximum wavelength of vitamin C can be seen in Figure 1.

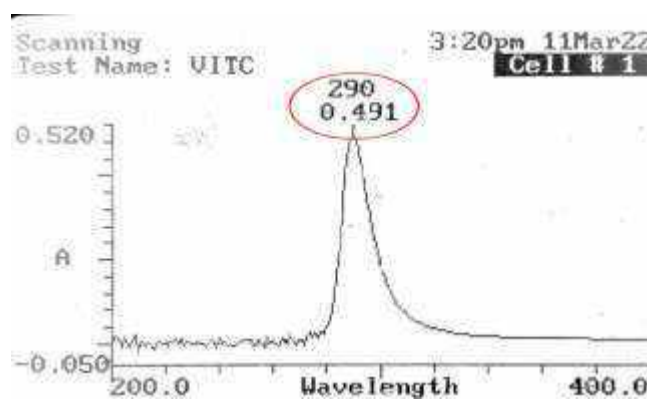


Figure 1. Maximum Wavelength

Determination of Vitamin C Standard Curve

The standard curve for vitamin C was performed using six concentration series, namely 10, 12, 14, 16, 18, and 20 (ppm), the regression equation $y = 0.0057x + 0.3116$ with a value (r^2) of 0.9981 was obtained. The standard curve for vitamin C can be seen in Figure 2.



Figure 2. Vitamin C Standard Curve

Determination of Precision

The determination of precision was carried out three times with the selected concentrations of 10 ppm, 12 ppm, and 14 ppm, the % RSD value was 1.71%, respectively; 1.42%; and 1.82%. The table of precision determination results can be seen in table 2.

Table 2. Determination of Precision

Replication	Concentration (ppm)	Absorbance at 290 nm	Rate (ppm)
1	10	0.370	10,246
2	10	0.369	10,070
3	10	0.371	10,421
Average		10,246	
SD		0.175	
RSD (%)		1.71%	
1	12	0.381	12,175

2	12	0.382	12,351
3	12	0.383	12,526
Average		12,351	
SD		0.175	
RSD (%)		1.42%	
1	14	0.394	14,456
2	14	0.397	14,982
3	14	0.396	14,807
Average		14,749	
SD		0.268	
RSD (%)		1.82%	

Determination of Accuracy

Determination of accuracy using the standard addition method was replicated three times. The analyte levels added were 2.92 ml (80%), 3.64 ml (100%), and 4.37 ml (120%). The result of % Recovery value is 101.22-102.03%, with an average % Recovery obtained is 101.70%. The data on the results of determining the accuracy can be seen in table 3.

Table 3. Determination of Accuracy

Range (%)	Replication	Average Concentration Standard (ppm)	nal Concentration (ppm)	% Recovery
80%	1	14,559	35,333	107.25%
	2		31,474	97.61%
	3		32,000	98.81%
	Average		101.22%	
100%	1	18,199	38,667	104.12%
	2		35,509	100.26%
	3		36,035	101.22%
	Average		101.87%	
120%	1	21,838	42,351	103.63%
	2		38,491	97.21%
	3		40,596	105.24%
	Average		102.03%	
			Total average	101.70%

Determination of Vitamin C Levels

Determination of vitamin C content using fresh fruit samples and candied guava crystals with each sample being replicated three times. The results obtained for vitamin C levels with an average of 1,820 mg/2.5g, while in candied fruit the results of vitamin C levels with an average of 5,902 mg/2.5g. The data on the results of the determination of vitamin C levels can be seen in table 4.

Table 4. Determination of Vitamin C Kadar Levels

Sample	Absorbance	Concentration End sample (ppm)	Rate Sample Vitamin C (mg/2.5g)	Rate Sample Vitamin C (%w/w)
Fruit fresh	0.424	19,719	1,972	0.08
	0.410	17,263	1,726	0.07
	0.412	17,614	1,761	0.07
		Average	1,820	0.07%
Sweets Fruit	0.643	58,140	5,814	0.23
	0.649	59,193	5,919	0.24
	0.652	59,719	5,972	0.24
		Average	5,902	0.24%

Data Analysis Processing

Processing of data analysis using parametric statistical tests, namely the paired sample t-test or paired t. The results of the homogeneity test on the results of the average vitamin C content with a significance value of 0.280, the normality test on the results of the average vitamin C content with a significance value for fresh fruit of 0.252 and candied fruit of 0.641, and a parametric test using paired t-test obtained a significance value of 0.001. The statistical test can be seen in table 5.

Table 5. Test Statistics

Statistic test	Results
Number of samples (N)	6
Homogeneity	0.280
Normality	a. Fresh fruit 0.252 b. Candied fruit of 0.641
Paired t test or paired sample t-test	0.001

DISCUSSION

Vitamin C Color Test

The qualitative analysis of vitamin C with 1% KMnO₄ reagent aims to determine the presence of an oxidation-reduction reaction between KMnO₄ and vitamin C in the sample (Jayanti et al., 2021). A positive result for the presence of vitamin C is indicated by the change of the sample solution to brown (Sari et al., 2021). The reaction between potassium permanganate (KMnO₄) and vitamin C which acts as an oxidizing agent is potassium permanganate (KMnO₄), which means that KMnO₄ is reduced in this reaction. Vitamin C acts as a reducing agent, that is, a substance that oxidizes vitamin C in reactions. Potassium permanganate (KMnO₄) is reduced by vitamin C, turning purple permanganate ions (MnO₄⁻) into a brown precipitate, indicating the formation of manganese ions (Mn²⁺). Vitamin C on the other hand, is oxidized by permanganate to dehydroascorbic acid (Chandra et al., 2019). According to the research of Sari et al (2021), the qualitative test of tomato HST from the 45th to the 72nd day showed positive results containing vitamin C, indicated by a change in the color of the sample solution to brown. The reaction of vitamin C with KMnO₄ can be seen in Figure 1.

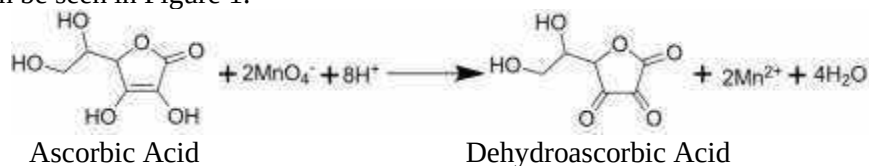


Figure 1. Reaction of Vitamin C with KMnO_4 (Yuliansi et al., 2020)

Determination of Vitamin C Wavelength

The maximum wavelength is the wavelength at which the substance provides maximum absorption (Apriliyana et al., 2018). The purpose of checking the maximum wavelength is to determine the absorption area that can be produced in the form of the absorbance value of a standard solution of vitamin C with the absorption measured by a UV-Vis spectrophotometer in the wavelength range of 200-400 nm (Sukmawati et al., 2018). Scanning determination of the wavelength of vitamin C obtained in the range of 200-400 nm in this study was 290 nm with a concentration of 20 ppm in distilled water. Research conducted by Chandra et al (2019), obtained 265 nm as the maximum wavelength for vitamin C. This difference is due to a shift in the absorption band of vitamin C. This can occur because the molecular structure of vitamin C binds the auxochrome group to the chromophore group, so that When the auxochrome group binds to the chromophore group, a red shift (bathochromic) occurs. Bathochromic shift is a shift of the absorption band to a longer wavelength with an increase in the intensity of absorption called the hyperchromic effect (Sayuthi and Kurniawati, 2017). The structure of vitamin C which shows the chromophore and auxochrome groups can be seen in Figure 2.



Figure 2. Vitamin C Structure Showing Chromophore and Auxochrome Groups (Yuliansi et al., 2020)

Determination of Vitamin C Standard Curve

The calibration curve is a standard method that can be used to determine the analyte concentration according to the Lambert-Beer rule (Sayuthi and Kurniawati, 2017). The determination of the calibration curve in this study was replicated three times using six concentration series, namely 10, 12, 14, 16, 18, and 20 ppm. A series of solution concentrations were measured for each absorbance at 290 nm, the maximum wavelength of vitamin C. The measurement results showed that the higher the concentration of the measured standard solution of vitamin C, the higher the absorbance obtained. This is because the higher the concentration, the higher the concentration of vitamin C compounds. Vitamin C standard solutions (x-axis) and absorbance (y-axis) are plotted to create a standard calibration curve, which is connected by a straight line (Sayuthi and Kurniawati, 2017). The terms of the correlation coefficient (r) obtained must be $-1 \leq r \leq 1$ (Yunita et al., 2019). Based on the results of the determination of the standard curve for vitamin C, the equation of the line is obtained, namely $y = 0.0057x + 0.3116$, with the correlation coefficient (r) is 0.999 and the coefficient of determination (r^2) is 0.9981. Based on research conducted by Yunita et al (2019), the equation of the line $y = 0.069x - 0.01$ with a correlation coefficient (r) = 0.999 produces linear results. This correlation coefficient meets the acceptable criteria, so the determination of vitamin C levels by UV-Vis spectrophotometry has good linearity.

Determination of Precision

In this study, the precision used was the repeatability category, namely by observing the absorbance of the standard solution of vitamin C at three concentrations, namely 10, 12, and 14 ppm and measuring the absorbance three times. The precision value is calculated using the standard deviation (SD) and relative standard deviation (RSD) or coefficient variation (CV). Careful criteria are given if the method gives the %RSD value less than equal to 2% ($\leq 2\%$) (Sayuthi and Kurniawati, 2017). The results obtained were 1.71%; 1.42%; and 1.82%, this indicates that the test method used to validate the method of measuring vitamin C levels in samples using UV-Vis spectrophotometry meets the requirements for acceptable % RSD values.

Determination of Accuracy

This study uses the standard addition method, meaning that the sample is analyzed and then a certain amount of analyte being examined is added to the sample, mixed, and analyzed again. Accuracy helps determine how close the measurement result is to the actual result. % recovery to express accuracy, so the smaller the difference between the two, the more accurate the analysis method (Yunita et al., 2019). The % recovery results obtained are 101.22-102.03%. The requirement for % recovery is acceptable if it is in the range of 80 – 100% of the actual level (Harmita, 2004). The % recovery value obtained in this study is in the range, so this method can be said to be very accurate.

Determination of Vitamin C Levels

Determination of the levels of vitamin C samples is the final step carried out in this study, by measuring the absorbance of the sample solution that has been prepared using UV-Vis spectrophotometry at the maximum wavelength that has been obtained, which is 290 nm. The results in this study the average level of vitamin C in 100 grams of crystal guava in fresh fruit is 72.8 mg/100 g, while candied fruit is 236.08 mg/100 g. The results obtained were that the levels of vitamin C in fresh fruit of crystal guava were smaller than candied fruit of crystal guava. In theory, the levels of vitamin C in candied guava fruit are smaller than fresh fruit, because vitamin C is easily oxidized by outside air and easily soluble in water (Kurniawati and Riandini, 2019).

In this study, there were several causes of higher levels of vitamin C in candied fruit than fresh fruit, including: crystal guava fruit that will be made into candied fruit does not undergo a heating process. The similarity of vitamin C levels in candied fruit is higher than that of fresh fruit. Niswah et al (2016) conducted a study on vitamin C levels on the effect of processing fresh manalagi mango into dried candied fruit. The yield of vitamin C levels in dried candied fruit was higher than that of fresh mango manalagi, with the yield on fresh fruit of 3.08 mg/100 g while the dried candied fruit was 5.28 mg/100 g. This can occur due to the addition of food additives such as citric acid, sugar, and lime. The addition of citric acid

can affect the content of vitamin C, This means that the higher the citric acid content used, the higher the vitamin C content. The shorter low temperature boiling process and the low lime solution content can affect the vitamin C content of dried mango candied. Fruit ripeness can also affect vitamin C content, in other words, the more ripe the fruit, the higher the vitamin C content (Niswah et al., 2016). Citric acid serves to maintain the content of vitamin C, which in acidic conditions can inhibit the process of oxidation reactions (Putri, 2016). Citric acid can be used to preserve food, because citric acid is a good natural preservative. Natural citric acid is widely contained in fruits such as various types of oranges, pineapples, starfruit, pears, and so on (Wardani, 2018).

Data Analysis Processing

The results obtained from the statistical test table for the homogeneity test and normality test, where the significance value is > 0.05 , as the basis for decision making, it can be concluded that the average results of vitamin C levels in fresh and candied fruit samples are the same or homogeneous and normally distributed. The results of the paired sample t test showed a 2-way (t-tailed) significance value for the variable mean levels of vitamin C samples in fresh fruit and candied fruit of 0.001. The significance value is $0.001 < 0.05$, so as the basis for making decisions in the paired t test above, it can be concluded that the average levels of vitamin C samples in candied fruit and fresh fruit are H_0 rejected, H_1 is accepted, which means that there is a difference in the mean levels of vitamin C significance between fresh fruit and candied crystal guava fruit.

CONCLUSION

Based on the results of the determination of vitamin C levels in fresh fruit and candied guava (*Psidium guajava* L.) using the UV-Vis spectrophotometric method, it can be concluded that the average vitamin C content obtained in fresh fruit samples is 1.820 mg/2.5 g or 72.8 mg/100 g and candied fruit samples of 5.902 mg/2.5 g or 236.08 mg/100 g. Based on the paired t-test showed that there was a significant difference in the average vitamin C levels between fresh fruit and candied fruit.

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ANALYSIS OF RHODAMINE B IN LIPSTICK SOLD ON MARKETPLACE X USING TLC METHOD (THIN LAYER CHROMATOGRAPHY)

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Abstract

Introduction: Rhodamine B is a chemical dye used for textile dyes and is prohibited by BPOM in cosmetics, especially lipsticks. This study aims to determine the presence of Rhodamine B contained in lipstick preparations sold in Marketplace X.

Method: A total of 13 lipstick samples sold on Marketplace X were selected based on predetermined criteria. This study used silica gel GF₂₅₄ stationary phase with several compositions and types of mobile phase, namely ethyl acetate:methanol:ammonia (15:6:3), ethyl acetate:methanol:ammonia (25:6:1), n-butanol:ethanol absolute:aquades (20:12:15), ethyl acetate:methanol:(ammonia+aquades) (15:3:3), ethyl acetate:methanol:ammonia (15:3:3), and n-butanol:ethanol absolute:aquades (55:20:25). The mobile phase was selected based on the criteria of R_f value of 0.2-0.8 and good separation with a value of R_s 1.5. The data were processed and presented in tabular form, and analyzed using descriptive analysis to determine the description of Rhodamine B.

Results: The optimal mobile phase with n-butanol ethanol absolute:aquadest (55:20:25) resulted in an average R_f value of 0.76 and an R_s value of 3.96. The results showed 2 from 13 samples were positive Rhodamine B (code L3 and L7) with a difference of R_f 0.2 with orange fluorescence stains. Consumers are expected to be vigilant in choosing cosmetic products by looking at the distribution permit number.

Conclusion: This study concludes that the optimal mobile phase for separating Rhodamine B were n-butanol ethanol absolute:aquadest (55:20:25). A total of 2 positive samples (code L3 and L7) from 13 lipstick samples.

Keywords: Rhodamine B, Lipstick, Thin Layer Chromatography, Optimization, Mobile Phase

INTRODUCTION

The more increase for cosmetic purposes, cosmetics with various prices and brands will appear. A marketplace is a platform that markets electronic products by bringing together many buyers and sellers to transact with each other (Apriadi et al., 2017). With the development of today's era, women have switched to using advanced technology for everything. One of them is how to buy goods through the Marketplace. According to the Central Statistics Agency in 2020, as many as 25.72% of sales accounts on Marketplace, and in 2019 sales of cosmetics sold on E-commerce increased by 8.87%, based on a survey in January 2018 it was found that the majority of online shopping consumers were women with a figure reached 65% (BPS, 2021). The use of cosmetics increases every year, data according to BPOM shows that the number of registered cosmetic products from 2017-2021 is 212,980 products. There was an increase in 2021 starting from July - December by 60.01% as many as 82,165 products (BPOM, 2021).

Rhodamine B is a substance that contains carcinogenic properties and in excess levels can cause liver damage. The use of lipsticks containing Rhodamine B can cause adverse effects such as irritation to inflammation in the lip area which results in decreased appetite. If it occurs in the long term it can cause cancer (Syamsuri, 2017). Evidence found in animal testing of Rhodamine B based on toxicity test has an LD₅₀ 2000 mg/kg causing strong irritation of the mucous membranes and toxicity tests with subcutaneous and oral injections to cause local sarcoma carcinogens, while in IV LD₅₀ 89.5 mg/kg kidney enlargement occurs, liver and spleen (Ristianingrum et al., 2018). BPOM has banned the use of Rhodamine B with another name red K10 or coloring agent CI 45170 in cosmetic preparations (BPOM RI, 2019).

METHOD

Tools and Materials

The tools used include TLC silica gel 60 F₂₅₄ plates (merck), oven (Ika), analytical balance (Ohaus), porcelain cup, measuring cup (Iwaki) 25 mL, volumetric flask (Iwaki) 100 mL, 25 mL, and 10 mL, UV lamp (CAMAG), chamber (CAMAG), water bath, stirring rod, watch glass, funnel (Iwaki), filter paper, and dropper, bulb pipette, spatula, beaker glass (Iwaki) 100 mL, micropipette 20 µL (Soccorex).

The materials used include: Lipstick, Rhodamine B p.a (Sigma-aldrich), methanol absolute pa (Supelco), ethyl acetate p.a (Supelco), ammonia p.a (Supelco), n-Butanol p.a (Supelco), ethanol absolute p.a (Supelco), aquadest, HCl p.a

Procedures

1. Sampling

The sample used in this research is lipstick sold on Marketplace X. A total of 13 samples of lipstick sold on Marketplace X were selected based on predetermined sampling criteria.

2. Mobile Phase Optimization of Samples

a. Mobile Phase Creation

The mobile phase used consists of:

- 1) n-Butanol : Ethanol absolute : Aquadest (20 : 12 : 15) (Fatimah *et al.*, 2016)
- 2) Ethyl acetate : Methanol : Ammonia (25 : 6 : 1) (Ratnaningtyas, 2013)
- 3) Ethyl acetate : Methanol : Ammonia (15 : 6 : 3) (Riyanti *et al.*, 2018)
- 4) Ethyl acetate : Methanol : (Ammonia+Aquadest) (15:3:3) (Jusnita dan Nandu, 2016)
- 5) Ethyl acetate : Methanol : Ammonia (15:3:3) (Jusnita dan Nandu, 2016)
- 6) n-Butanol : Ethanol absolute : Aquadest (55:20:25) (Riyanti *et al.*, 2018)

The method of making the mobile phase is done by inserting the mobile phase into a 30 mL volumetric flask with the stated ratio (Lero, 2021).

b. Preparation of Comparative Standard Solution

An amount of 10 mg of standard Rhodamine B dye was dissolved in a 100 mL volumetric flask with methanol pa to the limit mark. The solution was homogenized (100 µg/mL) (Elfasyari *et al.*, 2020).

c. Preparation of Test Solution

A total of 2 grams of the lipstick sample was poured into a porcelain cup, then 16 drops of 4 M concentrated HCl were added and 20 mL of methanol was added and then melted over a water bath until it melted. Furthermore, the liquid is filtered through filter paper that has been given 0.1 g of anhydrous sodium sulfate and the filtrate is taken. Then put in a water bath to be concentrated, then the filtrate is put into a vial. Then 2 ml of the filtrate was taken and 1 mL of Rhodamine B standard solution was added with a ratio of 2:1 and then put into the vial (Tjuana *et al.*, 2021).

d. Determination of Optimum Conditions

The selection of the mobile phase was carried out to obtain chromatography with circular, non-widening, and not tailing spots. Optimization was carried out by spotting the Rhodamine B comparison solution spot and then eluted with various mobile phases, namely n-butanol : ethanol absolute : aquadest (20 : 12 : 15), ethyl acetate : methanol : ammonia (25 : 6 : 1), ethyl acetate : methanol : ammonia (15 : 6 : 3), ethyl acetate : methanol : (ammonia+aquadest) (15:3:3), ethyl acetate : metahnol : ammonia (15:3:3), dan n-butanol : ethyl acetate : ammonia (55:20:25).

The mobile phase was first saturated in the chamber using filter paper for 10 minutes before the plate was inserted into the chamber. The TLC plate was cut to a size of 4x10 cm and was heated in an oven at 100°C for 30 minutes. Then, 3 L of the standard solution and the test solution were applied using a micropipette starting from a distance of 1 cm below the plate. Between the stains are spaced 1 cm. Then the plate is dried using a hair dryer. The TLC plate that has been dotted with the analyte is inserted into a chamber that has previously been saturated using the mobile phase. The plate was allowed to stand until optimally eluted, then removed, and then dried using room temperature.

Observe the color visually with 254 nm and 366 nm UV lamps, if the pink spots are visible and under ultraviolet light, they fluoresce pink or orange, it can be said that the sample contains Rhodamine B. The R_f value of the test solution is calculated and then compared with the R_f value of the solution. parent. Then it was replicated three times. Confirmed by using a spray reaction of concentrated HCl pa 4M. Re-observations were made on 254 nm and 366 nm UV

lamps with HCl spray as a reagent to produce orange fluorescence indicating samples containing Rhodamine B (Tjuana *et al.*, 2021).

3. Qualitative Analysis

a. Preparation of Test Solution

A total of 0.5 grams of the lipstick sample was poured into a porcelain cup, then 16 drops of concentrated 4 M HCl & 5 mL of methanol were added and then melted in a water bath until melted. Furthermore, the liquid is filtered through filter paper that has been given anhydrous sodium sulfate and the filtrate is taken. Then it is placed in a water bath to be concentrated, then the filtrate is put into a vial (Tjuana *et al.*, 2021).

b. Sample Identification

The TLC plate was cut to a size of 4x10 cm and was heated in an oven at 100°C for 30 minutes. Then smear the standard solution and the test solution using a micropipette as much as 3 µL starting from a distance of 1 cm below the plate. Between the stains are spaced 1 cm. Then the plate is dried using a hair dryer. The TLC plate that has been stained with the analyte is put into a chamber that has previously been saturated using the selected mobile phase, namely n-butanol : ethanol absolute : aquadest (20 : 12 : 15). The plate was allowed to stand until optimally eluted, then removed and then dried using room temperature. Observe the color visually under UV light of 254 nm and 366 nm, if the spots are pink and under ultraviolet light, they fluoresce pink or orange, it can be said that the sample contains Rhodamine B. The R_f value of the test solution was calculated and then compared with the R_f value of the mother liquor. Then it was replicated three times. It was confirmed by using a spray reaction of concentrated HCl pa 4 M. Re-observation was carried out on UV lamps at 366 254 nm and nm with HCl spray as a reagent, which resulted in orange fluorescence indicating samples containing Rhodamine B (Tjuana *et al.*, 2021).

RESULTS

A. Mobile Phase Optimization Results

The results of the R_f value and resolution obtained from the optimization of the six types of mobile phase composition are presented in the following table.

Table 1. R_f Value and Resolution of Mobile Phase Composition

Mobile Phase	Sample	R _f result		Resolution	Information
		R _f Rhodamine B (cm)	Nearest Spot R _f (cm)		
n-Butanol: Ethanol absolute: Aquadest (20:12:15)	+ control (Rhodamine B)	0.66			
	Replication 1	0.66	0.76	5.35	Homogeneous

	Replication 2	0.66	0.75	3.03	
	3 replication	0.66	0.77	3.53	
Ethyl acetate : Methanol: Ammonia (25:6:1)	+ control (Rhodamine B)	0.31	0.39	2.64	
	Replication 1	0.3	0.38	3.05	
	Replication 2	0.3	0.38	3.05	Homogeneous
	Replication 3	0.3	0.38	2.44	
	+ control (Rhodamine B)	0.8	0.84	3.53	
	Replication 1	0.79	0.84	5.35	
Ethyl acetate : Methanol: Ammonia (15:6:3)	Replication 2	0.8	0.84	3.03	Homogeneous
	3 replication	0.81	0.85	3.53	
	+ control (Rhodamine B)	TD	TD	TD	
Ethyl acetate: Methanol : (Ammonia+Aquades) (15:3:3)	Replication 1	TD	TD	TD	Inhomogeneous
	Replication 2	TD	TD	TD	
	3 replication	TD	TD	TD	
	+ control (Rhodamine B)	TD	TD	TD	
Ethyl acetate : Methanol: Ammonia (15:3:3)	Replication 1	TD	TD	TD	Inhomogeneous
	Replication 2	TD	TD	TD	
	3 replication	TD	TD	TD	
	+ control (Rhodamine B)	TD	TD	TD	
n-Butanol: Ethyl acetate: Ammonia (55:20:25)	Replication 1	TD	TD	TD	Inhomogeneous
	Replication 2	TD	TD	TD	
	3 replication	TD	TD	TD	
	+ control (Rhodamine B)	TD	TD	TD	
Conclusion	Of the 6 types of mobile phase mixtures, there are 3 types of homogeneous mobile phase mixtures that are used for the optimization of the mobile phase.				

Description: TD: Not Detected

Based on the results obtained, the best mobile phase was selected for qualitative analysis of the lipstick sample, namely the mobile phase mixture of n-Butanol : Etanol absolute : Aquades (20:12:15).

B. Results of Sample Identification with Selected Mobile Phase Mixture of n-Butanol : Ethanol absolute : Aquadest (20:12:15)

The results of the R_f value and the resolution obtained from the identification of samples with the selected mobile phase mixture of n-Butanol: Absolute ethanol: Aquades (20:12:15) are presented in the following table.

Table 2. R_f Value and Resolution of Identification Results of Lipstick Samples with N-Butanol
Mobile Phase: Absolute Ethanol: Aquadest (20:12:15)

Sample	Repetition	R _f Value	Average R _f	Difference R _f	R _f Difference Terms	Information
Control + Rhodamine B		0.72				
Sample 1	I	0.83	0.83	0.11	0.2	Positive
	II	0.83		0.11		Positive
	III	0.83		0.11		Positive
Control + Rhodamine B		0.76				
Sample 2	I	0.76	0.77	0	0.2	Positive
	II	0.76		0		Positive
	III	0.78		0.02		Positive
Control + Rhodamine B		0.73				
Sample 3	I	0.725	0.73	0.006	0.2	Positive
	II	0.725		0.006		Positive
	III	0.725		0.006		Positive
Control + Rhodamine B		0.6875				
Sample 4	I	0.7125	0.71	0.025	0.2	Positive
	II	0.7125		0.025		Positive
	III	0.7125		0.025		Positive
Control + Rhodamine B		0.78				
Sample 5	I	0.80	0.80	0.02	0.2	Positive
	II	0.80		0.02		Positive
	III	0.80		0.02		Positive
Control + Rhodamine B		0.68				
Sample 6	I	0.75	0.74	0.07	0.2	Positive
	II	0.74		0.06		Positive
	III	0.74		0.06		Positive
Control + Rhodamine B		0.64				
Sample 7	I	0.62	0.62	0.02	0.2	Positive
	II	0.62		0.02		Positive
	III	0.62		0.02		Positive
Control + Rhodamine B		0.79				
Sample 8	I	0.81	0.81	0.02	0.2	Positive
	II	0.81		0.02		Positive
	III	0.81		0.02		Positive
Control + Rhodamine B		0.66				

Sample 9	I	0.68	0.68	0.02	0.2	Positive
	II	0.68		0.02		Positive
	III	0.68		0.02		Positive
Control + Rhodamine B		0.75				
Sample 10	I	0.75	0.75	0	0.2	Positive
	II	0.75		0		Positive
	III	0.76		0.01		Positive
Control + Rhodamine B		0.74				
Sample 11	I	0.76	0.76	0.02	0.2	Positive
	II	0.76		0.02		Positive
	III	0.76		0.02		Positive
Control + Rhodamine B		0.74				
Sample 12	I	0.89	0.89	0.15	0.2	Positive
	II	0.9		0.16		Positive
	III	0.9		0.16		Positive
Control + Rhodamine B		0.75				
Sample 13	I	0.76	0.76	0.01	0.2	Positive
	II	0.76		0.01		Positive
	III	0.76		0.01		Positive
Conclusion	A total of 13 lipstick samples were positive based on the difference in R _f between the lipstick samples and the Rhodamine B . standard					

A. Observation Results of Lipstick Samples with Visible Light, UV Light 254nm and 366nm after Spraying 4M HCl

The results of the observation of lipstick samples with visible light and under UV light of 254nm and 366nm after being sprayed with 4 M HCl are presented in the following table.

Table 3. Results of Observation of Spots in UV Rays of 254nm and 366nm After Spraying with 4 M HCl

Sample	Visible Light	UV rays		Information
		254nm	366nm	
Sample 1	Pink	TT	TT	Negative
Sample 2	Pink	TT	TT	Negative
Sample 3	Pink	TT	TT	Negative
Sample 4	Pink	T	T	Positive, Fluorescent Orange
Sample 5	Pink	TT	TT	Negative
Sample 6	Pink	TT	TT	Negative

Sample 7	Pink	T	T	Positive, Fluorescent Orange
Sample 8	Pink	TT	TT	Negative
Sample 9	Pink	TT	TT	Negative
Sample 10	Pink	TT	TT	Negative
Sample 11	Pink	TT	TT	Negative
Sample 12	Pink	TT	TT	Negative
Sample 13	Pink	TT	TT	Negative
Conclusion: From the 13 lipstick samples, there were 2 positive lipstick samples containing Rhodamine B which were indicated by orange fluorescence stains.				

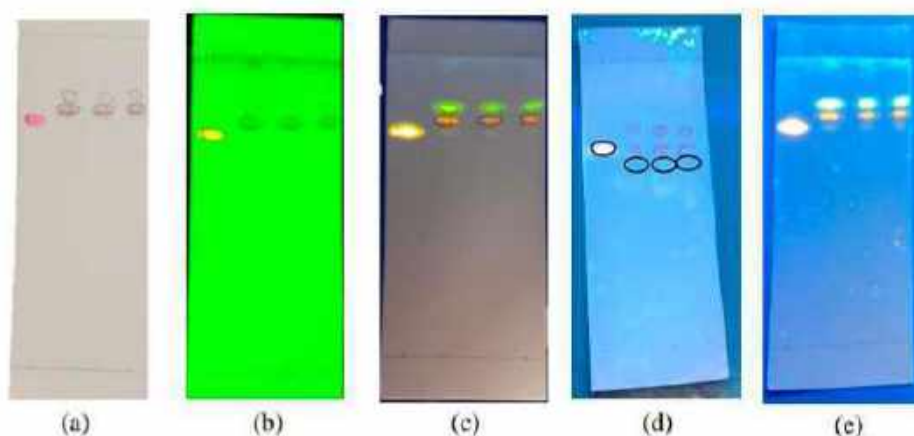


Figure 1. Results of Identification of Positive Stains for Lipstick Samples Code L3 (a) Visible Light (b) 254nm UV Light (c) 366nm UV Light (d) 254 nm UV Light after Spraying (e) 366nm UV Light after Spraying HCl 4 M

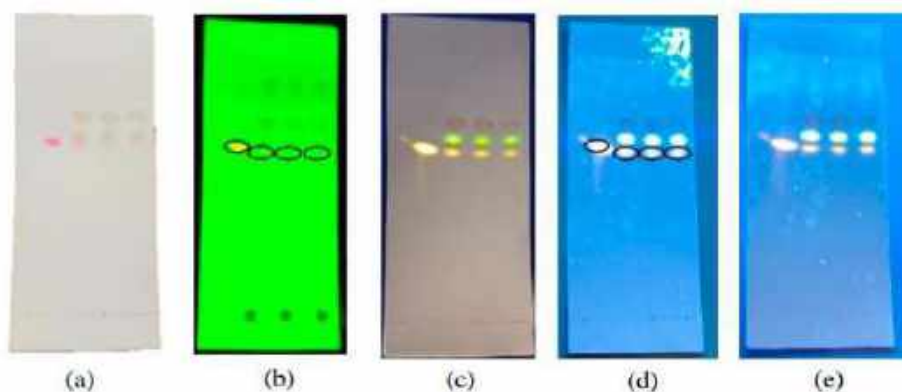


Figure 2. Results of Identification of Positive Stains for Lipstick Samples Code L7 (a) Visible Light (b) 254nm UV Light (c) 366nm UV Light (d) 254 nm UV Light after Spraying (e) 366nm UV Light after Spraying HCl 4 M

DISCUSSION

The sample used in this study is lipstick cosmetic preparations with the following criteria: red lipstick, the affordable price is around Rp. 1,000 – Rp. 25,000-star seller shops in the marketplace with a star rate of 4.0 – 4.9 the selection of prices and rates is because many women are interested in buying cosmetics at affordable prices and buying cosmetic products in stores that are widely purchased with good reviews, selected cosmetic packaging in composition using foreign language because if consumers lack understanding in foreign languages so they do not know the hazardous substances contained in the purchased product and the packaging, there is no distribution permit number from the Ministry of Health and a notification number from BPOM because the product has not been registered with BPOM so the product has not been carried out. product safety testing by BPOM.

The separation of Rhodamine B in lipstick samples is influenced by the type and composition of the mobile phase so to obtain an optimal separation, it is necessary to optimize the type and composition of the mobile phase (Ediningtyas, 2012). Optimization of the method is done by optimizing the length of the elution distance and the composition of the mobile phase that has been made (composition I, II, III, IV, V, and VI). A good R_f value is expected in the range of 0.2-0.8 (Gandjar and Rohman, 2012); A good resolution value if you think about a value of 1.5 and produces a round stain without tailings shaped like a ribbon (Wulandari, 2011).

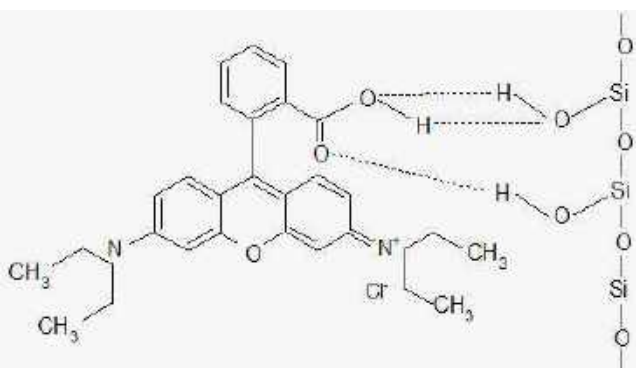


Figure 3. Hydrogen Bonding between Rhodamine and Silanol Group
----- = Hydrogen Bond (Ediningtyas, 2012)

The stationary phase used is silica gel GF₂₅₄ because silica gel has a small and uniform particle size so that resolution and efficient separation can be produced. The surface of the silica gel consists of Si-O-Si groups and Si-OH (silanol) groups. The presence of silanol groups can form hydrogen interactions with the analyte. The hydrogen interaction that occurs between Rhodamine B and the stationary phase is due to the presence of O atoms in Rhodamine B which can form hydrogen interactions with the stationary phase, so Rhodamine B will be retained in the stationary phase (Lero, 2021).

In addition to the analyte, the silanol group can also form hydrogen interactions with aquades which makes the silica gel deactivated so that before use, the silica gel needs to be heated at a temperature of 100°C for 30 minutes. This is done to reactivate the surface of the silica gel so that the silanol groups can interact with the analyte. GF254 shows that the fluorescent compound added to the plate has an excitation wavelength of 254 nm. The compound added is usually in the form of manganese or phosphorus-activated zinc silicate (Ediningtyas, 2012).

The composition of the mobile phase I was based on a previous study conducted by (Fatimah *et al.*, 2016) using a mixture ratio of n-butanol: ethanol: aquades (20: 12: 5) with a total volume of 30 mL. Optimization of the composition of the mobile phase needs to be done because the samples used are different. In a study conducted by Fatimah *et al.* (2016) the sample studied was a cake containing Rhodamine B. Optimization through the selection of the mobile phase aims to obtain an appropriate composition for the sample used in this study. The sample used in this study is lipstick sold in Marketplace X.

Based on Table 5.3, 6 types of mobile phase mixtures were made, each of which consisted of 3 types of solvents with different polarity indices. The composition of the mobile phase I, namely n-butanol: ethanol: aquades (20: 12: 5), has a polarity index of 6.22; the composition of the mobile phase II, namely ethyl acetate: methanol: ammonia (25:6:1) of 4.70; the composition of the mobile phase III is ethyl

acetate:methanol:ammonia (15:6:3) of 5.28; the composition of mobile phase IV is ethyl acetate:methanol:(ammonia+aquades)(15:3:3) of 5.32; the composition of the mobile phase V, namely ethyl acetate:methanol:ammonia (15:3:3) of 5.31; The composition of mobile phase VI is n-butanol:ethyl acetate:ammonia (55:20:25) of 5.55. The migration speed of the sample components depends on the physicochemical properties of the stationary phase, the mobile phase, and the sample components. The difference in polarity is expected to provide good separation results and can be used for qualitative analysis of the next sample. The mobile phase that has been made is saturated into the chamber. Saturation was carried out using filter paper for 10 minutes before carrying out the elution process. The purpose of saturation of the chamber is to equalize the vapor pressure of the mobile phase used so that the separation can run well (Wulandari, 2011).

The function is given the upper and lower limits of the plate as a marker of the distance traveled by the eluent. The lower limit of the plate is made so that it is not submerged by the eluent. The purpose of spotting is to use a micropipette so that the spotting is small, because in TLC a good spotting is kept as small as possible to avoid widening the stain and if too many samples are used it will reduce the resolution (Yuniarto & Rosalina, 2019). The widening of the stain can interfere with the R_f value because it allows peak crushing to occur. Highlighting is done on the bottom line that has been made. Then let it sit for a while to dry. Next, the plate is carefully inserted into a closed chamber containing the eluent with the mobile phase positioned below the line. This TLC uses the ascending method. After reaching the upper limit,

The composition of the mobile phase I was based on previous research conducted by (Fatimah *et al.*, 2016) using a mixture ratio of n-butanol: ethanol: aquades (20: 12: 15) with a polarity index of 6.22. The results of the separation of Rhodamine B in the lipstick sample after being eluted by the mobile phase showed that Rhodamine B could be eluted with an R_f value of 0.67 with symmetrical stains and produced a good resolution value of 1, 2, 3 replications, namely 5.35; 3.03; 3.53 of these results are said to have met the requirements of good resolution because the value obtained is 1.5.

The composition of the mobile phase II is based on a previous study conducted by (Ratnaningtyas, 2013) using a mobile phase ratio of ethyl acetate: methanol: ammonia (25:6:1) with a polarity index of 4.70. The results of the replication R_f values 1, 2, and 3 were 0.43; 0.42; and 0.42 with the resolution value of replication 1, 2, and 3 of 2.42; 2.53; and 2.55. The results of the separation of Rhodamine B in the lipstick sample after being eluted by the mobile phase showed that Rhodamine B could be eluted with the standard R_f value of Rhodamine B of 0.30625 and the R_f value of replication samples 1, 2, and 3 was 0.3 based on the results obtained that met the requirements. a good range of R_f values is 0.2-0.8. The resolution values obtained from each replication 1, 2, and 3 were 3.05; 3.05; 2, 4375 based on the results obtained, the resolution value has met the requirements, namely 1.5. However, the stains obtained from the separation with the mobile phase did not produce good stains, then the separation between Rhodamine B stains and other stains was too close together.

The composition of the mobile phase III is based on previous research conducted by (Riyanti *et al.*, 2018) on lipstick samples, namely by using a ratio of ethyl acetate: methanol: ammonia (15:6:3) with a polarity index of 5.28 resulting from the Rhodamin standard R_f obtained is 0.91. The results of the separation of Rhodamine B in the lipstick sample after being eluted by the mobile phase showed that Rhodamine B could be eluted with the standard R_f value of Rhodamine B of 0.8 and the R_f value of the replication samples 1, 2, 3 was 0.79; 0.8; 0.80 based on the results obtained have met the requirements of a good R_f value range of 0.2-0.8. The resolution value obtained from each replication 1, 2, and 3 was 5.35; 3.03; 3.53 based on the results obtained the resolution value has met the requirements, namely 1.5. However,

The composition of the mobile phase IV-VI is not a good mobile phase because the mixture of the composition is not homogeneous and is formed into 2 separate phases which are divided into an upper and lower layer, the top layer is clear, and the bottom layer is cloudy. This does not meet the requirements of a good eluent, which has sufficient and stable purity (Wulandari, 2011). The solution was separated into 2 phases due to the difference in polarity of each type of solvent, so it was not continued for the separation of Rhodamine B compounds in the lipstick sample because it would affect the separation of the analyte to be not good.

The optimal separation between Rhodamine compounds and other analytes was shown in the

mobile phase of a mixture of n-butanol: ethanol: and distilled water (20: 12: 5). Other analyte stains originating from the mobile phase or other compounds from the lipstick sample did not interfere with the Rhodamine B stain because the Rhodamine B peak was already separated from other nearby stains from the mobile phase or other compounds from the lipstick sample as indicated by the resolution value. which is more than 1.5. The mixed mobile phase of polar and non-polar solvents can separate compounds well because the polar part of the mobile phase will interact with the polar part of the analyte, while the non-polar part will interact with the non-polar part of the analyte (Ediningtyas, 2012).

Next, a qualitative analysis was carried out on the lipstick sample. The addition of HCl destroys the compounds present in the lipstick sample, in this case, the sample is also added with methanol to melt the waxy substance in the lipstick with the help of heating so that the filtrate from the sample can be obtained, also because methanol is a polar organic solvent. and has a low boiling point so that it can dissolve organic substances which are also polar (Yuniarto & Rosalina, 2019). Filtration with anhydrous sodium sulfate aims to reduce the water content of the heating product (Yuniarto & Rosalina, 2019). Heating the TLC plate in an oven with a temperature of 100°C for 30 minutes aims to remove water molecules contained on the TLC plate,

To compare the R_f value and color, a standard comparison solution for the positive control was made. The positive control consisted of Rhodamine B. The eluent used was the eluent that was selected after the previous optimization was n-butanol: absolute ethanol: aquades with a ratio of 20:12:15. The eluent was allowed to saturate for ± 30 minutes for complete separation. The reason for closing the chamber is to ensure that the conditions in the chamber are saturated with solvent vapors (Lero, 2021). To obtain this condition, filter paper is placed in the chamber until it is moistened with solvent. Saturated conditions in the chamber prevent evaporation of the objective solvent from saturation to homogenize the mixture and remove water vapor/other gases in the absorbent phase which will hinder the eluent rate (Yuniarto & Rosalina, 2019).

Then calculate the R_f value, if the identification of the R_f value has the same value then the compound can be said to have the same or similar characteristics. Meanwhile, if the R_f value is different, the compound can be said to be a different compound. The greater the R_f value of the sample, the greater the movement distance of the compound on the thin layer chromatography plate. Then, the addition of a stain-seeking reagent by spraying with 4M HCl is needed to produce colored or fluorescent compound derivatives (Wulandari, 2011).

In general, a combination of aromatic compounds and some unsaturated compounds can absorb UV light (Wulandari, 2011). These compounds can be analyzed using thin layer chromatography with a stationary phase which is then detected by examination under 254 nm UV light. The results of the identification of Rhodamine B are listed in Table 5.4. showed that among the 13 lipstick samples, Rhodamine B was found in sample codes 3 and 7 where the UV light at 254 nm showed a yellow fluorescence sample, and visually observed the stain on the sample code that appeared on the TLC plate was pink. Rhodamine B will give yellow or orange fluorescence when observed at UV light at 254 nm and 366 nm, and pink when viewed visually, in addition, the results are declared positive if the color of the sample spots,

The R_f value of the lipstick sample with sample code 3 (0.71 cm), and sample code 7 (0.62 cm) is almost parallel to the R_f value of the Rhodamine B reference standard solution in sample code 3 of 0.69 cm, and the sample code 7 is 0.74 cm. While in Table 5.15. shows the sample codes 1, 2, 4, 5, 6, 8, 9, 10, 11, 12, and 13 the R_f value shows a difference of 0.2 with the reference standard solution which is parallel to the R_f value of the Rhodamine B reference standard solution, but after sprayed with a spray reagent using 4M HCl the stain does not fluoresce orange when observed under UV light at 254 nm it is said that the stain is not a Rhodamine B stain. The results obtained are the difference between the R_f value of the sample and the reference standard solution 0,

It was concluded that the samples with codes 3 and 7 were positive for Rhodamine B dye and when observed after spraying with 4M HCl then observed under UV light of 254 nm with orange fluorescence. The results of sample codes 1, 2, 4, 5, 6, 8, 9, 10, 11, 12, and 13 were not positive for Rhodamine B, seen from the visual test results that the stains that appeared were not pink and in ultraviolet light. 254 nm and 366 nm did not show the presence of orange fluorescence and the difference between the R_f value of the sample and the reference standard solution was 0.2, the result was declared negative.

CONCLUSION

Based on the results of the Mobile Phase Optimization research using the TLC (Thin Layer Chromatography) Method on the Analysis of Rhodamine B Content in Lipstick Cosmetic Preparations Sold in Marketplace X, it can be concluded that:

1. The best mobile phase was selected, namely n-butanol: ethanol: aquadest (20:12:15) because the R_f value and resolution obtained had met the requirements, namely for an R_f value of 0.2-0.8 and the best resolution value was 1.5 and resulted in stains that were not widened and not tailing, the separation between Rhodamine B stains was separated from other stains.
2. In the lipstick sample which was analyzed qualitatively using the TLC method with the best selected mobile phase, namely n-butanol: ethanol: aquadest (20:12:15) obtained as many as 2 positive samples from 13 samples, namely samples with codes 3 and 7 where the samples were there is no notification number from BPOM with the difference in the R_f value that is close to the comparison standard R_f value, namely the price difference 0.2. The R_f value of the lipstick sample with the code of sample 3 (0.71) and sample 7 (0.62). That is, the sample has violated the rules that have been made by BPOM which prohibits the addition of harmful ingredients to cosmetics, one of which is Rhodamin B based on BPOM No. 23 of 2019 regarding the technical requirements for cosmetic ingredients, Rhodamine B preparations are substances that are prohibited to be added to cosmetics.

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ANALYSIS OF RHODAMIN B IN EYESHADOW SOLD ON MARKETPLACE X USING TLC METHOD (THIN LAYER CHROMATOGRAPHY)

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Abstract

Introduction: The synthetic dye Rhodamine B is used for coloring paper, textiles, wool, silk, and as a reagent. Rhodamine B is prohibited for food coloring and cosmetics, as it irritates the eyes and skin, poisoning, impairs liver function, and causes cancer. The study aimed to determine the presence of Rhodamine B in eyeshadow sold in the marketplace X

Method: Identification of Rhodamine B compounds using TLC with mobile phase optimization. The design of this research is experimental with a purposive sampling technique. Sample in pink, red, and deep red, solid powder, imported or local products, no composition label listed, price from Rp. 5,000 – Rp. 50,000, no Expired date recorded, and no Notification number from National Agency of Drug and Food Control. R_f and Resolution value data are processed using descriptive statistics in the form of tables.

Result: The optimal motion phase is n-butanol : Ethanol : Aquadest (20:12:15), samples that showed positive Rhodamine B produced round/elliptical and tailless stains. Visually, it offers light pink colors, and under UV light, 254 and 366 show yellow or orange. Sample 4 with an average R_f value of 0.64 and Resolution of 3,07. Sample 8 with an average R_f value of 0.66 and Resolution 3,57 and sample 9 with an average of 0,79 and Resolution 2,03.

Conclusion: This study concluded that 3 out of 10 samples contain Rhodamine B.

Keywords: Rhodamine B, Eyeshadow, Thin Layer Chromatography, Mobile Phase Optimization

INTRODUCTION

The pandemic caused people's shopping patterns to change from offline to online by 80%. Data obtained from the Coordinating Ministry for Economic Affairs in 2020 shows an increase in online transactions for body care products, especially cosmetics and spas (Kemenperin, 2021). BPOM reports that there are 14 types or 27,299 cosmetics sold online in North Jakarta and as many as 26 types or 188,395 cosmetics found in South Jakarta with a search range of one to two months (BPOM, 2020). Based on sampling and testing conducted in July 2020-September 2021, 18 cosmetic products contain prohibited and dangerous ingredients which are dominated by Hydroquinone and prohibited dyes, namely Red K3 and Red K10 (BPOM, 2021).

The 2011 BPOM regulation explains that Rhodamine B is prohibited from being used as food coloring and cosmetics because it causes irritation if it is exposed to the eyes, skin, poisoning, impaired liver function and cancer (Herdini and Wahyidianan, 2019). The increasing need for cosmetics and technological developments support the expansion of sales marketing in an easy and practical way. The electronic system or call the marketplace is one of the media providers of buying and selling goods or services. So many may sell illegal products or contain prohibited and dangerous ingredients.

Several previous studies related to cosmetic preparations containing Rhodamine B have been carried out, such as in the study conducted by Jusnita and Nandu (2017) of 25 samples there were 4 positive samples of Rhodamine B with a standard R_f value of Rhodamine B of 0.8 samples. D2 0.8; D3 0.8; E5 0.8; and E6 0.8. The mobile phase consists of a mixture of ethyl acetate: methanol: [ammonia-water] (15:3:3). Research conducted by Afriyeni and Utari (2016) of the 5 test samples contained 1 positive sample of Rhodamine B with an R_f value of 0.66 and a standard R_f value of 0.67. The mobile phase mixture used ethyl acetate: methanol: ammonia 9% (15:3:3). Research by Nafiq *et al* (2020) of 9 samples of 4 samples resulted in positive results. Rhodamine B has an R_f value of 0.82, B code sample is 0.76, D sample is 1, E code sample is 0.74 and H has an R_f value of 0.72. The eluent used was a mixture of n-butanol: ethyl acetate: ammonia ratio (55:20:25).

This study takes eyeshadow samples in marketplace X because no one has examined samples from marketplace X. Based on this description, researchers are interested in conducting research to determine the presence of Rhodamine B in eyeshadow sold in marketplace X.

METHOD

Tools and Materials

Analytical Chromatography silica gel GF254 Merck, CAMAG UV cabinet dual wavelength 254 and 366, CAMAG Scientific Twin Trough Chamber for 20×10 cm Plates, Analytical Scales (Ohaus), Oven (IKA) 100 mL measuring cup (Pyrex), 10 mL measuring cup (Pyrex), 100 mL volumetric flask (IWAKI), 25 mL volumetric flask (IWAKI), 10 mL volumetric flask (IWAKI), Socorex 2—20 µL Micropipette, Stirring Rod, Watch Glass, Funnel, Filter Paper, Volume Pipette, Dropper Pipette, Eyeshadow samples sold in marketplace X, Rhodamine B (Sigma), Methanol (Pro analysis), Aquades, Ethyl acetate (Pro analysis), Ammonia (Pro analysis), Hydrochloric acid (Pro analysis), N-butanol (Pro analysis), Ethanol (Pro analysis).

Qualitative Analysis

Preparation of Test Solution

A total of 100 mg of the sample was added 1 drop of 4M HCl. Then add 5 mL of methanol (Pro analysis) and homogenize. Then the sample solution was separated with filter paper and put the filtrate into the ampoule (Fauziah *et al.*, 2020).

Preparation of standard Solution

A total of 10 mg of standard Rhodamine B was weighed and then dissolved in a 100 mL volumetric flask with methanol (Pro analysis) up to the calibration limit mark. The solution was shaken homogeneously (100 g/mL) (Elfasyari *et al.*, 2020).

Identification of Rhodamine B using TLC

To obtain chromatographic results with round/elliptical and tailless spots in the stationary phase, prior to spotting the plate, optimization of the mobile phase with various compositions and concentrations was carried out:

1. n-Butanol : Ethyl Acetate : Ammonia (55:20:25) (Nafiq *et al.*, 2020)
2. Ethyl acetate: Methanol : Ammonia (15:6:3) (Riyanti *et al.*, 2018)
3. Ethyl acetate: Methanol : Ammonia (15:3:3) (Afriyeni and Utari, 2016)
4. Ethyl acetate : Methanol : Ammonia + Water ratio (15:3:3) (Jusnita and Nandu, 2017)
5. Ethyl acetate: Methanol: Ammonia (25:6:1) (Ratnaningtyas, 2013)
6. n-butanol : Ethanol : Aquadest (20:12:15) (Fatimahet *et al.*, 2016)

Rhodamine B standard spotting and eyeshadow samples on a thin layer chromatography plate by:

1. Using a TLC plate measuring 4 x 10 cm, it was previously dried by heating it in an oven at 100 °C for 30 minutes.
2. The parent solution and the test solution (sample) were spotted using a micropipette as much as 3 µL with a distance of 1 cm from the bottom of the plate and a distance between the spots of 1 cm.
3. Dry the spots using a hairdryer.
4. The TLC plate containing the sample is dipped into a chamber with the mobile phase in it having been pre-saturated using filter paper. The plate was allowed to stand until completely eluted and removed, dried at room temperature.
5. Observe the color of the stain visually and under ultraviolet light. If the color of the stain is pink and below UV 254 and 366 nm it fluoresces yellow or orange, it proves the presence of Rhodamine B dye, the R_f value is calculated (Nafiq *et al.*, 2020).
6. The plate with a positive sample of Rhodamine B was sharpened by a color reaction using an HCl spray reaction, it will turn pink if it reacts. Then calculate the R_f value that appears on the plate that has been sprayed (Annas and Sapriyanto, 2017).

Data analysis

Data analysis in this study used descriptive statistical tests by processing the R_f value data and sample resolution in the form of images and tables. The results of data processing in the form of images and tables are described or interpreted to clarify information regarding the description of Rhodamine B content

in eyeshadow preparations sold in marketplace X.

RESULTS

The results of the optimization of the mobile phase in this study were carried out by measuring and calculating the Rf value and resolution in the variation of the mobile phase mixture as shown in table 1.

Table 1. Optimization of Mobile Phase Mixture

(P1) mobile phase n-butanol : Ethanol : Aquadest (20:12:15)				
Sample	Rf Value	Difference Rf	Resolution	Description
Rhodamine B	0,7	-	-	Homogeneous mobile phase
I	0,74	0,04	9,37	
II	0,73	0,03	6,43	
III	0,74	0,04	8,04	
(P2) mobile phase Ethyl acetate : Methanol : Ammonia (15:6:3)				
Sample	Rf Value	Difference Rf	Resolution	Description
Rhodamine B	0,75	0,04	2,33	Homogeneous mobile phase
I	0,75	0,05	2,29	
II	0,75	0,04	1,5	
III	0,76	0,04	1,5	
(P3) fase gerak Etil asetat : Metanol : Amoniak (25:6:1)				
Sample	Rf Value	Difference Rf	Resolution	Description
Rhodamine B	0,41	0,08	1,20	Homogeneous mobile phase
I	0,41	0,08	1,44	
II	0,40	0,09	0,93	
III	0,40	0,06	1,11	
(P4) mobile phase Ethyl acetate : Methanol : Ammonia (25:6:1)				
Sample	Rf Value	Difference Rf	Resolution	Description
Rhodamine B				The mobile phase forms 2 inhomogeneous layers
I	-	-	-	
II				
III				
(P5) mobile phase Ethyl acetate : Methanol : Ammonia + water (15:3:3)				
Sample	Rf Value	Difference Rf	Resolution	Description
Rhodamine B				The mobile phase forms 2 inhomogeneous layers
I	-	-	-	
II				
III				
(P6)mobile phase Ethyl acetate : Methanol : Ammonia (15:3:3				
Sample	Rf Value	Difference Rf	Resolution	Description
Rhodamine B				The mobile phase forms 2 inhomogeneous layers
I	-	-	-	
II				
III				

The optimization of the mobile phase in this study also shows the shape and color of the stain from the eyeshadow sample. The results of the stains seen visually, under UV light 254 and 366 nm on the TLC plate can be shown in Figure 1.

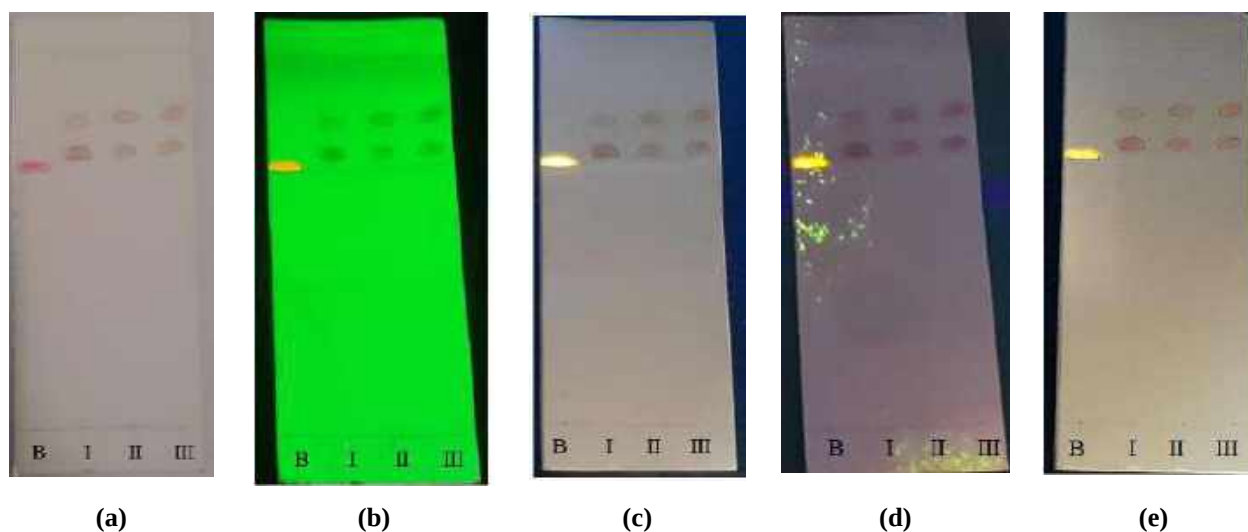


Figure 1. Spots of mobile phase n-butanol : Ethanol : Aquadest (20:12:15)

Information : (a) Visual

(b) under UV light 254

(c) under UV light 366

(d) under UV light 254 after spraying HCl

(e) under UV light 366 after spraying HCl

B : Standard

I : Replication 1

II : Replication 2

III: Replication 3

Figure 2 shows the results of the stains seen visually on the TLC plate.

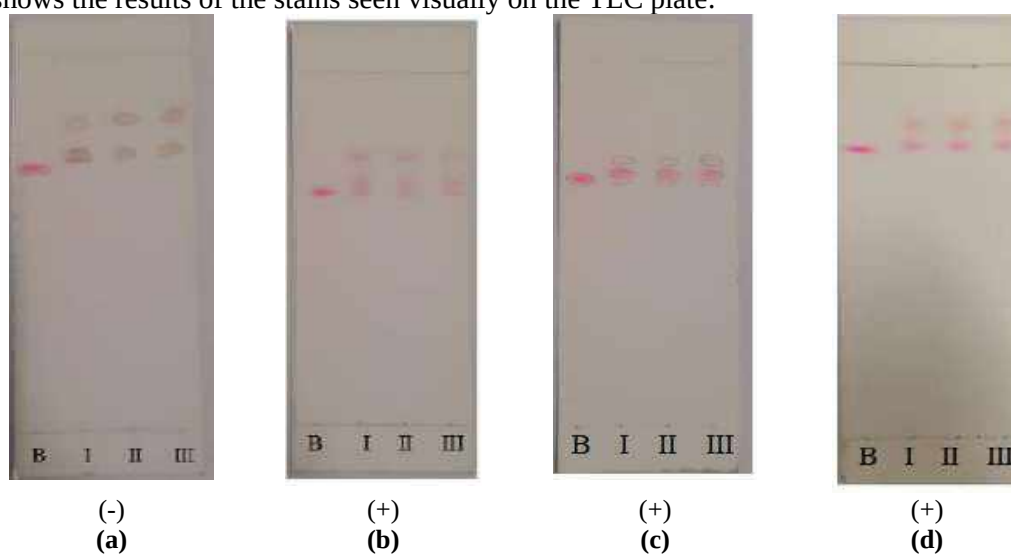


Figure 2. Visually positive sample stains

Information : (a) Negative sample

(b) Sample 4

(c) Sample 8

(d) Sample 9

B : Standard

I : Replication 1

II : Replication 2

III: Replication

Figure 2 shows visually the stains are seen based on the shape and color of the stain, as for the positive results of samples containing Rhodamine B indicated by a bright pink color and the shape of the stain is round/ellipse. Meanwhile, the negative results of samples containing Rhodamine B are shown without a red color bright young.

The positive and negative results on samples containing Rhodamine B in UV 254 light can be seen in Figure 3.

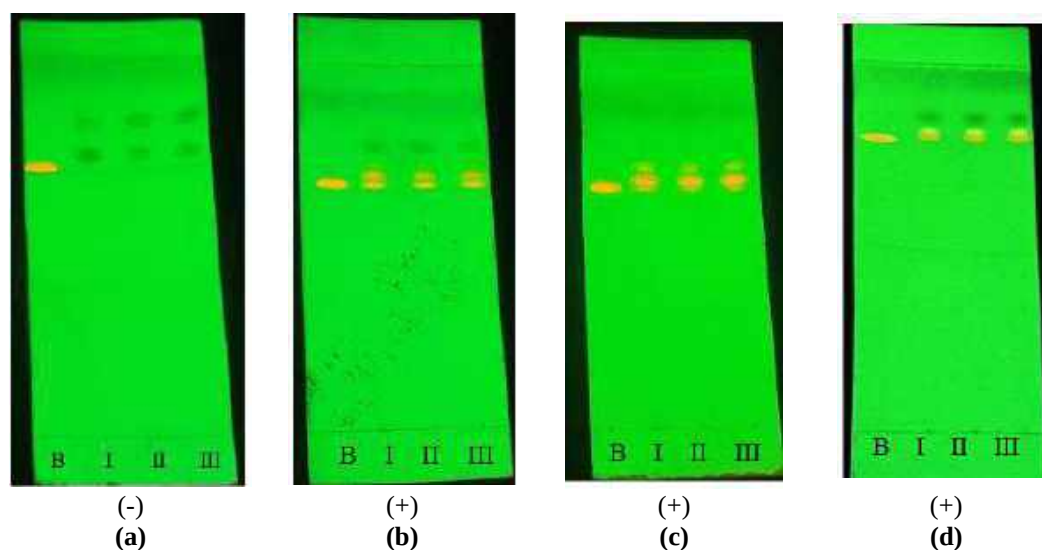


Figure 3. Spots of positive sample stains under UV light 254

Information : (a) Negative sample	B : Standard
(b) Sample 4	I : Replication 1
(c) Sample 8	II : Replication 2
(d) Sample 9	III: Replication

Figure 3 shows the stains on the sample that are positive for Rhodamine B under UV 254 light, which looks yellow or orange fluorescence and the shape of the stain is round/elliptical, while the spots on the sample that are negative for Rhodamine B are indicated by stains that do not fluoresce yellow or orange.

The positive and negative results on samples containing Rhodamine B in UV 366 light can be seen in Figure 4.

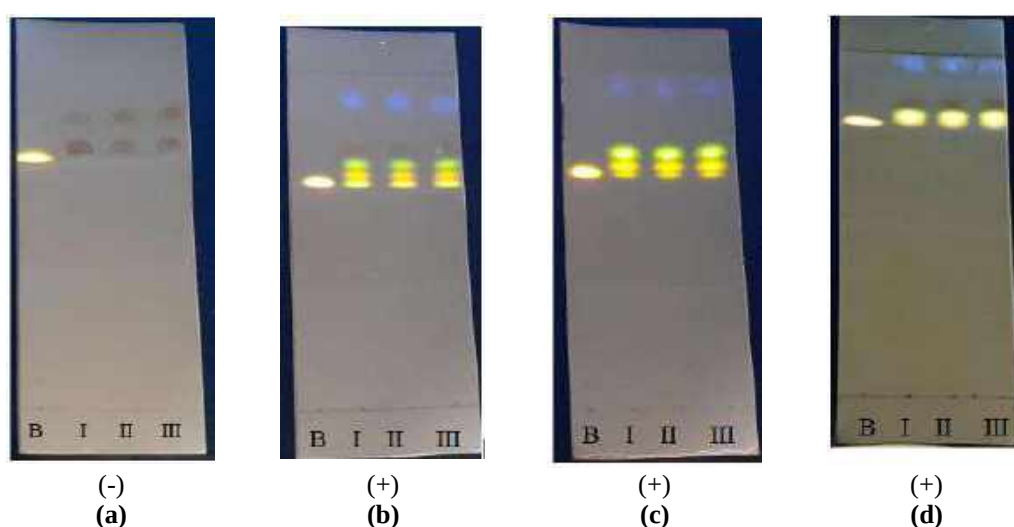


Figure 4. Spots of positive sample stains under 366 . UV light

Information : (a) Negative sample	(c) Sample 8
(b) Sample 4	(d) Sample 9

B : Standard

II : Replication 2

I : Replication 1

III: Replication

Figure 4 shows that the stains on the sample that are positive for Rhodamine B under UV 366 light appear to have yellow or orange fluorescence and the shape of the stain is round/elliptical, while the spots on the sample that are negative for Rhodamine B are indicated by stains that do not fluoresce yellow or orange.

The results of the calculation of the R_f and Resolution values in this study are shown in table 2.

Table 2. Qualitative Test Results Eyeshadow Samples using the mobile phase n-butanol : Ethanol : Aquadest (20:12:15)

Rep. Sample	Visual	UV 254 nm	UV 366 nm	R _f value	R _s value	Difference R _f	Desc
1	Rhodamine B	Pink	Yellow fluorescence	0,7	-	-	(-)
	I	Faded pink	Not fluorescence	0,74	9,37	0,04	
	II	Faded pink	Not fluorescence	0,73	6,43	0,03	
	III	Faded pink	Not fluorescence	0,74	8,04	0,04	
2	Rhodamine B	Pink	Yellow fluorescence	0,68	-	-	(-)
	I	Faded red	Not fluorescence	0,73	3,53	0,04	
	II	Faded red	Not fluorescence	0,73	2,44	0,04	
	III	Faded red	Not fluorescence	0,83	3,05	0,15	
3	Rhodamine B	Pink	Yellow fluorescence	0,69	-	-	(-)
	I	Faded red	Not fluorescence	0,73	0	0,04	
	II	Faded red	Not fluorescence	0,73	0	0,04	
	III	Faded red	Not fluorescence	0,74	0	0,05	
4	Rhodamine B	Pink	Yellow fluorescence	0,65	-	-	(+))
	I	Pink	Yellow fluorescence	0,65	2,69	0,00	
	II	Pink	Yellow fluorescence	0,64	4,01	0,01	
	III	Pink	Yellow fluorescence	0,64	2,52	0,01	
5	Rhodamine B	Pink	Yellow fluorescence	0,73	-	-	(-)
	I	-	-	-	-	-	
	II	-	-	-	-	-	
	III	-	-	-	-	-	
6	Rhodamine B	Pink	Yellow fluorescence	0,66	-	-	(-)

7	I	Faded red	Not fluorescence	Not fluorescence	0,7	7,03	0,04	(-)
	II	Faded red	Not fluorescence	Not fluorescence	0,7	7,03	0,04	
	III	Faded red	Not fluorescence	Not fluorescence	0,71	13,02	0,05	
	Rhodamine B	Pink	Yellow fluorescence	Not fluorescence	0,65	-	-	
	I	Pink	Not fluorescence	Fluorescent orange	0,73	2,03	0,08	
	II	Pink	Not fluorescence	Fluorescent orange	0,74	2,02	0,09	
8	III	Pink	Not fluorescence	Fluorescent orange	0,74	2,02	0,09	(+)
	Rhodamine B	Pink	Yellow fluorescence	Fluorescent orange	0,68	-	-	
	I	Pink	Yellow fluorescence	Fluorescent orange	0,67	4,01	0,02	
	II	Pink	Yellow fluorescence	Fluorescent orange	0,66	4,02	0,01	
	III	Pink	Yellow fluorescence	Fluorescent orange	0,66	2,69	0,01	
	Rhodamine B	Pink	Yellow fluorescence	Fluorescent orange	0,78	-	-	
9	I	Pink	Yellow fluorescence	Fluorescent orange	0,79	2,03	0,01	(+)
	II	Pink	Yellow fluorescence	Fluorescent orange	0,79	2,03	0,01	
	III	Pink	Yellow fluorescence	Fluorescent orange	0,79	2,03	0,01	
	Rhodamine B	Pink	Yellow fluorescence	Not fluorescence	0,75	-	-	
	I					-	-	
	II	-	-	-	-	-	-	
10	III					-	-	(-)

DISCUSSION

In this study, the sample used was eyeshadow cosmetic preparations sold in marketplace X by optimizing the mobile phase using the thin layer chromatography method. Eyeshadow samples were taken from stores with star criteria and star+ is a store that has the best performance and service and sales. Observation of the stain for the thin layer chromatography method can be seen from the shape of the stain which is round/elliptical. Optimization of the mobile phase is carried out to obtain accurate measurement results. Obtaining a mobile phase with a suitable mixture of solutions for the separation of samples and other compounds results in the shape of the stain and the color of the stain is clear. Optimization of the mobile phase is determined from the physicochemical properties provided that it has sufficient purity, stability, low viscosity, vapor pressure that is not too low or too high, and the lowest possible toxicity.

The variation in concentration of the solvent mixture used affects the polarity of the mobile phase. A mixture of mobile phases consisting of very polar and very non-polar solvents will produce a mobile phase that is less mixed or demixed, causing the stains that appear to be uncalculated, the R_f value is not calculated, the stain is tailless, not round, and wide (Wulandari, 2011). The immiscibility of the mobile phase is due to the difference in polarity between the mobile phase and the sample which results in the stain not being completely eluted, the closer the polarity of the mobile phase to the compound to be tested, the better the separation, as in principle, like dissolved like. The nature of Rhodamine B affects the selection of the mobile phase because Rhodamine B has a carboxyl group with a lone pair of electrons and an amine group so that

intermolecular hydrogen bonds will form with a solvent that has a polar nature, so that Rhodamine B compounds will easily dissolve in polar solvents, so a mixture of mobile phases is used. a mixture of solutions with polar properties that will produce a good separation of Rhodamine B with other compounds (Nafiq *et al.*, 2020).

The selected mobile phase will be inserted into the chamber for saturation, the type of chamber used is a twin trough chamber which has better advantages compared to chamber N and can control saturation and humidity in the chamber. Saturation using filter paper for approximately 15 minutes, on each side of the twin trough chamber to control the process of separating compounds that are very susceptible to changes in humidity. The use of a saturated chamber will have a lower R_f value than an unsaturated chamber with the same handling conditions (Wulandari, 2011). The stationary phase uses silica GF 254 because on the surface of the silica gel it has Si-OH (silanol) and Si-O-Si (siloxane) groups. The stationary phase was dried first by heating at a temperature of 100°C for 30 minutes to remove the water content that was absorbed in the plate. Improper spotting will result in stains with scattered, tailed, non-round/elliptical spots and produce double peaks. The sample volume is at least 0.5 µL – 10 µL (Wulandari, 2011).

Methanol is used as an organic solvent that has polar properties and a low boiling point that will dissolve organic substances that have polar properties well. The use of HCl serves to destroy other compounds contained in the eyeshadow sample and to clarify the red color of Rhodamine B and HCl can stabilize the Rhodamine B compound contained in the eyeshadow sample, so that it does not turn into a neutral form from the ionized form (Nafiq *et al.*, 2020). To obtain a clear solution without disturbing substances, a filtering process is carried out.

In this study, to determine the appropriate mobile phase indicated by the shape and color of the stain on the TLC plate of the eyeshadow sample visually, under UV light 254 and 366 nm, the R_f value, the difference in the R_f value, the resolution value and the homogeneity of the solvent mixture were calculated as mobile phase. The R_f value was used to see the migration of compounds on the TLC plate. The difference in R_f is used to determine the distance between the Rhodamine B standard and the stain that is declared positive and the Resolution value is used to see the ability of the analyte to separate Rhodamine B compounds from other compounds in the eyeshadow sample. The results of the optimization of the mobile phase selected a mixture of (P1) n-butanol: Ethanol: Aquadest (20:12:15) with a clear, homogeneous solution, producing stains with an R_f value that entered the requirement range of 0.2 – 0.8, the difference between the R_f standard of Rhodamine B with sample 0.2 and Resolution value 1.5 compared to other mobile phase mixtures P1 has a better resolution or separation value and has a clear and homogeneous solution mixture. The results of the study can be seen in table 1.

Stains containing Rhodamine B visually showed stains parallel to the Rhodamine B standard having a light pink color. The results of stains that do not contain Rhodamine B are shown by the color of the stains being the same as the color of the sample solution and not bright pink. Observation of stains under UV light 254 and 366 resulted in yellow or orange fluorescence stains, samples with non-fluorescent stains showed no Rhodamine B content in the sample. HCl spraying is used to clarify the color of stains from samples that are positive with stains that fluoresce under UV 254 and 366 yellow or orange, spraying can also indicate new stains that appear (Nafiq *et al.*, 2020). The results of the optimization of the mobile phase mixture which were observed visually under UV 254 and 366 are shown in Figure 1.

The test results from ten samples of eyeshadow sold in marketplace X, there are three samples with positive results indicated by samples 4, 8, and 9 in Figure 2, Figure 3, and Figure 4. The stains produce a round/elliptical shape with no tail. The stains visually observed were bright pink and yellow fluorescence under UV 254 light and orange under UV 366 light. Optimal results will be obtained if spotting the sample on the plate with the smallest and narrowest spot size possible, the more sample volume that is applied will decrease the sample resolution value. A good separation is obtained if a thin, tall, and slender peak is obtained (Rohman, 2020). The results of the qualitative test using the mobile phase of n-butanol : Ethanol : Aquadest (20:12:15) are shown in table 2. Tests on samples 1, 2, 3, 5, 6, 7, and 10 showed negative test results observed from the shape of the stains resulting in round/elliptical stains and no tails. Visually observed, it did not show a bright pink stain on the stain, and under UV light 254 nm and 366 nm did not show a yellow or orange fluorescence stain which resulted in the sample not showing the presence of Rhodamine B compounds. Sample 1 showed an R_f value that entered the range requirements for qualitative analysis. 0.2 – 0.8 and the terms value R_s 1.5.

CONCLUSION

In this study, it can be concluded that Rhodamine B content analysis by optimizing the mobile phase using the thin layer chromatography method on eyeshadow preparations sold in marketplace X, namely:

1. Spots with a positive result, visually showed a bright pink color similar to the color of the standard Rhodamine B stain. Observations under UV 254 and 366 with positive samples resulted in yellow or orange fluorescence stains. HCl spray was used to clarify the color of stains containing Rhodamine B.
2. The optimal mobile phase is the mobile phase with a mixture of n-butanol: Ethanol: Aquadest ratio (20:12:15).
3. The ten eyeshadow samples studied were three samples that showed positive results containing Rhodamine B.

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EXAMINATION OF HYDROQUINONE CONTENT IN FACE WHITENING CREAM SOLD IN BEKASI CITY WITH UV SPECTROPHOTOMETRY METHOD

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Abstract

Introduction: Hydroquinone is often misused as a whitening agent in cosmetics. According to the Regulation of the Food and Drug Supervisory Agency of the Republic of Indonesia Number 23 of 2019, hydroquinone has been banned as a bleach in cosmetics (BPOM RI, 2019). The purpose of this study was to determine the levels of hydroquinone in facial whitening cream sold in Bekasi City.

Method: This research is a non-experimental type of research and sampling through the purposive sampling method. Qualitative test with organoleptic test and color test with FeCl₃ reagent. Quantitative analysis with UV spectrophotometry.

Results: Hydroquinone has a maximum wavelength of 294 nm. The results of the linearity test were shown by the correlation coefficient (r) = 0.9986, the accuracy test obtained a hydroquinone recovery value of 92.36% and the precision test with an RSD value of 1.69%. The results of the analysis showed that from 5 samples there were 4 positive samples containing hydroquinone with an average level of WL 5.25%, WC 1.29%, SC 3.59%, and HM 1.45%.

Conclusion: Based on these data, it can be said that 4 out of 5 samples of facial whitening cream contain hydroquinone that does not meet the requirements set by BPOM.

Keywords: Hydroquinone, Face Whitening Cream, UV Spectrophotometry

CHIMERIC ANTIGEN RECEPTOR T-CELL GENES TARGET ON LYMPHOMA: A COMPREHENSIVE BIOINFORMATIC ANALYSIS

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Abstract

Introduction: Selective and reliable surface antigen expression is needed for CAR T cell cancer clearance. Antigens targeted by CAR-T cells in lymphoma are abundant although enigmatic. Therefore, these multiple gene targets can be potentially developed into biomarkers, theragnostics, and drug target.

Method: Utilizing Bioinformatics tools, such as: GeneMANIA, STRING, cBioportal, ShinyGO, MetaCore software to reveal several gene pathways and functions, biomarker, and potentially drug targeted.

Results: GeneMANIA and STRING show multiple genes interactions among CD19, CD20, CD22, and CD30. cBioPortal reveal the mutation frequency, mutation and amplification. ShinyGO disclose fold enrichment, enrichment FDR and pathways. Metacore inform pathways, disease biomarker network, enrichment, and potential drug target. Metacore offers 50 possible gene pathways. Only prominent and certain pathways are expounded herein.

Conclusion: Using several comprehensive bioinformatics tools, softwares, and analyses, we can comprehend and observe numerous pathways, interactions, biomarker, and potential treatments.

Key words: Chimeric antigen receptor T-cell, CAR-T cells, lymphoma, bioinformatics.

Introduction

Lymphomas are the third most prevalent tumor developing from the lymphoreticular system in the head and neck area. They are a diverse range of hematological malignancies with common lymphoid-cell origins and a clinically and biologically heterogeneous group of malignancies that arise from mature T- or B-lymphocytes in secondary lymphoid organs. [1]

Lymphomas are widely and informally divided into Hodgkin lymphomas (HLs) and non-Hodgkin lymphomas (NHLs) based on differences in their clinical presentation, treatment, prognosis, and number of neoplastic cells, among other characteristics. Similar to Hodgkin's disease, NHL comprises roughly 5 percent of head and neck cancers and manifests in a variety of ways.[2]

More than 80 types of adult lymphoid neoplasms (B-cell, T-cell, and Hodgkin lymphomas) are described by their morphology, immunophenotype, genetic lesions and molecular profiles, clinical characteristics, and cellular origin in the 2017 revision of the World Health Organization classification.[3]

Most B-cell lymphomas depend on a B-cell receptor (BCR) for survival, and antigen activation of lymphoma cells through BCR signaling seems to be critical for lymphoma pathogenesis.

Pathogenesis of non-Hodgkin's lymphoma

Immunosuppression, lack of control of latent EBV infection, and chronic antigen stimulation are essential pathogenetic processes. B and T lymphocytes are immune system cells that fight infections. B-cells make antigen-binding antibodies, while T-cells identify cell-presented antigen. Immunosuppression raises the risk of NHL. Most risk factors for malignant lymphomas involve dysregulation or reduction of T-cell function (HIV/AIDS, organ transplantation), which allows Epstein Barr virus (EBV)-driven B-cell proliferation and transformation.[4] Chronic antigenic stimulation promotes B-cell proliferation, which raises the risk of random genetic errors, especially immunoglobulin gene rearrangements. In circumstances when a virus is the foreign stimulant, the virus may infect a normal cell and integrate viral DNA into the host genome, changing the cell into a self-replicating malignant cell. [5] Antigenic stimulation can lead to

compensatory T-cell downregulation, or immunosuppression.[6] Lymphoma tumour cells are malignant precursor lymphocytes stopped in differentiation. Up to 90% of NHL cases have chromosomal translocations, usually balanced reciprocal recombinations. Translocations, with or without chromosomal deletions and mutations, can activate oncogenes or inactivate tumour suppressor genes.[7]

Pathogenesis of Hodgkin's lymphoma

Hodgkin lymphoma (HL) is a unique disease since the tumour cells, Hodgkin and Reed-Sternberg (HRS) cells, represent a minor component of the tumour mass. HRS cells are clonal B cells that have lost their phenotypic. HRS cells must have developed methods to help mature B cells without B cell receptors survive apoptosis. [8] HRS cell apoptosis escape and transcriptional reprogramming appear crucial to disease etiology. Epstein-Barr virus (EBV) produces oncogenic genes in certain HRS cells. EBV likely affects reprogramming and survival through dysregulating signaling networks and transcription factors, including NF-kappa-B. [9, 10] All HRS cells activate NF-kappa-B, and gene abnormalities impacting this pathway are prevalent in EBV-negative HL. HRS cells survive by recruiting immunological and stromal cells and inhibiting local immune response. Despite many unsolved concerns, our knowledge of this complex disease has grown in the previous two decades.[11]

Theragnostics

Over the past 50 years, theragnostics improvements in lymphoma have significantly improved the prognosis for the majority of patients. [12] The availability of effective treatment presents new obstacles, including improving the assessment of a therapeutic response, reducing the likelihood of relapse, and minimizing the patient's exposure to toxicities. [13] Advanced anatomical and functional imaging techniques are now widely utilized, and staging recommendations have evolved, with a focus on PET imaging.[14]

Hodgkin lymphoma (HL) is an uncommon cancer that affects primarily young adults and, to a lesser extent, the elderly. Classical HLs, which represent the majority of cases, and nodular lymphocyte-predominant HLs are the two major disease types based on their clinical and histological characteristics. [15] A young age of onset; rare malignant cells, which are large multinucleated cells derived from B lymphocytes (known as Hodgkin and Reed-Sternberg (HRS) cells); a high cure rate, even when the patient presents with advanced metastatic spread; and a particular sensitivity to radiation therapy characterized HL. Non-Hodgkin lymphomas (NHLs) are significantly more prevalent, ranking as the fifth most prevalent malignancy in the United States. Their incidence has steadily increased over the past three decades for causes that are not entirely clear. Approximately 85% of NHLs are of B-cell origin (B-NHLs) and contain a wide variety of malignancies with distinct clinical and biological histories.[16]

Lymphoma treatment varies on the lymphoma subtype and illness stage. Various immunotherapy treatments have demonstrated encouraging outcomes, and some have become the standard of care. The US Food and Drug Administration (FDA) has licenced monoclonal antibodies (mAbs), immune checkpoint inhibitors (ICIs), antibody-drug conjugates (ADCs), immunomodulatory drugs (IMiDs), and genetically modified chimeric antigen receptor T cells (CAR T cells) for the treatment of lymphoma.[17]

CAR T Cells Therapy

CAR-T cell therapy is a groundbreaking novel cancer treatment pillar based on reprogramming a patient's own T cells with a CAR construct that targets cancer antigens ex vivo. Following that, patients are infused with modified CAR-T cells to initiate an appropriate immune response and potentially eradicate tumour cells. [18] Concisely, CAR-T cells are genetically engineered, cancer-killing T cells.

The hinge, the transmembrane (TM) domain, the single-chain variable fragment (scFv), and the intracellular signaling domain are the four main components of CAR constructs. In 1987, a Japanese group reported the CAR concept.[19] In 1989, Zelig Eshhar further studied and developed it at the Weizmann Institute, establishing the first-generation CARs.[20]

The first chimeric antigen receptor (CAR) was created by exchanging the endogenous T cell receptor (TCR) variable region with the VL and VH regions of an anti-2,4,6-trinitrophenyl (TNP) antibody. This was done while preserving the TCR extracellular constant C-region, as well as the transmembrane and intracellular signaling domains of T cells.[21] Anti-CD19 CAR-T cell therapy's efficacy against B cell

malignancies led to FDA approval in 2017.[22]

CAR-T is the first FDA-approved lymphoma cellular treatment. In this individualised therapy, the patient's own T cells are genetically transduced with lentiviral or retroviral vectors *ex vivo* to express a CAR that allows CAR-positive T cells to bypass MHC presentation for antigen recognition and activation of T cells with fused intracellular CD3 and costimulatory signalling domains.[23] Axicabtagene ciloleucel (Axi-cel) and tisagenlecleucel are licenced to treat relapsed, refractory aggressive B cell non-Hodgkin lymphoma, including high-grade B cell lymphoma, DLBCL, transformed follicular lymphoma, and PMBCL (Axi-cel only). FDA has already licenced lisocabtagene maraleucel (liso-cel) in early 2020.[24]

However, life-threatening CAR-T cell toxicities, manufacturing time, limited efficacy against solid tumours, inhibition and resistance in B-cell malignancies, financial burden, antigen escape, limited persistence, trafficking restrictions, limited tumour infiltration, the immunosuppressive microenvironment, and severe CAR-T cell toxicities should be addressed. [25, 26]

Target antigen CAR T

Safe and effective CAR T cell cancer clearance needs selective and dependable surface antigen expression. Advanced CAR T cell products target B lineage antigens and haematological malignancies. CD19, CD22, CD20, BCMA (B cell maturation antigen) in multiple myeloma, and CD30 in Hodgkin lymphoma are B lymphocyte antigens.[27-31]

Antigens targeted by CAR-T cells in first clinical studies in solid tumors include B7H3, HER2 (human epidermal growth factor receptor 2), CEA (carcinoembryonic antigen), PSMA (prostate-specific membrane antigen), ROR1 (receptor tyrosine kinase like orphan receptor 1), EGFRvIII (epidermal growth factor receptor variant III), interleukin (IL)13R α 2, disialoganglioside GD2, and neural cell adhesion molecule L1 (NCAM-L1, CD171). [32-37]

CD20, CD22, and kappa-light chain for B-cell lymphomas, and CD30 for Hodgkin and T-cell lymphomas are being studied in several clinical studies. [38] CD19 is a more prevalent target antigen than CD20 or CD22 due to its frequent, wider, and higher expression in B-cell leukaemia and lymphoma.[39]

Aims of Study

Using bioinformatics, the researchers in this study hope to identify the gene that CAR T cells in lymphoma are targeting. An examination using a Venn diagram revealed that there are four target antigens for CAR T in lymphoma. These target antigens CAR T are CD19, CD20, CD22, and CD30. It is envisaged that by doing an exhaustive bioinformatics study, we would be able to comprehend the pathways, interactions, and other information regarding the four CAR T antigen targets, as well as the biomarker and possible medications.

Methods

Utilizing the cBioportal web tool [40], genes coexpressed with CD19, CD20, CD22, and CD30 in lymphoma were downloaded. The network of gene–gene interactions were supervised utilizing the Gene Multiple Association Network Integration Algorithm (GeneMANIA; <https://www.genemania.org/>; accessed on July 04, 2022) [41] and the Search Tool for the Retrieval of Interacting Genes Database (STRING v.10.0; <https://string-db.org/>; accessed on July 04, 2022) was utilized to generate a protein–protein interaction (PPI) network [42]. ShinyGO 0.76 (<http://bioinformatics.sdstate.edu/go/>; accessed on July 01, 2022) was utilized as an enrichment analysis to link several gene lists with underlying molecular pathways and functional categories such as gene ontology (GO) and other databases [43]. Pathway analysis was governed utilizing MetaCore software (GeneGo, San Diego, CA) which is a systems biology analysis series to recognize adjusted gene pathways and functions, biomarker, and potentially drug targeted [44].

Results and Discussion

The "CAR T Cell target genes treatment for lymphoma" database is compiled using credible sources (Table 1). Using Venn Diagram on Figure 1 [45], analysis was performed on the four databases. The outcomes of the intersection of the four were CD19, CD20, CD22, and CD30.

Table 1. Source of Database

Total CAR-T Cell target genes therapy for lymphoma	References
8	[46]
26	[47]
24	[48]
125	[49]

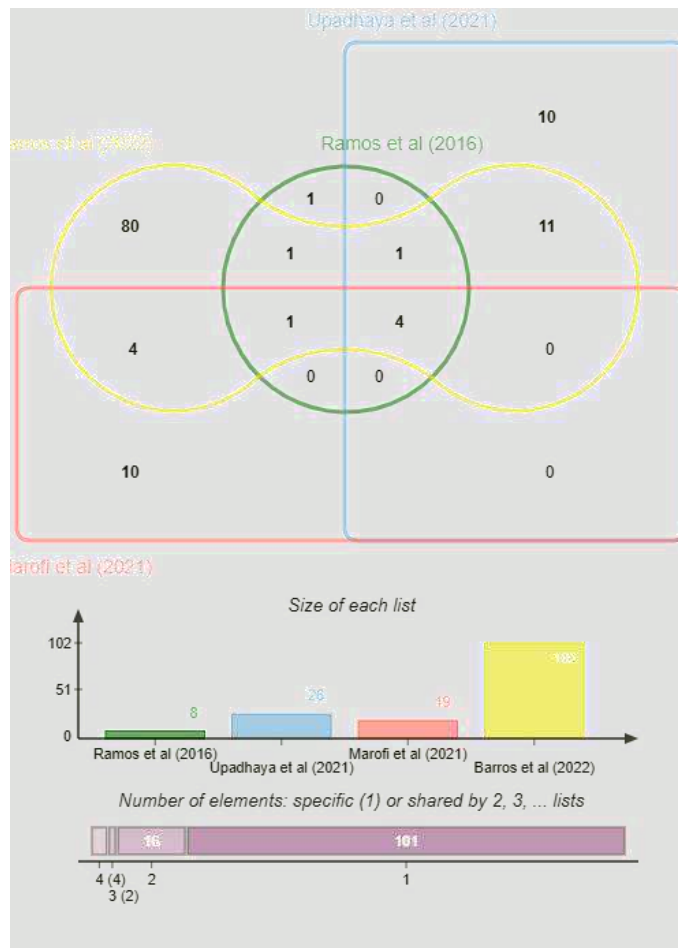


Figure 1. Venn Diagram

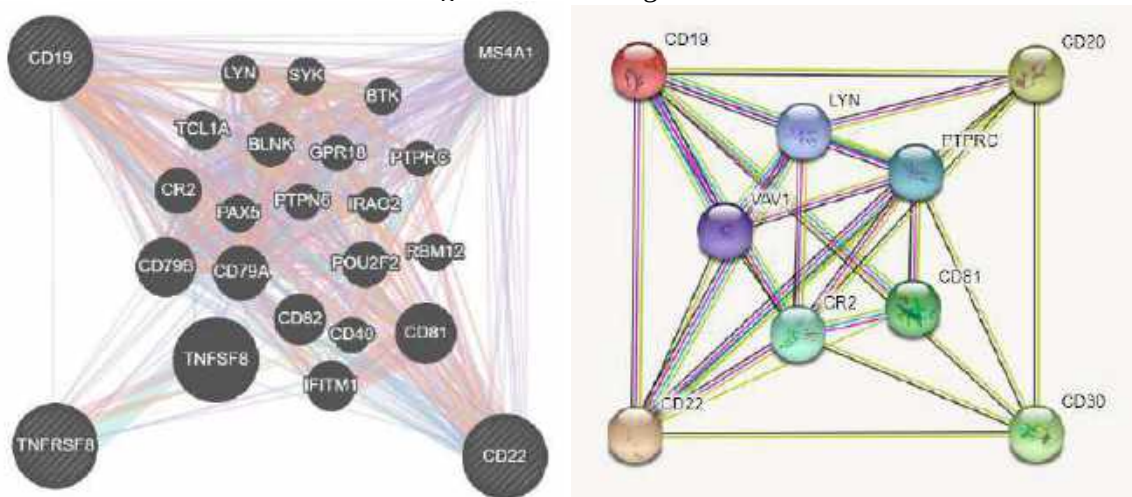


Figure 2. Multiple genes interaction (GeneMANIA on left and STRING on right)

From genemania software, it is known the multiple genes interaction among between CD19, CD20 (=MS4A1), CD22, and CD30 (=TNFRSF8). Among them it is comprehended that the physical interactions 77.64%, co-expression 8.01%, predicted 5.37%, co-localization 3.63%, genetic interactions 2.87%, pathway 1.88%, and shared protein domains 0.60%.

From local network cluster (STRING) software, it is known that from cluster (CL:15956) CD22 mediated BCR regulation and (CL:15952) antigen activates B cell receptor (BCR). It has also several biological processes (Gene Ontology), such as: regulation of B cell receptor signaling pathway (GO:0050855), B cell receptor signaling pathway (GO:0050853), positive regulation of alpha-beta T cell proliferation (GO:0046641), and B cell proliferation (GO:0042100). It has also several cellular component (Gene Ontology), such as: membrane raft (GO:0045121), external side of plasma membrane (GO:0009897), side of membrane (GO:0098552), extracellular exosome (GO:0070062), integral component of plasma membrane (GO:0005887).

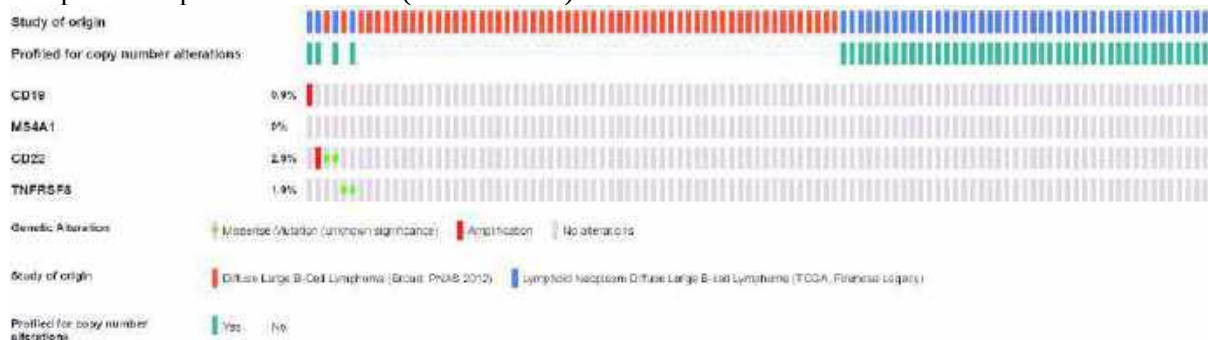


Figure 3. Mutation Frequency
Source: cBioPortal

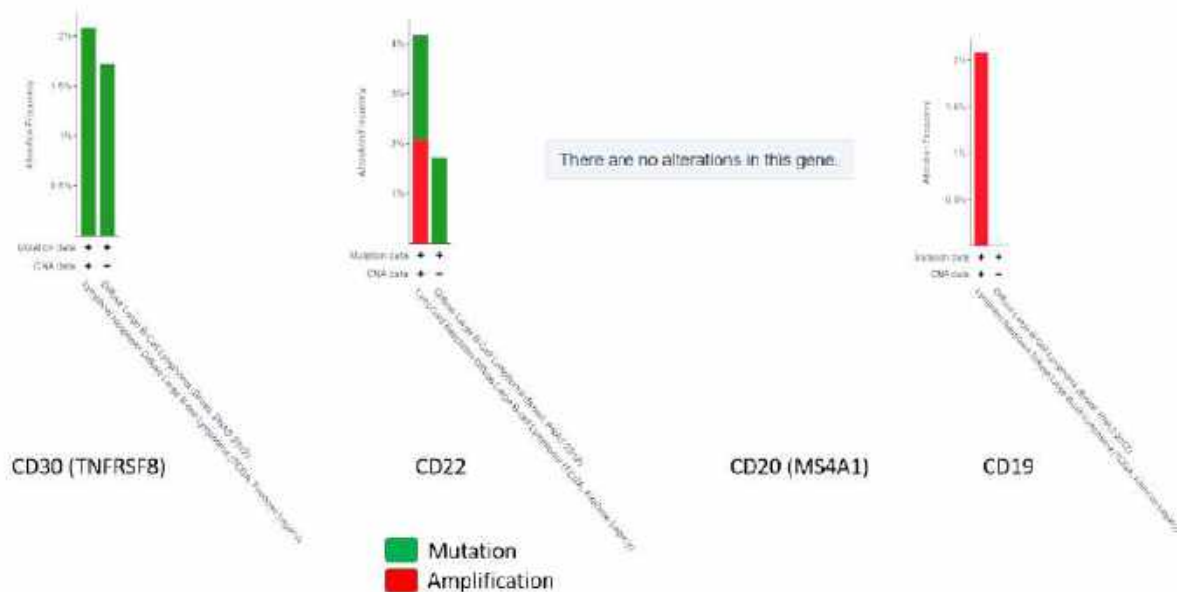


Figure 4. Mutation and Amplification
Source: cBioPortal

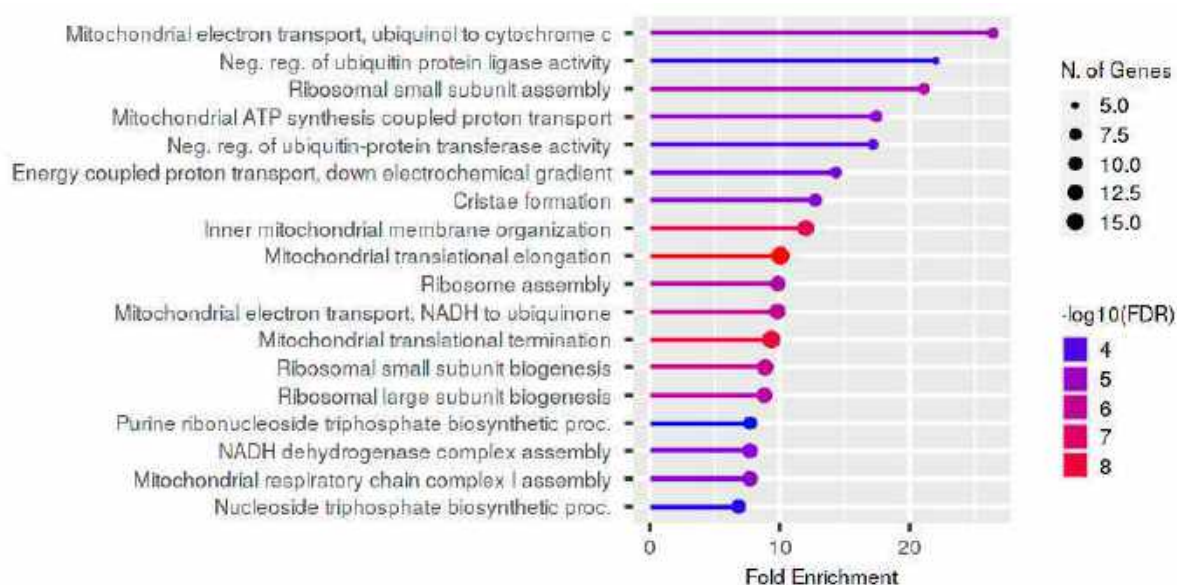


Figure 5. Fold Enrichment
ShinyGO 0.76 (URL: <http://bioinformatics.sdstate.edu/go/>)

Enrichment FDR	nGenes	Pathway Genes	Fold Enrichment	Pathways (click for details)
3.4E-51	48	112	24.5	SRP-dependent cotranslational protein targeting to membrane
2.3E-48	48	128	21.5	Protein targeting to ER
1.1E-47	48	132	20.8	Establishment of protein localization to endoplasmic reticulum
3.9E-45	48	147	18.7	Nuclear-transcribed mRNA catabolic process, nonsense-mediated decay
1.0E-48	53	169	18	Cytoplasmic translation
7.0E-43	48	162	17	Protein localization to endoplasmic reticulum
6.4E-39	50	217	13.2	Viral transcription
7.0E-39	51	230	12.7	Translational initiation
1.7E-36	51	256	11.4	Nuclear-transcribed mRNA catabolic proc.
8.1E-36	52	278	10.7	Viral gene expression
5.8E-34	60	433	7.9	Protein targeting
1.2E-31	57	421	7.8	Establishment of protein localization to membrane
2.7E-40	86	808	6.1	Translation
4.7E-39	86	839	5.9	Peptide biosynthetic proc.
7.5E-35	88	1005	5	Amide biosynthetic proc.
2.1E-34	88	1020	4.9	mRNA metabolic proc.
1.0E-33	87	1018	4.9	Peptide metabolic proc.
1.5E-36	95	1126	4.8	RNA processing

Figure 6. Enrichment FDR and Pathways
ShinyGO 0.76 (URL: <http://bioinformatics.sdstate.edu/go/>)

From the cBioPortal for Cancer Genomics database, with combined study (106 samples) in two studies, we can comprehend the pattern of mutation frequency (Figure 3), mutation and amplification ((Figure 4), fold enrichment (Figure 5), enrichment FDR and pathways (Figure 6), CD19 co-expression genes pathways (Figure 8), CD19 Enrichment by pathways map (Figure 9), CD20 and CD22 co-expression genes potential pathways (Figure 11).



Figure 7. CD30 co-expression genes pathways (left) and disease biomarker network (right)
Source: Metacore

We can comprehend the top 10 potential pathways from CD30 co-expression genes such as: transcription role of AP-1 in regulation of cellular metabolism until immune response IL-4-induced regulators of cell growth, survival, differentiation, and metabolism, and potentially disease network, such as: Parkinson disease and colonic neoplasms (Figure 7).

#	Network	GO processes	Total nodes	Seed nodes	Pathways	p-Value	zScore	gScore
1	U1C, PLEKHA7, OSTF1, COMMD6 (Acr), CDW52	macromolecule metabolic process (64.4%; 3.63e-06), anaphase-promoting complex-dependent catabolic process (6.7%; 1.61e-06), metabolic process (73.3%; 1.902e-05), nitrogen compound metabolic process (62.2%; 8.739e-05), response to lipid hydroperoxide (4.4%; 1.165e-04)	60	18	0	6.040E-34	49.14	49.14
2	Ubiquitin, Secum, RMRP, RASSF1, DGCR14	mitotic cell cycle process (31.2%; 3.829e-12), cell cycle (41.7%; 7.524e-12), mitotic cell cycle (33.3%; 1.382e-11), cell cycle process (35.4%; 2.716e-11), regulation of cell cycle process (31.2%; 4.264e-10)	60	15	5	1.410E-27	42.19	48.44
3	RNA polymerase II, FDX1L, HACA snoRNP, G/D snoRNP, 6330673E17Bk	adaptive immune response (33.3%; 5.195e-11), transcription by RNA polymerase II (21.4%; 1.750e-08), nucleobase-containing compound biosynthetic process (31.0%; 3.072e-08), cell surface receptor signaling pathway (45.2%; 4.277e-08), snRNA pseudouridine synthesis (7.1%; 4.598e-08)	60	15	0	9.650E-28	42.66	42.66
4	MorR1-ps1, GAS5-AS1, C6orf49, PFN1P2, Fela	cytoplasmic translational initiation (11.1%; 1.509e-05), oxidative phosphorylation (14.8%; 1.920e-05), negative regulation of chemokine (C-X-C motif) ligand 2 production (7.4%; 2.421e-05), positive regulation of translational fidelity (7.4%; 3.226e-05), negative regulation of translational initiation in response to stress (7.4%; 4.146e-05)	60	15	0	1.410E-27	42.19	42.19
5	DERP6, CDC122, UBL5, PK-gamma (liver), PPP1R7	regulation of skeletal muscle contraction by regulation of release of sequestered calcium ion (4.5%; 4.656e-05), nitrobenzene metabolic process (4.5%; 6.511e-05), regulation of skeletal muscle contraction by calcium ion signaling (4.5%; 6.511e-05), detoxification of nitrogen compound (4.5%; 6.672e-05), cellular detoxification of nitrogen compound (4.5%; 6.672e-05)	60	15	0	2.950E-27	41.31	41.31

Figure 8. CD19 co-expression genes pathways (Source: Metacore)

Msps	Total	pValue	Min FDR	p-value	FDR	In Data	Network Objects from Active Data
Regulation of metabolism: Insulin regulation of glucose metabolism	57	9.954E-02	5.269E-01	9.954E-02	5.269E-01	2	PHK gamma (liver), PHK gamma
Transcription: Epigenetic regulation of gene expression	57	9.954E-02	5.269E-01	9.954E-02	5.269E-01	2	Histone H3, Sitund
GTP metabolism	54	9.085E-02	5.269E-01	9.085E-02	5.269E-01	2	NDPK B, NDPK B
HIF2 regulation of oxidative stress response	54	9.085E-02	5.269E-01	9.085E-02	5.269E-01	2	Ubiquitin, DJ-1
Role of IL-2/IL-17 cytokine axis in apoptosis	53	8.800E-02	5.269E-01	8.800E-02	5.269E-01	2	Calgranulin A, Calgranulin B
Immune response: BCRF-induced signaling	51	8.240E-02	5.269E-01	8.240E-02	5.269E-01	2	Ubiquitin, RPS8
Cell adhesion: Role of tetraspanins in the integrin-mediated cell adhesion	50	7.964E-02	5.269E-01	7.964E-02	5.269E-01	2	Calmyrin, ICAM3
Apoptosis and survival: Regulation of apoptosis by mitochondrial signaling	108	7.726E-02	5.269E-01	7.726E-02	5.269E-01	3	RASSF1, Pin1, Flot
HIF-1 in gastric cancer	49	7.691E-02	5.269E-01	7.691E-02	5.269E-01	2	Ubiquitin, LAMR1
Transcription: 14-3-3/SMRT complex-mediated epigenetic gene silencing	48	7.691E-02	5.269E-01	7.691E-02	5.269E-01	2	GPS2, Ubiquitin

Figure 9. CD19 Enrichment by pathways map (Source: Metacore)

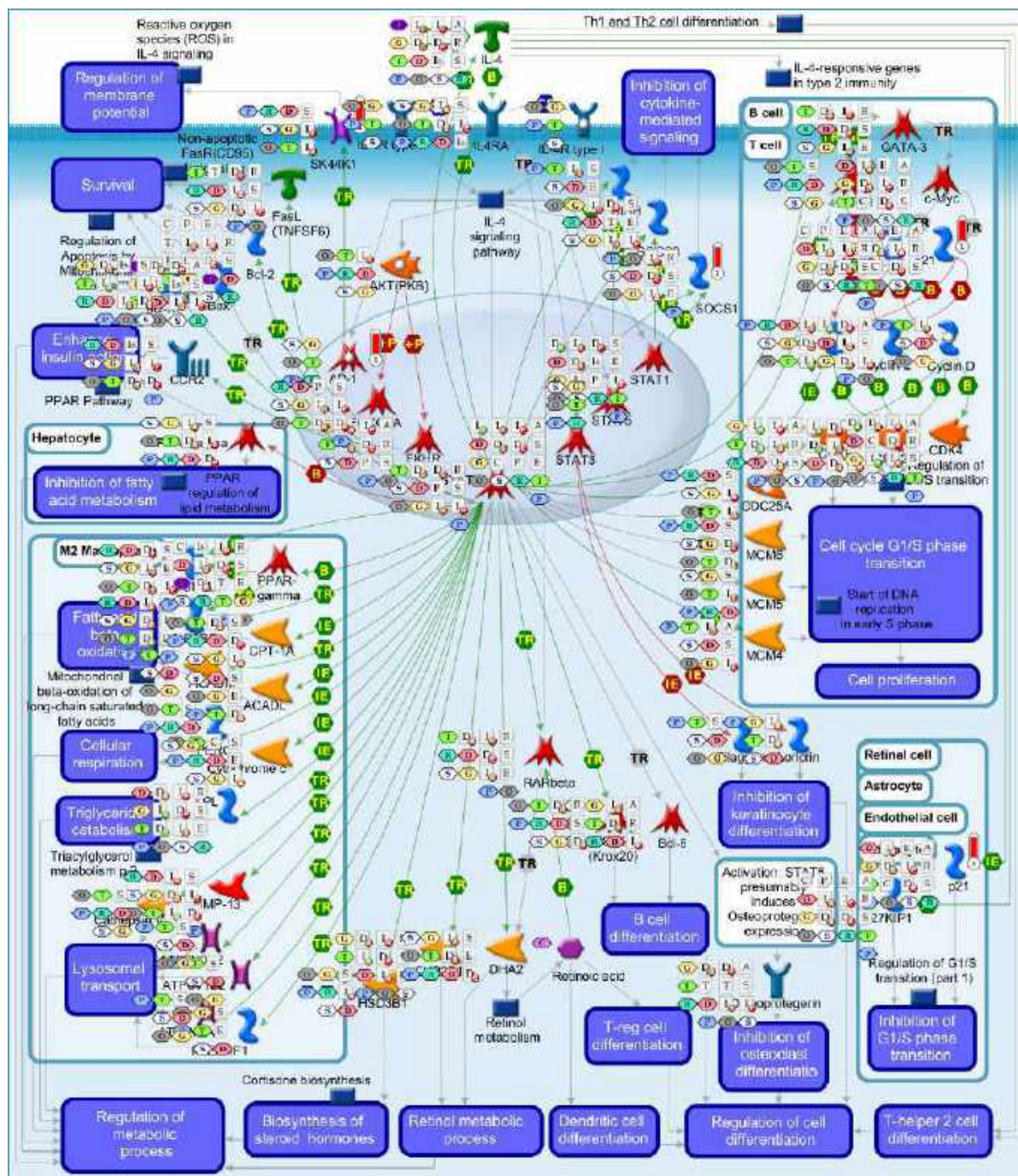


Figure 10. Immune response_IL-4-induced regulators of cell growth, survival, differentiation, and metabolism

Interleukin-4 (IL-4) has many biological functions. IL-4 recognizes two receptor complexes (IL-4R type I and II) constituted of a common receptor alpha subunit (IL4RA) and a cell-specific component [50]. IL-4 activates transcription factors after receptor binding through a sequence of phosphorylation events [51]. STAT6 is important for IL-4-mediated responses [52]. IL-4 binding to IL-4R type I affects type 2 immune cell survival, proliferation, and differentiation [53]. In non-hematopoietic cells, IL-4 communicates through the IL-4R type II receptor and regulates a wide range of cellular processes, integrating immune responses with tissue metabolism [54].

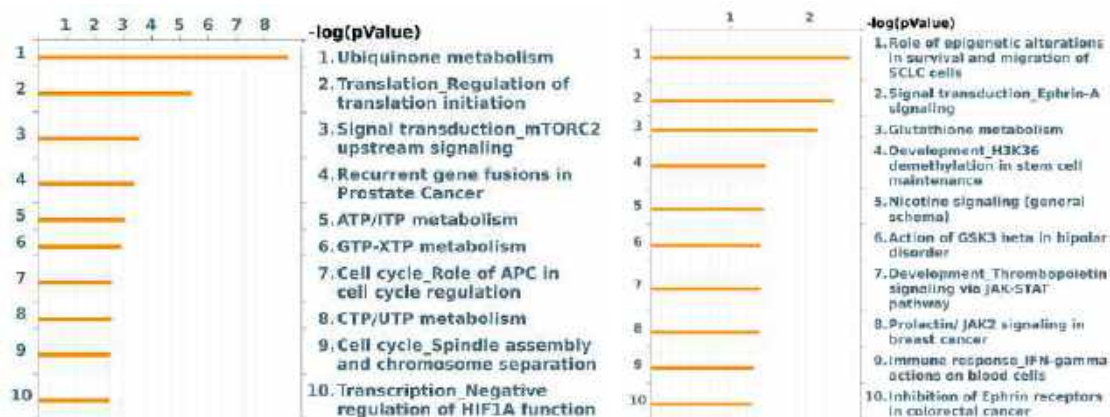


Figure 11. CD20 (left) and CD22 (right) co-expression genes potential pathways

Source: Metacore

Potential Therapy

In lymphoma, especially on relapsed or refractory follicular type, there are several therapies that have reached a certain phase of clinical trials. For example: (1) epigenetic therapy agents, in the form of: Tazemetostat (phase II); (2) PI3K inhibitors, in the form of: Idelalisib (phase II), Duvelisib (phase II), Copanlisib (phase II), Umbralisib (phase II), Zandelisib and Zandelisib and Rituximab (phase Ib); (3) CAR T Cells, in the form of: Axicabtagene ciloleucel (Phase II), Tisagenlecleucel (Phase II); (4) Bi-specific Antibodies, in the form of Mosunetuzumab (Phase I), Glofitamab (Phase I), Epcoritamab (Phase I or II); (5) BCL2 Inhibitors, in the form of Venetoclax (Phase I), Venetoclax + rituximab, venetoclax + bendamustine + rituximab, and bendamustine + rituximab (phase II); (6) BTK Inhibitors, in the form of Ibrutinib (phase II); (7) Checkpoint Inhibitors, such as: Nivolumab (phase II)[55].

Based on CD19 and CD22 co-expression genes, there was potentially pathways drug targeted (Figure 12 and Figure 13).

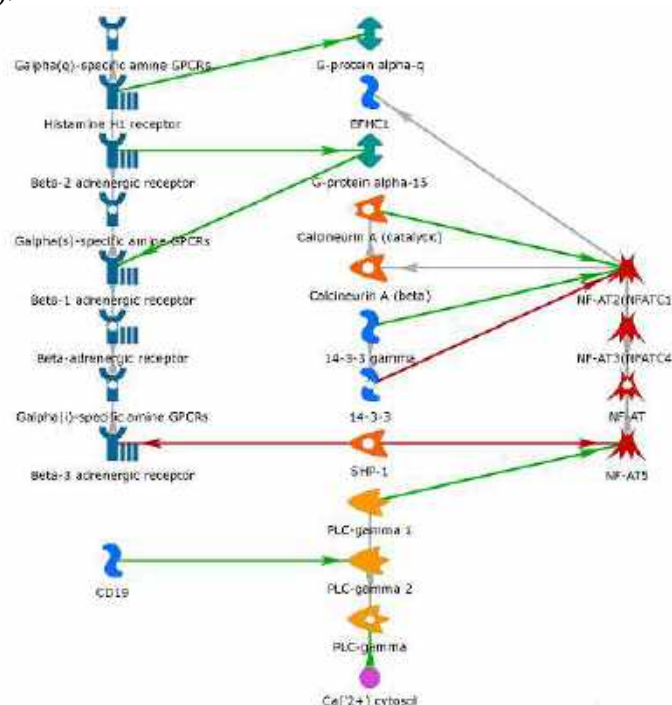


Figure 12. CD19, NF-AT2(NFATC1), NF-AT5, G-protein alpha-15, Ca(2+) cytosol.

Source: Metacore

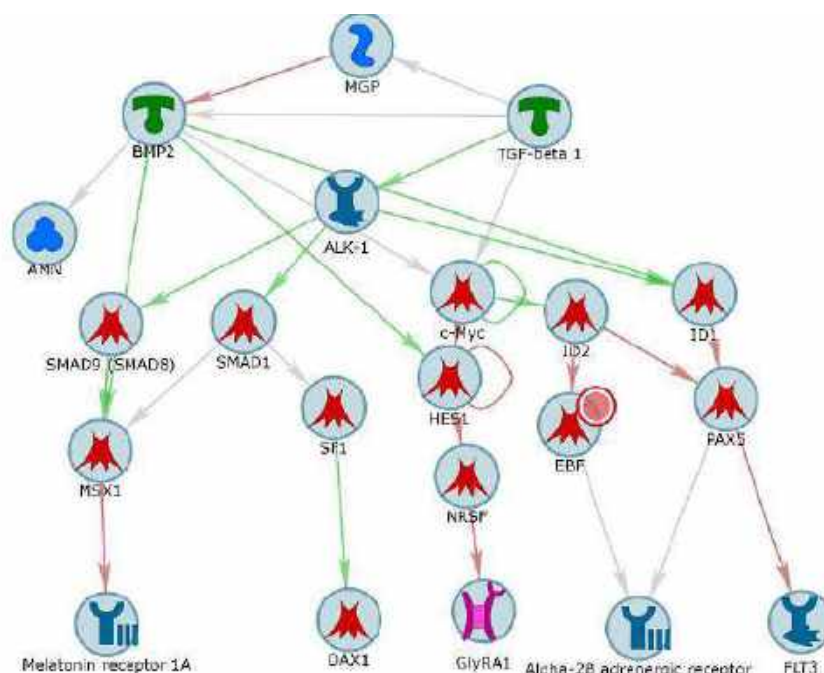


Figure 13. CD22 - Signal transduction_ALK-1. Source: Metacore

For CAR-T drug target interaction based on several co-expression genes, we should consider this fundamental schema (Figure 14). From Metacore database, we will comprehend several potentially drug targets, as shown on Figure 15.

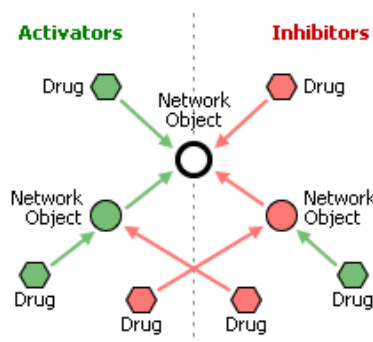


Figure 14. Activators – Inhibitors
Source: Metacore

Drug Object	#	Drug	Mechanism	Reference
CD22	1	N-Acetylcysteine acid esterase/ase-inhibition	receptor binding	7577844, 6963234, 8732208, 11512885, 12464712, 1446666, 1617946
Inhibitors				
CD20	1	* Veluturumab	binding	15102686
	2	* Docetaxel	binding	18815300
	3	* SB-1057	binding	
	4	* TRU-015	binding	
	5	Chintrastat	binding	
CD30(TNFRSF8)	1	* MOX1451	binding	
	2	* Bortezomib	binding	
	3	Ibrutinib	binding	1894384
	4	Ibrutinib	binding	12097203
CD22	1	* Epratuzumab	binding	1582536
	2	* Bortezomib	binding	12437671
	3	Ibrutinib	binding	18815323
CD19	1	* Ibrutinib	binding	
	2	* Ibrutinib	binding	12310380

Figure 15. Part of Drug Target
Source: Metacore

Conclusion

Using several comprehensive bioinformatics tools, softwares, and analyses, we can comprehend and observe numerous pathways, interactions, biomarker, and potential treatments. Further research and discovery based on wet-labs, artificial intelligence (AI), multiomics technologies, deep-learning, and machine learning are expected to validate this bioinformatics discovery and foster the development of theragnostics in the treatment of lymphoma.

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KNOWLEDGE, PERCEPTION AND ATTITUDE ABOUT CLIMATE CHANGE: A CROSS SECTIONAL STUDY AMONG GENERAL PEOPLE IN BANGLADESH

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Abstract

Bangladesh's topography makes it particularly susceptible to climate change. To tackle the impending tragedy of climate change, infrastructural, institutional, and financial capacity of the country must be increased. People's perception of climate change varies greatly based on their social backgrounds. Thus, understanding public perceptions of climate change may help improve government policies, adaption, and community activities. To understand Bangladeshi people's knowledge, perception, and attitude about climate change, an online survey among the general population was conducted using a structured questionnaire methodology. The study participants are mostly from Dhaka (70.25%) and have a moderate level of education (79.75% undergraduate). Most of them have heard of climate change and acknowledge the negative effects of climate change. Over half of participants are willing to give up personal gains to help solve current climatic and environmental issues. The majority of them, however, don't consider environmental factors while purchasing things and have never participated in climate change-related environmental protection initiatives. In Bangladesh, the general public's knowledge, attitude, and perception of climate change varies considerably. While knowledge and perceptions are high, attitude towards climate change is quite different. Along with promoting awareness, people should be encouraged to participate in climate change-related activities.

Key words: Climate change, Knowledge, Attitude, Perception, General people, Bangladesh

Introduction

Due to its topography, Bangladesh is more susceptible to dangers that may rise due to climate change than other countries. Bangladesh is exposed to climate-related calamities due to its place at the mouth of the drainage basin of the powerful Ganges-Brahmaputra-Meghna River systems[1]. As a result, flooding, cyclones, high rainfall, and drought are all common occurrences in Bangladesh. From 1999 to 2018, Bangladesh has suffered 191 climate-related severe events, placing it in seventh place on the 2020 Global Climate Risk Index[2]. For South and Southeast Asia, the IPCC's (Intergovernmental Panel on Climate Change) latest report sketched a future that is irrevocable and certain. There will be more and more regular and intermittent flooding episodes as the monsoon becomes stronger[3]. There would be a significant impact on agriculture and food security in coastal region of Bangladesh as a consequence of increased susceptibility to floods and salinity[4]. People's lives and the future generations of this nation are in danger because of climate change-related natural catastrophes.

This imminent disaster of climate change is calling for strengthening the infrastructures, institutional capacity and financial preparation as well as setting up a communal effort to combat it. However, the fact that people's views and perceptions concerning climate change vary widely and are difficult to forecast across a wide range of sociocultural backgrounds should not be overlooked. It is people's attitudes and views about climate change that determine how the general public perceives the issue. A person's innate ability to monitor his or her immediate surroundings is seen as a strength, and individuals who have personally experienced the negative effects of climate change, such as increased air pollution and temperature extremes, are more likely to see these threats. How worried individuals are about climate change depends on where they live, whether it is an urbanized or rural region, and if they have a history of dealing with extreme weather conditions.

Some argue that climate change is a direct result of human activity, while others think that it is an action of God as a retribution for the sins of humanity. Then there are some people who actually do not believe that climate change is in fact, real. There are several sociodemographic elements that influence how people perceive and understand climate change. In addition to sociodemographic factors, religious activity

is also connected to climate change perspectives. Now, people can only cope with climate change only if they are aware of it and prepared to deal with its effects. The news, the online world, and people's daily experiences with severe weather events such as flooding, cyclones, and drought all contribute to the informal acquisition of climate change knowledge. Hence it is quite possible that people's daily encounters with severe climatic and weather events might influence their views on climate change. Research on views and perceptions of Bangladeshis about climate change has previously been conducted among university students, indigenous people and at the household level [5,9], but this study was aimed at obtaining baseline data on the general population's knowledge, perception, and attitude toward climate change. Knowledge, perception, and attitude are the emphasis of this study. In light of its status as one of the world's most vulnerable LDCs (Least Developed Countries), research like this is critical to people's understanding of climate change. This research hypothesized that Bangladeshis today have minimal to moderate understanding of climate change and believe in its existence rather than rejecting it as a hoax, irrespective of their age, education level, occupation, living area (rural vs. urban), etc. It was discovered that this was definitely the case after concluding the investigation.

Materials and Methods

Aim of the study

The primary objective of this study is to gather baseline data on the general public's knowledge, perception, and attitude about climate change in Bangladesh.

Study design and setting

To understand Bangladeshi people's knowledge, perception and attitude about climate change, an investigative and anonymized survey among the general population was conducted. A structured questionnaire methodology was used, and an online survey was conducted to gather both quantitative and qualitative data. In order to ensure a high level of accuracy, the survey questions were written in English. Since exact questionnaires relevant for this research, especially in the context of general Bangladeshi citizens, were unavailable, an original, custom questionnaire was devised based on the literature review and media material, and an expert examined the questionnaire. Likert-type answers like "Yes," "No," or "Did not notice" or categorical options were used for most of the climate change knowledge and perception questions. Some questions were deleted or rephrased for better clarity as a result of expert feedback. At last, there were 35 questions in total in the final structured questionnaire[S1 File], divided into following four sections:

1. Sociodemographic information (10 questions)
2. Climate change knowledge related questions(7 questions)
3. People's perception regarding climate change (11 questions)
4. People's attitude towards climate change(7 questions)

Participants' socio-demographic variables and their knowledge, perceptions and attitude about climate change were gathered using the final questionnaire.

Questionnaire validation

In order to ensure that the questionnaire was appropriate for this study, the help of an appropriate expert was enlisted to assess its suitability for the job. The expert concluded that the questions and responses were relevant to the objectives of the investigation.

Study population and inclusion criteria

Bangladeshis who were in the country at the time of data collection were the target population of the study. The total population was around 166,961,248 as per Worldometer elaboration of the latest United Nations data[6]. To be included in the survey, applicants had to be Bangladeshi nationals at the time of the survey. Convenient and snowball sampling was used in this research. The google form link for the survey was put on different social media platforms and the participants were requested to share it their networks

online.

Data collection technique

Survey participants were asked to fill a Google form link and to spread the word about it to their friends and family through social media platforms like Facebook, Messenger, and WhatsApp. The form was also emailed to a number of additional people. Anyone could submit the form from September 17 until November 22, 2021. There were 800 total responses.

Sample size calculation

At a 95 percent confidence level, with a margin of error of 5 percent, 800 answers were collected. It was predicted that around 50% of the population would be aware of climate change. 385 individuals were needed to estimate this percentage with a 95 percent confidence interval. An overall sample size of 800 people was deemed to be appropriate for this investigation.

Statistical analysis

Microsoft Excel 2020 was used to clean, modify, sort, and analyze the data. Afterwards, using Microsoft Excel, descriptive statistics (i.e., frequencies, percentages, means, and standard deviations) and first-order analysis (i.e., chi-square tests, Fisher's exact tests) were conducted. Frequency tables were used to summarize and illustrate sociodemographic characteristics and responses of participants. Cross-tabulation and chi-square tests were used to examine the relationship between sociodemographic characteristics and knowledge of climate change. When the p-value was less than 0.05, all statistical tests were declared significant at a 95% confidence level.

Outcome measures

In order to evaluate respondents' degree of knowledge, attitudes, and perceptions regarding climate change, those who stated they had heard of it were asked about their source of knowledge, proceeded by questions about its causes and effects. Additionally, all respondents were asked climate-related questions to ascertain their attitude and perspective toward climate change, regardless of their level of knowledge about climate change

Results

The frequency distribution of respondents by their profile characteristics such as age, greatest degree of education, nation, and district is shown in Table 1. Of the total 714 participants, 89.25 percent were between the ages of 16 and 25. The mean age of the participants was 22.63, with a standard deviation (SD) of 6.45 years. It was found that a sizable proportion of respondents were young people from schools, colleges, universities, or other educational institutions, followed by persons in their early adulthood and middle age, who account for 7.75 percent and 2.75 percent of the study population, respectively. Only two (0.25 percent) individuals were above 60 and classified as elderly. In terms of highest level of education, respondents were more likely to be undergraduates, with a percentage of 79.75 percent (638), which explains the research population's high proportion of young individuals. Among the remainder, 91 (11.38%) were postgraduates, followed by 63 (7.88%) with a higher secondary education and just 8 (1%) with a secondary education. There were more inhabitants of Dhaka city than of other districts; around 70.25 percent (562) of the 800 respondents chose Dhaka as their present district of residence. This survey includes participants from different districts in Bangladesh, although they are overwhelmingly Dhaka city dwellers. Finally, all 800 respondents resided in Bangladesh, as the objective of this study was to elicit data on the general public's knowledge, perception, and attitude about climate change in Bangladesh.

TABLE 1: Characteristics of the participants (n = 800)

Characteristics	Frequency(800)	Percentage(100)
Age		
16-25	714	89.25%
26-40	62	7.75%
41-60	22	2.75%
>60	2	0.25%
Highest level of Education		
Higher Secondary	63	7.88%
Postgraduate	91	11.38%
Secondary	8	1.00%
Undergraduate	638	79.75%
Country		
Bangladesh	800	100.00%
District		
Manikganj	13	1.625%
Jessore		
Khulna	7	0.875%
Pabna	8	1%
Barguna	6	0.75%
Barisal	1	0.125%
Bhola	13	1.625%
Bogra	2	0.375%
Brahmonbaria	7	0.875%
Chandpur	6	0.75%
Chittagong	12	1.5%
Comilla	18	2.25%
Cox's Bazar	11	1.375%
Dhaka	9	1.125%
Dinajpur	562	70.25%
Faridpur	3	0.25%
Feni	4	0.5%
Gajipur	1	0.125%
Habiganj	12	1.5%
Jessore	6	0.75%
Jhalakhati	6	0.75%
Joypurhat	1	0.125%
Khulna	6	0.75%
Kushtia	11	1.375%
Madaripur	1	0.125%
Mymensingh	2	0.375%
Naogaon	3	0.25%
Narail	6	0.75%
Narayangonj	1	0.125%
Pabna	1	0.125%
Patuakhali	6	0.75%
Pirojpur	1	0.125%

Rajshahi	2	0.375%
Rangamati	13	1.625%
Rangpur	2	0.375%
Satkhira	19	2.375%
Sirajganj	1	0.125%
Sylhet	1	0.125%
Tangail	2	0.375%
Thakurgaon	8	1%
	6	0.75%

The frequency and percentage distributions of respondents' knowledge on climate change are shown in Table 2. The vast majority (n =800) of research participants (99 percent) were aware of climate change and gained knowledge from various sources, while just 1% had never heard of it. Additionally, a significant proportion of participants (99.75 %) believe that climate change has occurred in the recent decade, and 96.38 percent answered that they understand what the phrase "climate change" means. The majority of knowledgeable participants (31.75 percent) stated that they learned about climate change via newspapers, media, and teachers. When questioned about the causes of climate change, answers ranged from deforestation, fast urbanization and lifestyle change, and excessive carbon emission to industrial effluents, black smoke from vehicles, rapid urbanization and lifestyle change, and excessive carbon emission (0.13 percent). Over half of respondents (87 percent) believed climate change would have negative consequences, while 13% said it would have both negative and good consequences. In terms of the source gas of greenhouse effect, Carbon di-oxide, Nitrous oxide, C.F.C and Ozone were the most commonly mentioned(23%) .

TABLE 2: Participants' knowledge about climate change

VARIABLES	FREQUENCY(800)	PERCENTAGE(100)
Have you ever heard of climate change?	Total	Percentage
Yes	792	99%
No	8	1%
Do you think there has been climate change in last 10 years?		
No	2	0.25%
Yes.	798	99.75%
Do you know what the term climate change refers to?		
Maybe a little	28	3.50%
Not at all	1	0.13%
Yes	771	96.38%
How did you know about climate change?		
Newspaper	7	0.88%
Newspaper; Family members/ Relatives; Others	1	0.13%
Newspaper; Neighbors; NGO workers; Family members/ Relatives	14	1.75%
Newspaper; NGO workers; Family members/ Relatives	4	0.50%
Newspaper; Others	2	0.25%

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Newspaper; Teachers; Family members/ Relatives	10	1.25%
Newspaper; Teachers; Neighbors; Family members/ Relatives	3	0.38%
Newspaper; Teachers; Neighbors; NGO workers; Family members/ Relatives	15	1.88%
Newspaper; Teachers; Neighbors; NGO workers; Others	2	0.25%
Newspaper; Teachers; NGO workers; Family members/ Relatives	3	0.38%
Newspaper; Teachers ;Others	1	0.13%
Newspaper; Television	32	4.00%
Newspaper; Television; Family members/ Relatives	24	3.00%
Newspaper; Television; NGO workers	7	0.88%
Newspaper; Television; NGO workers; Family members/ Relatives	18	2.25%
Newspaper; Television; Others	20	2.50%
Newspaper; Television; Teachers	254	31.75%
Newspaper; Television; Teachers ;Family members/ Relatives	164	20.50%
Newspaper; Television; Teachers ;Family members/ Relatives; Others	1	0.13%
Newspaper; Television; Teachers; Neighbors	2	0.25%
Newspaper; Television; Teachers; Neighbors; Family members/ Relatives	46	5.75%
Newspaper; Television; Teachers; Neighbors; Family members/ Relatives ;Others	12	1.50%
Newspaper; Television; Teachers; Neighbors; NGO workers	9	1.13%
Newspaper; Television; Teachers; Neighbors; NGO workers; Family members/ Relatives	25	3.13%
Newspaper; Television; Teachers; Neighbors; NGO workers; Family members/ Relatives; Others	4	0.50%
Newspaper; Television; Teachers; NGO workers	2	0.25%
Newspaper; Television; Teachers; NGO workers; Family members/ Relatives	46	5.75%
Newspaper; Television; Teachers; NGO workers; Family members/ Relatives; Others	1	0.13%
Newspaper; Television; Teachers; NGO workers; Others	9	1.13%
Newspaper; Television; Teachers; Others	5	0.63%
Others	4	0.50%
Teachers	26	3.25%
Teachers; Neighbors	1	0.13%
Television	9	1.13%
Television; Teachers	16	2.00%
Television; Teachers; NGO workers; Family members/ Relatives; Others	1	0.13%

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What could be the reason behind climate change?		
Deforestation	29	3.63%
Deforestation; Black smoke of vehicles; Excessive carbon emission	7	0.88%
Deforestation; Black smoke of vehicles; Rapid urbanization and change in lifestyle	11	1.38%
Deforestation; Black smoke of vehicles; Rapid urbanization and change in lifestyle; Excessive carbon emission	139	17.4%
Deforestation; Excessive carbon emission	39	4.88%
Deforestation; Industrial effluents	1	0.13%
Deforestation; Industrial effluents; Black smoke of vehicles	1	0.13%
Deforestation; Industrial effluents; Black smoke of vehicles; Rapid urbanization and change in lifestyle	6	0.75%
Deforestation; Industrial effluents; Black smoke of vehicles; Rapid urbanization and change in lifestyle; Excessive carbon emission	28	3.5%
Deforestation; Industrial effluents; Excessive carbon emission	1	0.13%
Deforestation; Industrial effluents; Population growth	7	0.88%
Deforestation; Industrial effluents; Population growth; Black smoke of vehicles	2	0.25%
Deforestation; Industrial effluents; Population growth; Black smoke of vehicles; Excessive carbon emission	8	1%
Deforestation; Industrial effluents; Population growth; Black smoke of vehicles; Rapid urbanization and change in lifestyle	11	1.38%
Deforestation; Industrial effluents; Population growth; Black smoke of vehicles; Rapid urbanization and change in lifestyle; Excessive carbon emission	148	18.5%
Deforestation; Industrial effluents; Population growth; Excessive carbon emission	1	0.13%
Deforestation; Industrial effluents; Population growth; Rapid urbanization and change in lifestyle	5	0.63%
Deforestation; Industrial effluents; Population growth; Rapid urbanization and change in lifestyle; Excessive carbon emission	25	3.13%
Deforestation; Industrial effluents; Rapid urbanization and change in lifestyle; Excessive carbon emission	19	2.38%
Deforestation; Population growth; Black smoke of vehicles	6	0.75%
Deforestation; Population growth; Black smoke of vehicles; Excessive carbon emission	2	0.25%
Deforestation; Population growth; Black smoke of vehicles; Rapid urbanization and change in lifestyle; Excessive carbon emission	78	9.75%
Deforestation; Population growth; Excessive carbon emission	14	1.75%
Deforestation; Population growth; Rapid urbanization and change in lifestyle	2	0.25%
Deforestation; Population growth; Rapid urbanization and change in lifestyle; Excessive carbon emission	21	2.63%
Deforestation; Rapid urbanization and change in lifestyle	7	0.88%

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Deforestation; Rapid urbanization and change in lifestyle; Excessive carbon emission	166	20.8%
Excessive carbon emission	4	0.5%
Industrial effluents; Black smoke of vehicles; Rapid urbanization and change in lifestyle; Excessive carbon emission	1	0.13%
Industrial effluents; Population growth; Black smoke of vehicles	1	0.13%
Industrial effluents; Population growth; Black smoke of vehicles; Rapid urbanization and change in lifestyle; Excessive carbon emission	6	0.75%
Population growth; Black smoke of vehicles	2	0.25%
Rapid urbanization and change in lifestyle	2	0.25%
What is the effect of climate change?		
Both	104	13%
Negative	696	87%
Positive	0	0%
According to your knowledge, which gas causes the greenhouse effect?		
C.F.C	13	1.625%
C.F.C; Ozone	12	1.5%
Carbon di-oxide	128	16%
Carbon di-oxide ;C.F.C	75	9.375%
Carbon di-oxide; C.F.C; Ozone	139	17.375%
Carbon di-oxide; C.F.C; Ozone; Water	6	0.75%
Carbon di-oxide; Nitrous oxide	43	5.375%
Carbon di-oxide; Nitrous oxide; C.F.C	71	8.875%
Carbon di-oxide; Nitrous oxide; C.F.C; Ozone	183	22.875%
Carbon di-oxide; Nitrous oxide; C.F.C; Ozone; Water	31	3.875%
Carbon di-oxide; Nitrous oxide ;C.F.C ;Water	1	0.125%
Carbon di-oxide; Nitrous oxide; Ozone	31	3.875%
Carbon di-oxide; Nitrous oxide; Ozone; Water	1	0.125%
Carbon di-oxide; Ozone	62	7.775%
Carbon di-oxide; Ozone; Water	1	0.125%
Nitrous oxide; C.F.C	2	0.25%
Ozone	1	0.125%

Table 3 summarizes respondents' perception on climate change. More than 90% of the participants believed there has been an increase in the episode of cyclone, sea waves and drought in last 10 years.

Additionally, 98% of respondents stated that they had seen changes in rainfall patterns over the previous decade and 90% stated that there was a lack of fresh water owing to an increase in salinity. The majority of respondents strongly agreed that climate change is responsible for rising occurrences of extreme weather events (80%), and more than 95% believe that climate change is having an influence on people's health and agricultural production. Around 56% strongly agreed with the assertion that climate change is the catalyst for natural ecological crises. Most of the participants (99%) believed that the sea level has risen as a result of climate change. Finally, over 47% of respondents agreed strongly that climate change raises the threat of infectious illnesses.

TABLE 3: Participants' Perception towards climate change

VARIABLES	FREQUENCY(800)	PERCENTAGE(100)
Do you think the episode of cyclone increased in last 10 years?	Total	Percentage
Didn't notice	14	1.75
No	4	0.5
Yes	782	97.8
Do you think the episode of sea waves increased in last 10 years?		
Didn't notice	54	6.75%
No	4	0.50%
Yes	742	92.75%
Do you think the episode of drought increased in last 10 years?		
Didn't notice	45	6%
No	14	2%
Yes	741	93%
Did you notice any change in rainfall pattern in last 10 years?		
Didn't notice	12	2%
No	4	1%
Yes	784	98%
Do you think the increase in salinity caused scarcity of fresh water?		
Don't know	74	9%
No	8	1%
Yes	718	90%
Climate change increases extreme weather		
Agree	155	19%
Don't know	7	1%
Strongly agree	638	80%
Climate change affects human health		
Agree	280	35%
Disagree	1	0%
Don't know	7	1%
Strongly agree	512	64%
Climate change affects agricultural production		
Agree	307	38%
Strongly agree	493	62%
Climate change initiates natural		

ecological crisis		
Agree	342	43%
Don't know	10	1%
Strongly agree	448	56%
Climate change causes sea-level rise		
Agree	247	31%
Don't know	7	1%
Strongly agree	546	68%
Climate change increases threat of infectious diseases		
Agree	369	46%
Disagree	14	2%
Don't know	39	5%
Strongly agree	378	47%

Table 4 summarizes respondents' attitude on climate change. The vast majority of respondents (98 percent) believed that human activities (rather than natural factors) are the primary cause of climate change. Among the 800 respondents, 627 (79 percent) agreed that both developed and developing countries should have a greater share of the responsibility for climate change, while 16 percent said the former should bear the responsibility. The majority of respondents (88 percent) stated that while climate change cannot be totally averted, it can be mitigated through endeavor, and an even bigger proportion (93 percent) indicated that they would join actual efforts to mitigate climate change if called upon. On a more positive note, 97 percent of them indicated that they are extremely willing to forego some personal gain in order to address present climate change-related problems. When asked if they consider environmental factors before making a purchase, the percentages revealed a very different picture, with 77 percent of respondents responding that they typically pay little heed to such information. Additionally, 49% of respondents stated that they had never had the opportunity to participate in any environmental protection activities related to climate change, followed by 42% who had previously participated in such activities.

TABLE 4: Participants' Attitude towards climate change

VARIABLES	FREQUENCY(800)	PERCENTAGE(100)
Do you believe human activities (compared to natural factors) are the main cause of climate change?	Total	Percentage
No	7	1%
Uncertain	7	1%
Yes	786	98%
Do you think the developed countries or developing countries need to take a greater responsibility for climate change?		
Both	627	79%
The former	128	16%
The latter	4	1%
Unable to explain clearly	41	4%
Do you think climate change can be avoided?		
Absolutely	44	6%
It can be mitigated through endeavor	696	88%
Not possible	49	5%
Unable to explain clearly	11	1%
If someone called for, will you join the actual efforts to mitigate climate change?		

No	2	0.25%
Uncertain	52	6.5%
Yes	746	93.25%
Are you willing to sacrifice some individual benefit to solve existing problems?		
Not very willing	21	2.625%
Not willing at all	2	0.25%
Very willing	777	97.125%
Do you consider any environmental factor before your purchase?		
I do check these information activities every time before buying	181	23%
Usually paid little attention to such information	619	77%
Did you participate in any environmental protection activities related to climate change ever before?		
Didn't get a chance	388	49%
No	76	10%
Yes	336	42%

Table 5 compares respondents' knowledge of climate change by their age and highest level of education. In the multivariate study, education level and age were shown to be strongly linked with climate change knowledge. This suggests that when respondents are classified according to their age and education level, there is a statistically significant difference in their knowledge. Respondents with an undergraduate degree and those at the young age group (16-25) were more likely to have knowledge about climate change than others, presumably due to the knowledge gained through educational institutions and their effectiveness in obtaining information about climate change through technology.

TABLE 5: Relationship between socio-demographic variables and knowledge of climate change

Education Level	Knowledge of Climate change		Percentage		Result of Chi square test
	No	Yes	No	Yes	
Higher Secondary	3	60	37.5%	7.58%	$\chi^2 = 21.5104$
Postgraduate	1	90	12.5%	11.36%	$df = 3$
Secondary	1	7	12.5%	0.88%	$P = .000082$
Undergraduate	3	635	37.5%	80.18%	
Total	8	792	100%	100%	
Age-group	Knowledge of Climate change		Percentage		Result of Chi square test
	No	Yes	No	Yes	
Young (16-25 years)	3	727	37.5%	91.79%	$\chi^2 = 96.1948$
Early adulthood (26-40 years)	1	49	12.5%	6.19%	$df = 3$
Middle age (45-60 years)	3	15	37.5%	1.89%	$P < 0.00001$
Old age (>60 years)	1	1	12.5%	0.13%	
Total	8	792	100%	100%	

P indicates Probability value in the table, and χ^2 and *df* refers to the chi square and degree of frequency, respectively.

1. Discussion

This study is a quantitative endeavor to measure the general public's knowledge, perceptions, and attitude toward climate change in Bangladesh. The bulk of participants were in their twenties and have a rudimentary degree of formal education. They possess extensive knowledge and comprehension of the occurrence, causes, and consequences of climate change. Additionally, they have a strong understanding of climate change and the affiliated diversity in severe weather events, temperature, rainfall, salinity, and sea level, along with the impact of these changes on human health and agriculture, as well as the occurrence of ecological crises and the spread of infectious diseases. Knowledge, perception, and attitude study had been previously conducted among university students and CDC (Centers for Disease Control and Prevention) health professionals in Philippines and China, respectively[7,8]. In Bangladesh, Knowledge and perception study about climate change among university students and Knowledge and perception study about climate change and its impact on human health has been conducted in past[5,9]. In this study, the authors followed a somewhat identical method, but the sample size was bigger, more representative, and included more diversified population from Bangladesh. Over 95 percent of the 800 participants were aware of 'climate change,' which is a very promising indicator. They were also aware of the detrimental consequences of climate change. According to this study, the majority of people who were aware of climate change cited mass media like television, newspapers, and teachers as their sources. Very few persons reported hearing about climate change via non-governmental organization (NGO) workers, family members/relatives, and others. This demonstrates the importance of mass media and discussions with teachers in educational institutions. Service providers, such as non-governmental organization workers and elder members of families, require improved access to resources that provide knowledge of climate change such as television, internet etc. There appears to be a sectoral divergence in the government system's dealing with environmental concerns. In general, the analysis demonstrates that mass media coverage, particularly newspaper and television coverage, and opinion leaders, particularly teachers, are the primary sources of knowledge regarding climate change at the moment.

The majority of the participants thought that the climate change had occurred in the decade before the survey. This notion is borne out by statistics collected at the national level on Bangladesh's climate. According to data from the Bangladesh Meteorological Department (BMD), any 10-year period between 1951 and 2011 had greater temperatures and unpredictable rainfall than the prior ten years[10]. Additionally, a sizable proportion of respondents cited deforestation, rapid urbanization and lifestyle change, and excessive carbon emissions as the primary causes of climate change, along with mentioning carbon di-oxide, nitrogen oxide, carbon monoxide, carbon dioxide, and ozone as the primary suspects of the greenhouse effect. The participants' other less scientific explanations, such as black smoke from automobiles, may be explained by their poor education levels and limited exposure to scientific truths. In summary, this implies that they are aware of how anthropogenic actions in their immediate environment contribute to climate change and have expressed an interest in learning more about it.

The general public's perceptions of climate change are likewise aligned with the findings at the national scale. Over Bangladesh, the annual maximum temperature is growing by 0.09 °C, while the annual minimum temperature is rising by 0.10 °C. The yearly lowest temperature increases at a faster rate than the annual maximum temperature[11]. Over 95% of respondents reported noticing changes in rainfall patterns throughout the previous decade, which is corroborated by the fact that annual average rainfall over Bangladesh is rising by around 10.6 millimeters per decade, whereas monsoonal rainfall is falling by 7.6 mm per decade. The frequency of severe rainfall in June, July, and August has grown during the previous few decades[12]. Additionally, participants believed that extreme weather phenomena such as cyclones, sea waves, and droughts had risen in frequency during the previous decade. Cyclone frequency has been growing in each and every decade since the 1970s, according to the BMD (Bangladesh Meteorological Department) database[13]. Another evidence-based study corroborates our participants' perceptions of salt intrusion, increased water salinity, and lack of fresh water as a result of this[14]. Participants unanimously agreed that agricultural food crop productivity had decreased over the previous decade and that climate change had had a detrimental effect on population health. Additionally, they concurred that climate change is to blame for an increase in the threat of infectious illnesses and the onset of natural ecological disasters. These discoveries have far-reaching consequences for food security and livelihoods. Concerning sea level rise, the participants' perceptions are backed up by past records on sea level rise in 3 coastal districts. The

results indicate that the mean annual water level has increased by 4.5 millimeters per year in about 20% of the overall area of these three districts, which is ascribed to global sea level rise caused by human activity[15].

When it comes to attitude towards climate change, the majority of participants feel that human actions (rather than natural processes) are the primary cause. This is one of the study's most significant findings because it demonstrates that they are aware of how manmade activities in their nearby territory contribute to climate change. They believe that while the harm caused by climate change cannot be reversed, climate change may still be mitigated by effort. Additionally, they stated that both developed and developing nations must work cooperatively to combat climate change. LDCs such as Bangladesh are not entirely self-sufficient in terms of mitigating the harmful effects of climate change. Thus, in light of present circumstances, this appears to be the most sensible strategy to addressing climate change. On a more positive note, a sizable proportion of participants shown a strong readiness to forego some personal gain in order to fix current environmental issues. However, when it came to everyday actions, the data revealed a different picture, as the majority of respondents claimed that they paid little attention to environmental factors prior to making a purchase. This clearly demonstrates the need of increasing public knowledge about environmentally friendly products and the building of a green economy in Bangladesh. This discovery is especially beneficial for policy making and government planning. Additionally, the majority of participants stated that they had never had the opportunity to participate in any environmental protection activities connected to climate change, which might be a result of the general public's lack of access to and enthusiasm for environmental conservation activities. This demonstrates a clear sectoral divide in the way the government system addresses environmental concerns, as unmistakably, environmental protection activities require increased promotion to reach out to and engage the majority of Bangladesh's population, in order for these initiatives to be successful in mitigating the damage caused by climate change. In this study, young individuals (aged 16-25) on undergraduate level of education were more likely to have a stronger understanding of climate change than others. Additionally, other research found that respondents' understanding of climate change was highly dependent on their degree of education[8,9]. As a result of our study, it can be concluded saying that education and educational institutions are critical for improving awareness of climate change and associated adaptation concerns.

The research offers a number of strengths. Participants in the study were drawn from a wide range of climate change-prone regions. Climate-vulnerable places in Bangladesh might benefit from the findings of this study. Using these insights, researchers can devise new methods for changing people's and communities' behavior to better adapt to climate change and reduce the environmental problems it causes. A future cohort research might use these data as a starting point. The research also has a few drawbacks. In the first place, because the research was cross-sectional, causality cannot be established, and findings may not be extrapolated to other time periods. A longitudinal study is essential here. In addition, the study relied on online self-reporting, which is prone to social acceptance bias as well as memory bias. Last but not least, the study adopted snowball sampling approach that might be responsible for selection bias. As a result, a random sampling method should be used to conduct additional inquiry.

Conclusion

The knowledge of climate change far exceeded the expectation (99 percent), and the general public's perception of altering climate was similarly greater (99.75 percent). Attitudes concerning climate change appear to depict a quite different image, as seen by the general public's lack of active engagement in environmental protection activities in Bangladesh. The general public needs to be increasingly involved in such field-level actions to solve climate change-related environmental challenges. Level of education is a major factor in how people understand climate change and how it affects the surrounding environment. On the basis of these fundamental findings, policymakers may establish effective policies to safeguard the environment from the severe effects of climate change for the general population in Bangladesh and comparable LDCs. Educating children about climate change in schools might be a potential avenue for future study to safeguard future generations[16].

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PLATELET RICH PLASMA THERAPY IN DIABETIC MICE

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Abstract

Introduction:

Pancreas damage will have an impact on the occurrence of diabetes mellitus (DM). DM is characterized by a state of hyperglycemia. Platelet rich plasma (PRP) contains various types of growth factors and their application in medicine is known to have a repair response to tissue damage. The aim of this study was to describe the effect of PRP therapy on reducing blood glucose levels in diabetic mice.

Method: This study used 15 male mice and were separated into 3 groups: group I (normal), group II (DM), group III (DM + 0.1 mL PRP). The effect of diabetes in mice was carried out by intraperitoneal injection of streptozotocin (45 mg/kgBW). PRP was injected by intraperitoneal one time a week for 4 weeks. Blood glucose levels are measured using a glucometer and test strips (Autocheck™)

Results: The results showed that there was a decrease in blood glucose levels in group III treated with 0.1 mL PRP, which was 217.6 mg/dL compared to group II (317.15 mg/dL) and group I (113.5 mg/dL).

Conclusion: PRP therapy 0.1 mL can reduce blood glucose levels of diabetic mice, but it takes a higher dose of PRP and administration of therapy for a long period of time to significantly reduce blood glucose levels of diabetic mice compared to the normal group.

Key words : Blood Glucose Levels, Diabetic, Platelet Rich Plasma

INTRODUCTION

DM sufferers continue to increase along with the increasing level of the economy and changes in lifestyle. WHO estimates that the number of people with diabetes in Indonesia will increase from 8.4 million in 2000 to 21.3 million in 2030, while the IDF estimates that in 2009 there will be an increase in people with diabetes from 7.0 million to 12 million in 2030 (World Health Organization, 2016). Diabetes Mellitus (DM) is a metabolic disorder in the body characterized by hyperglycemia due to abnormal insulin secretion in pancreatic beta cells. Hyperglycemia can cause insulin resistance which will interfere with the transport of GLUT-4 from cells to the plasma membrane, thereby inhibiting the process of glucose regulation to enter cells (Serang and Felisia, 2017).

Streptozotocin (STZ) triggers pancreatic cell damage due to toxic substances. STZ will enter and circulate in cells through GLUT-2 in the plasma membrane. GLUT-2 is associated with Liver and Kidney organs which will be damaged by STZ. The toxic effects of STZ such as swelling of the pancreas, degeneration of cells of the islets of Langerhans, insulin production will be inhibited and decreased, causing Diabetes Mellitus (Zulkarnain, 2013).

Platelet Rich Plasma (PRP) is a product produced from fresh whole blood which contains growth factor components. PRP is obtained by centrifugation to obtain PRP. Platelet levels in PRP are 20 times higher than in circulating blood. PRP can increase the rate of cell proliferation because it has bioactive molecules known to be effective as growth factors in tissues (Zulkarnain, 2013).

Based on research by Zarin et al (2019), PRP therapy can increase insulin secretion in pancreatic cells in STZ-induced mice. According to research by El Tahawy (2017), PRP can stimulate the formation of new lobes and trigger tissue repair, ductal cells, and acinar cells in the pancreas. Based on previous studies, it's necessary to conduct research on PRP therapy in diabetic mice to see the effect of PRP therapy on reducing blood glucose levels in diabetic mice.

METHOD

This study was conducted in the laboratory of the John Paul II Health Academy, Pekanbaru. Subjects used were male mice which were divided into 3 groups: group I (normal), group II (DM), group III (DM+PRP 0.1 mL). Mice were acclimatized for 7 days with standard food and drink. After

acclimatization, blood glucose levels of mice were measured before the induction of diabetes.

Induction of diabetes in mice was carried out by injecting streptozotocin 1 time at a dose of 45 mg/kgBW in groups II and III. Mice were fasted before being injected intraperitoneally with STZ. Blood glucose levels were measured after 3 days of STZ injection and mice with glucose levels >200 mg/dl were considered to have diabetes. Group III DM mice (>200 mg/dl) were given 0.1 ml of PRP for 28 days.

The preparation procedure for PRP used 10 ml of venous blood from male donors taken and accommodated in a tube containing 3.2% citrate buffer. The blood in the tube was centrifuged at 1200 rpm. Plasma and buffy coat from the first centrifuge were transferred to a tube without anticoagulant, then the second centrifuged at 4000 rpm. The results of the second centrifuge will show a plasma layer on top and pellet deposits (containing a small amount of red blood cells and platelets) at the bottom. Remove 1/3 of the liquid plasma at the top. The remaining little plasma and pellets in the tube are PRP.

PRP therapy 0.1 ml was injected intraperitoneally in group III, once a week for 28 days. Monitoring of blood glucose levels was carried out for 7 days, 14 days, 21 days, and 28 days for each group. Blood glucose levels were measured using a glucometer. Blood glucose level data in each group were analyzed descriptively and statistically using SPSS to see the effect of repairing pancreatic tissue by PRP, which was characterized by a decrease in blood glucose levels in DM.

RESULTS

The glucose level of mice in each group at the time of acclimatization was at a normal value, which was <200 mg/dL. Furthermore, groups II and III were injected with STZ to induce diabetes in mice. After 3 days of STZ administration (before PRP therapy), there were increased glucose levels (>200 mg/dL) in group II (240.8 mg/dL) and group III (242.4 mg/dL) compared to group I (113.5 mg/dL). Mice with blood glucose levels >200 mg/dL were given 0.1 mL of PRP injection per week for 4 weeks. The results showed that decreased blood glucose levels in group III treated with 0.1 mL PRP, which was 217.6 mg/dL compared to group II (317.15 mg/dL) and group I (113.5 mg/dL).

Table 1. Average blood glucose levels of mice during acclimatization, before and after 0.1 mL PRP therapy.

Group	Mean $\pm$ SD Blood glucose level (mg/dL)			The difference before and after
	acclimatization	Before PRP treatment	after PRP treatment	
I (Normal)	111,8 $\pm$ 11,71	110,8 $\pm$ 13,57	113,5 $\pm$ 14,9	2,7
II (DM)	110,8 $\pm$ 19,64	240,8 $\pm$ 8,41	317,15 $\pm$ 48,07	76,35
III (DM+PRP 0,1mL)	109,2 $\pm$ 31,14	242,4 $\pm$ 27,18	217,6 $\pm$ 14,04	24,8

Blood glucose level data were analyzed using the Kolmogorov-smirnov test with p value >0.05 and that means blood glucose level data from 3 groups were normally distributed. The homogeneity test with p value >0.05 indicated that variants of all groups did not have significant differences or the homogeneity test was fulfilled. The results of the oneway ANOVA test with p value <0.05, and this could be interpreted as there were significant differences in blood glucose levels between the 3 groups. The post-Hoc LSD test was performed to compare the mean differences between groups. The results of the LSD test can be seen in Figure 1.

Based on statistical analysis, there were significant differences between each group concerning the blood glucose level of mice. Mice treated with STZ (group II) had blood glucose levels of 317.15 $\pm$ 48.07 mg/dL, significantly higher than Group I (normal) 113.5 $\pm$ 14.9 mg/dL. The 0.1 mL PRP treatment group in DM mice (Group III) was able to significantly reduce blood glucose levels by 217.6 $\pm$ 14.04 mg/dL compared to group II.

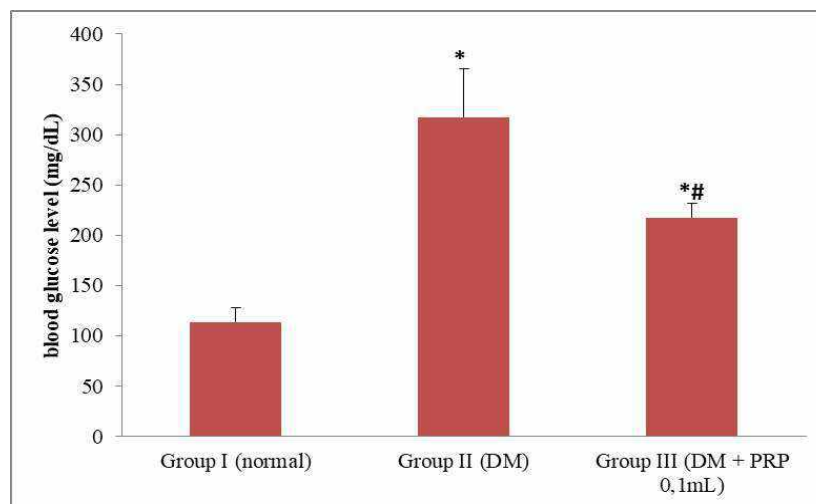


Figure 1. Mean and standar deviation of blood glucose levels in mice after PRP therapy. Group I (normal group) 113.5 ± 14.9 mg/dL; group II (DM) 317.15 ± 48.07 mg/dL; group III (DM + 0.1 mL PRP) 217.6 ± 14.04 mg/dL. * $p < 0.05$ compared with Group I, # compared with Group II

DISCUSSION

Induction of diabetes in this study was carried out using STZ injection 45 mg/kg BW. The results of STZ injection in groups II and III showed an increase in blood glucose levels >200 mg/dl when compared to the normal group. hyperglycemia in mice after STZ induction was characterized by blood glucose levels of 200-400 mg/dl (Saputra et al., 2018). Research by Munjiati et al (2021), the use of a single dose of STZ can increase the blood glucose levels of rats to 383.38 mg/dl. STZ is the most commonly used agent to induce diabetes mellitus in experimental animals. STZ works by forming free radicals that can damage pancreatic beta cells, so insulin production can be disrupted (Yuliana, 2021).

pancreas damage has an impact on increasing blood glucose levels. PRP therapy 0.1 ml can reduce blood glucose levels in group III (217.6 ± 14.04 mg/dl) compared to group II (317.15 ± 48.07 mg/dl). PRP therapy has been used to repair tissue damage. Hadi et al (2019) said that giving PRP can stimulate cell proliferation, so that PRP is often applied for therapy in wound healing or tissue repair (Ahmed et al., 2017; Irianto et al., 2021).

The normal group was used as a comparison of healthy mice that did not have pancreatic tissue damage. Synthetic insulin was not used in this study because it has no effect on repairing pancreatic tissue but only fulfills the need for insulin that cannot be produced by the body of people with diabetes. The decrease in blood glucose levels in group III indicated an improvement in pancreatic tissue damage. This study is in line with the study of Zarin et al (2019) that the use of PRP can increase insulin secretion in the pancreas, which is characterized by a decrease in blood glucose levels. Glucose levels decrease in group III was still significantly different from group I (normal), so that higher doses of PRP therapy and longer therapy were needed to improve pancreatic tissue, which was characterized by a decrease in blood glucose levels comparable to the normal group.

CONCLUSION

PRP therapy 0.1 ml has a repair effect on the pancreatic tissue of Diabetic mice which was characterized by a decrease in glucose levels in Diabetic mice, but higher doses and longer therapy were needed.

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SERUM CHOLINESTERASE LEVEL ON OIL PALM FARMERS IN PALAS, PEKANBARU

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Abstract

Introduction: Organophosphate pesticide is one of the most commonly used pesticides in agriculture especially oil palm to control pests. Pesticide exposure through oral, skin, and inhalation can affect oil palm farmers' health. This study aims to analyze the serum cholinesterase levels of oil palm farmers.

Method: Cross-sectional study design was conducted in this research. The population were 16 farmers who worked in spraying and fertilizing. Data were collected by examining cholinesterase, interviewing the oil palm farmers regarding, age, work period, pesticides dose, wind direction during spray application and the use of personal protective equipment (PPE). Serum cholinesterase levels was measured by using an automated photometric technique.

Results: This study found that 5 oil palm farmers (31.25%) had low cholinesterase levels or had mild poisoning. The lowest cholinesterase level was found in sample M with a value of 1,837,325 U/L. Based on the results of the questionnaire data, all oil palm farmers (100%) had a working period of 5 years and did not wear complete Personal Protective Equipment (PPE). 5 oil palm farmers (31.25%) used pesticide dose exceeding the directions indicated on the pesticide label and 5 oil palm farmers sprayed against the wind.

Conclusion: Cholinesterase levels can be affected by several factors. Use of Personal Protective Equipment (PPE) during the pesticide spraying is important to protect oil palm farmers and prevent nervous system disorder.

Keywords: Pesticides, Serum Cholinesterase, Oil Palm Farmers

INTRODUCTION

Pesticides are chemical substances containing a mixture of several substances commonly used to control and eradicate pests on plants (Yapo et al., 2017). The chemical content used in pesticides is not less than 45,000 formulations. There are less than 900 types of pesticides used to eradicate pests. There are 500 types of pesticides registered and permitted to circulate in Indonesia, and 13 types of pesticides have been found that can have a very dangerous impact on health (Marisa and Pratuna, 2018).

Pesticides from the organochlorine, organophosphate, carbamate, and pyrethroid groups are pesticides that are commonly used in agriculture to eradicate and control bodily insects software (Raini, 2007). Pesticides can poison the human body and animals through the mouth, skin, and respiration (through inhalation). The level of poisoning in the body due to exposure to pesticides can be known from the examination of Cholinesterase in the blood. activity cholinesterase is a number of enzymes that play a role in maintaining the balance of the nervous system in plasma and red blood cells (Hardi, Ikhtiar and Baharuddin, 2020). Enzyme Cholinesterase has the ability to hydrolyze acetylcholine and convert it into choline and acetic acid (Marisa and Pratuna, 2018).

Organochlorine, organophosphate, carbamate, and pyrethroid pesticides can bind to the Cholinesterase, so that this enzyme becomes inactive and acetylcholine accumulation in the blood occurs (Raini, 2007). This causes Cholinesterase in the blood to be disturbed. Accumulation acethylcholine that is not hydrolyzed in the blood can cause nervous system disorders in the form of a cholinergic continuous. This disorder is a sign or symptom of poisoning that occurs not only in the nerve endings but also in the nerve fibers. Cases of pesticide poisoning in agricultural workers are more than 168,000 people die every year (Hardi, Ikhtiar and Baharuddin, 2020).

According to data from the World Health Organization (WHO, 2017) cases of poisoning from the use of pesticides are increasing, especially in developing countries such as Southeast Asia, Africa, America including Central America and Latin America. Pesticide poisoning in farmers occurs due to lack of attention to the use of Personal Protective Equipment (PPE) in using pesticides (Marisa and Pratuna, 2018). Currently there is no research that discusses Cholinesterase in oil palm farmers, especially in Palas, Pekanbaru city. Therefore, researchers are interested in conducting research on Cholinesterase as the impact of pesticide exposure on farmers, especially vegetable farmers in Palas Pekanbaru City.

METHODS

This cross-sectional study was conducted in oil palm farmers on Palas, Pekanbaru from March to June 2022.

Inclusion criteria

Purposive sampling techniques was used in this study. The inclusion criteria of farmers must be: (a) male or female who had been working longer than 5 years, (b) Age range 20–50 years old, (c) Personal Protective Equipment (PPE) usage, (d) did not have liver disease. For each farmer who meets our inclusion criteria, a fact sheet containing professional information, pesticide application conditions, and clinical aspects has been completed. After information, informed consent, a promise of confidentiality and a return of information, a blood sample for the cholinesterase test was made.

Tools and materials

This study used Centrifugator, Spectrophotometer BS4BN013 E, Vacutainer Red Syringe, Sterile Gauze, Tourniquet, 70% Alcohol Cotton, Cuvette, Micropipette 10 µL, 1000 µL, Blue Tip, Yellow Tip, Serum Cup, Test Tubes, Test Tube Racks, Pasteur Pipettes and Personal Protective Equipment (PPE). Materials were used in this study consist of serum sample, Cholinesterase kit Reagent 1 (Phosphate Buffer), Reagent 2 (substrate: Butyrylthiocholine), Reagent 3 (Dibucaine Chlorhydrate), Reagent inhibitor, Aquades

Samples

The blood collected by venipuncture in dry Vacutainer tubes cholinesterase. Samples were immediately conveyed to the laboratory in coolers containing ice. Tubes without anticoagulants were centrifuged at 3000 rpm for 3 min for analysis. Serum and red blood cells were separated using a pasteur pipette. The serum is transferred to a labeled test tube and the red blood cells are discarded.

Dosage

Serum cholinesterase was evaluated by photometry with kinetic reading. The dosage principle is as follows: Cholinesterase catalyzes the hydrolysis of butyrylthiocholine to butyrate and thiocholine. Thiocholine reacts with 5,5'-dithiobis-2-nitrobenzoic acid (D.T.N.B) to form nitro-2- mercapto-5-benzoate. The concentration of the product formed which is measured at 405 nm is proportional to the enzymatic activity of the cholinesterase present in the sample to be assayed. Based on *cholinesterase* values, the severity of *cholinesterase* is grouped into three categories, namely mild poisoning with high levels *cholinesterase* 1,401 – 3,500 U/L, moderate poisoning with *cholinesterase* 701 – 1,400 U/, and severe poisoning with *cholinesterase* < 700 U/L (Banday *et al.*, 2015) for a reaction temperature of 37°C.

RESULTS

Socio-demographic and professional characteristics of the sample

The study population consisted of 16 farmers. Based on the results of the questionnaire data, all farmers (100%) have a working period of 5 years. We identified that 100% of the farmers did not wear the personal protective equipment (PPE) during pesticide spraying, such as gloves, hat, mask and boots. Pesticide dose has a direct effect on the danger of pesticide poisoning. A total of 5 farmers (31.25%) used pesticide doses exceeding the rules stated on the pesticide label. Wind direction can affect cholinesterase levels in farmers blood. We identified 5 farmers (31.25%) spraying against the wind (Table 1).

Table 1. Characteristics of the samples

Characteristics	Variables	Number	%
Sex	Male	16	100
	Female	0	0
Working period	<5 years	0	0
	≥5 years	16	100
Use of PPE	Complete	0	0
	Not complete	16	100
Pesticide dosage	Right dose	11	73,33%
	Over dose	5	31,25%
Spraying direction	Against the wind	11	73,33%

Based on cholinesterase levels to normal values, the severity of cholinesterase poisoning is grouped into three categories, namely mild poisoning with cholinesterase levels of 1,401–3,500 U/L, moderate poisoning with cholinesterase levels of 701–1,400 U/L, and severe poisoning with cholinesterase levels <700 U/L. (Banday et al., 2015). In table 2, the results of the cholinesterase examination showed that 11 farmers (68.75%) had normal cholinesterase levels and 5 farmers (31.25%) had cholinesterase levels below normal limits or had mild poisoning. The lowest cholinesterase levels were found in sample M with a value of 1,837.325 U/L.

Table 2. Cholinesterase activity levels

Sample Code	Cholinesterase Levels	Poisoning Level
Sample A	6,852.412 U/L	Normal
Sample B	4,134.558 U/L	Normal
Sample C	4,252.424 U/L	Normal
Sample D	6,762.280 U/L	Normal
Sample E	5,290.108 U/L	Normal
Sample F	4,869.488 U/L	Normal
Sample G	6,489.569 U/L	Normal
Sample H	4,210.824 U/L	Normal
Sample I	1,968.133 U/L	Mild Poisoning
Sample J	6,762.280 U/L	Normal
Sample K	2,747.898 U/L	Mild Poisoning
Sample L	8,417.027 U/L	Normal
Sample M	1,837.325 U/L	Mild Poisoning
Sample N	2,734.031 U/L	Mild Poisoning
Sample O	3,494.385 U/L	Mild Poisoning
Sample P	5,578.996 U/L	Normal

DISCUSSION

The sample criteria used are 20-50 years old, have a working period of more than five years, and do not use Personal Protective Equipment (PPE) in the form of gloves and masks when working. The sample used for cholinesterase examination is serum from the veins of oil palm farmers.

Pesticides are substances or chemicals that are often used in agriculture to prevent and kill plant pests that have the potential to interfere with agricultural products (Meokasan, Prabaningrum and Adiyoga, 2014). Pesticides can enter the body through the skin, inhalation and oral route, so that to determine the level of poisoning or exposure to pesticides in the body, it is necessary to examine cholinesterase on farmers. Blood cholinesterase activity is the amount of active cholinesterase enzyme in blood plasma and red blood cells that play a role in maintaining the balance of the nervous system.

Based on the results of the study in table 2, it was found that the levels of cholinesterase in 11 samples (68,75%) were normal and 5 samples (31.25%) had decreased levels of cholinesterase in the blood which were categorized as mild poisoning. Cholinesterase levels can be disturbed by several factors when spraying organophosphate pesticides because organophosphates will bind to the cholinesterase enzyme so that the cholinesterase becomes inactive. The factors are working period, Personal Protective Equipment (PPE), pesticide dose and spraying wind direction.

The results showed that 31.25% of respondents experienced a decrease in cholinesterase in the body. One of the factors that cause a decrease in cholinesterase levels in the body is the accumulation of pesticides in the body. Cholinesterase which is supposed to decompose acetylcholine will bind to the active substances contained in pesticides, especially the organophosphate group. When cholinesterase binds to organophosphate, acetylcholine cannot be broken down, resulting in the accumulation of acetylcholine (Agustina et al., 2018).

Based on the results of the questionnaire data, all farmers (100%) have a working period of 5 years. The duration of action can affect the level of cholinesterase in the blood. The longer the working period of a farmer, the lower the level of cholinesterase in the farmer's blood. This happens because the working period of farmers who have been spraying pesticides for a long time can cause poisoning due to exposure

to pesticides so that the amount of pesticide poison that enters the body will accumulate (Hardi et al., 2020).

In this study, all farmers did not use complete Personal Protective Equipment (PPE). Personal Protective Equipment (PPE) is a tool that is able to protect workers because it can protect workers from possible risks and potential hazards that can arise in the workplace. Incomplete use of PPE can increase the risk of exposure to pesticides and have a negative impact on farmers' health because chemicals can be absorbed by the skin causing poisoning caused by pesticides (Tutu et al., 2020)

Pesticide dose has a direct effect on the danger of pesticide poisoning. A total of 5 farmers (31.25%) used pesticide doses exceeding the rules stated on the pesticide label. Therefore, when mixing pesticides, you should pay attention to the dosage listed on the label. Doses that exceed the rules will harm the sprayer itself (Hardi et al., 2020)

Wind direction can affect cholinesterase levels in farmers' blood. In this study, 5 farmers sprayed against the wind. Farmers must pay attention to the wind direction when spraying. If farmers do not pay attention to the direction of the wind and spray with the opposite wind direction, the level of pesticide exposure will be even greater and can reduce cholinesterase levels in farmers blood (Tutu et al., 2020)

The impact if there is a decrease in cholinesterase levels in the body is a nervous system disorder in the form of an increase in cholinergic activity continuously because acetylcholine is not hydrolyzed. This disorder is known as a sign or symptom of poisoning which does not only occur in nerve endings but also occurs in nerve fibers (Hardi, Ikhtiar and Baharuddin, 2020).

CONCLUSION

Based on this study 11 farmers had *cholinesterase* levels (68.75%) and 5 farmers (31.25%) had *cholinesterase* below normal limits. The *cholinesterase* level in farmers was 1,837.325 U/L. It is necessary to sensitize and evaluation of these farmers as recommended by the WHO (1979) for subjects exposed to organophosphates and carbamates. Further studies should be conducted on a larger number of farmers in order to have a more representative conclusion.

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DECREASE IN THE NUMBER OF GERMS AFTER WASHING HANDS BASED ON VARIATIONS IN THE CONTACT TIME OF THE USE OF HAND SANITARY CHLORHEXIDINE 2 % GEL

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Abstract

Introduction : Hand washing is a standard precautionary requirement to prevent infectious diseases and the implementation of health protocols during the Covid-19 pandemic. The use of hand sanitizers is a practical way for people to use in special situations to replace hand washing with soap and running water. Hand sanitizer gel contains chlorhexidine 2% and alcohol 70% is widely used as an active ingredient in antimicrobials. This study aims to determine the comparison of the number of germs before and after washing hands with the use of chlorhexidine hand sanitizer 2% gel based on variations in contact time of 15 seconds and 30 seconds.

Method : The type of research used is true experiment pre- post test design research design. The sampling technique used purposive sampling of 30 and analyzed with a dependent T-test with a 95% confidence level.

Results : The results of the study obtained the average number of germs before washing hands was 2.3×10^2 CFU/cm², after washing hands a contact time of 15 seconds of 1.3×10^2 CFU/cm² and a contact time of 30 seconds of 0.7×10^2 CFU/cm². Decreased the number of 15-second contact time germs by 46.16% and 30-second contact time by 70.98%. Based on the dependent T test, it showed a significant difference in the number of germs before and after hand washing, the contact time variation was 15 seconds and the contact time variation was 30 seconds (p value = 0.00).

Conclusion: Conclusion of contact time of 30 seconds gel hand sanitizer containing chlorhexidine 2% and alcohol 70% effectively lower the number of germs. Chlorhexidine and alcohol have a similar mechanism of action, so there is a combination of antiseptics between the two substances.

Key Words: Hand Sanitizer Gel, Chlorhexidine, Germ Numbers

INTRODUCTION

The Indonesian government is increasingly strictly implementing several health measures to keep people productive during the pandemic by implementing the 3M health protocol, namely the use of masks, maintaining distance and washing hands with soap (Ministry of Health, 2020). According to the Task Force for Handling COVID-19, the risk of transmitting COVID-19 without 3M behavior can reach 100% but by washing hands the risk of contracting it decreases by 35% (SATGAS, 2020). Wash hands with soap (CTPS) to prevent transmission because in daily activities the hands are often contaminated with microbes, so that the hands can be an intermediary for the entry of microbes into the body (Desiyanto and Djannah, 2013). Based on PMK No.27 concerning Guidelines for Infection Prevention and Control in Health Service Facilities, there are 11 standards of vigilance in health facilities, one of which is hand hygiene using soap and running water (Ministry of Health, 2017). One of the benefits of CTPS is that it can and prevents the incidence of diarrhea up to 50% (Fazriyati, 2013). Similar to the study conducted by Purnomo R.A (2016) that 69.4% of respondents did not experience the incidence of diarrhea because they had a good handwashing habit.

Reporting from the viva news portal, the Indonesia Water Institute (IWI) conducted a study of 1,296 respondents in 2020 and showed that as much as 67% there was an increase in handwashing activities from normal conditions before the COVID-19 pandemic (Viva, 2020). There are several obstacles in handwashing activities with water and soap such as traveling, the location of handwashing stations that are not strategic, the unavailability of soap and water and a busy schedule (CDC, 2002). Therefore, hand sanitizer without soap and water was created, namely hand sanitizer. Hand sanitizers are divided into 2, namely gel and spray (Diana, Hendrarini and Narto, 2013)

One of the disadvantages of hand sanitizer spray products is that washing them must be done quickly because it only takes 15 seconds to dry and is prone to evaporate into the air (Diana, Hendrarini and Narto, 2013). People in general like the use of gel hand sanitizers because it causes a cold feeling in the skin and is not too hasty to use because it takes 30 seconds to dry (Asngad, R and

Nopitasari, 2018). According to who hand hygiene in health care guidelines state that hand sanitizers take 20-30 seconds and there are a number of studies on antimicrobials in vivo on alcohol activity about the effects of antiseptic handrubs, it is found that washing hands for 30 seconds can reduce bacterial flora. A survey conducted by the Central Statistics Agency (BPS) on September 7-14, 2020 on 90,967 respondents obtained results of 77.71% of the use of hand sanitizers as disinfectants when outside the home (Nasional Tempo, 2020). Hand sanitizer is an antiseptic substance in which there is 60-95% alcohol so that it can be used as an antimicrobial active ingredient in a hand sanitizer product (CDC, 2020). The CDC also recommends choosing a hand sanitizer with an alcohol content of at least 60% (CDC, 2020). In addition to alcohol, there are also non-alcoholic active ingredients in the hand sanitizer product, one of which is chlorhexidine. The kandunan hand sanitizer used in this study was 70% alcohol and 2% chlorhexidine.

Chlorhexidine is a bactericide that works by damaging cell walls and cell outer membranes, resulting in intracellular leakage and ultimately cytosol coagulation (Adham et al., 2015). Chlorhexidine and alcohol have a similar mechanism of action, so there is a combination of antiseptics between the two substances.

In previous studies that have been conducted by Yosi et al., in 2019 regarding the synergistic effect of the combination of chlorhexidine and alcohol on the inhibitory power of *Staphylococcus aureus* growth, the results of the combination of chlorhexidine-alcohol were stronger than chlorhexidine and alcohol alone.

Students are used as research respondents because they have dense activities and often come into contact with objects and equipment while on campus such as laboratory tools and materials, elevator buttons and other practical activities. The number of activities makes students less likely to have time to wash their hands with soap, so students often use hand sanitizers as an alternative to washing their hands because hand sanitizers can be used as an antiseptic of microorganisms. Then to see the effectiveness of using hand sanitizers as an alternative antiseptic in reducing microorganisms on the hands that can cause infection, one of which is by calculating the number of germs.

METHOD

The research was conducted at the Bacteriology Laboratory, Department of Medical Laboratory Technology, Health Polytechnic of Jakarta III in January 2022 – July 2022. The population in this study were 30 respondents of the Jakarta Police Iii Semester 4 Medical Laboratory Technology Students. According to the sample size for an experimental study according to sugiyono (Soares, 2013). With sampling techniques using purposive sampling based on predetermined inclusion and exclusion criteria.

The principle of calculating the number of germs using the saucer count method is that if the surviving microbial cells are grown on the media so that the microbial cells will multiply and form colonies that can be seen directly with the eyes without the use of a microscope. Researchers took a palm swab sample before washing hands using a cotton swab in sterile 0.9% NaCl. Then take a sample after washing hands using hand sanitizer with a contact time of 15 seconds and 30 seconds. Then the sample was inoculated on PCA media and incubated 37°C for 24 hours. Colonies of growing germs are calculated using *colony counters*.

RESULTS

Tabel 1. Respondent Characteristics Results

Questions	Answers	Persentase(%)
Handwashing Counseling	Yes	100%
	No	0%
Benefits and Impacts of Handwashing	Yes	100%
	No	0%
Wash Hands Before and After Practicum	Yes	100%
	No	0%
Wash Hands With Soap Or Hand Sanitizer	Hand Sanitizer	70%
	Soap	30%

Frequency of HandWashing in a Day	1-5 times	64%
	6-10 times	34%
Types of Hand Sanitizers used	Gel	66%
	Spray	34%

In table 1, it shows that all respondents have received handwashing counseling so that they understand the benefits and impacts of washing hands, then respondents wash their hands 1-5 times a day (64%) and use hand sanitizers more often (70%) in the form of gels (66%).

Tabel 2 Results of the Analysis of the Average Number of Germs

Sample Group	N	Mean (CFU/cm ²)
Before CTPH	30	2,3 x 10 ²
After CTPH contact time 15 detik	30	1,3 x 10 ²
After CTPH contact time 30 detik	30	0,7 x 10 ²

Ket. CTPH : Cuci Tangan Pakai Hands sanitizer

Tabel 2 the average number of germs in the non-handwashing sample group was 2.3 x 10² CFU/cm², then the decrease in the sample group after hand washing was a 15-second contact time variation of 1.3 x 10² CFU/cm². The results of the analysis of the average decrease in the number of germs based on variations in contact time can be seen in the following table:

Table 3 Decrease in the Number of Germs Before and After Washing Hands with Hand Sanitizer Gel Based on Contact Time Variations of 15 and 30 seconds

Sample Group	Mean (CFU/cm ²)	Decrease in the number of colonies (%)
Decrease in the number of germs before and after hand washing 15-second contact time variation	1,1 x 10 ²	46,16%
Decrease in the number of germs before and after hand washing contact time variation 30	1,7 x 10 ²	70,98%

In table 3, the results obtained with the largest decrease in the number of colonies were in the variable group with a contact time variation of 30 seconds, which was 70.98%

DISCUSSION

The selection of respondents to this study was TLM students in semester 4 of Health Polytechnic of Jakarta III because the consideration of the lecture schedule that was being carried out was more crowded than other semester students. In addition, the intensity of the practicum of 4th semester students is more. In accordance with the background of hands sanitizer, it is effective as an antiseptic alternative product in respondents who have a busy schedule and do not have enough time to wash their hands with soap and running water.

The application of handwashing behavior among TLM students in semester 4 of Health Polytechnic of Jakarta III is influenced by several factors including the knowledge and behavior of respondents. It can be seen in table 1 that all respondents already know how to wash their hands properly, understand well the benefits and impacts of washing hands, and have a good habit of washing hands regularly because respondents have a high awareness of their own vigilance against microorganism infections. Students wash their hands while doing activities or finish doing activities. This is in line with a survey that has been conducted by the Central Statistics Agency for the period 13-20 July 2021 showing that almost 75% of community members have washed their hands frequently.

Interestingly, the observation results also showed that the majority of respondents washed their hands more often with hand sanitizers compared to soap. This is in line with a survey conducted by the Central Statistics Agency in 2020 with the results that as many as 77.71% of people use hand sanitizers more often when outside the home because it is more practical. Then the observation results also showed that the majority of respondents used gel-shaped hand sanitizer because it caused a cold

feeling in the skin and easily dried up so that they felt comfortable (Asngad, R and Nopitasari, 2018).

Table 2 shows the highest average number of germs that do not wash their hands at 2.3×10^2 CFU/cm² and the average number of the smallest germs is with a contact time variation of 30 seconds, which is 0.7×10^2 CFU/cm². This result is in line with previous studies on the comparison of the effectiveness of hand washing with soap and hand sanitizer that the number of germs before washing hands is higher than after washing hands (Amaliyah, 2019). As is known, the hand is the part of the body that is most often used for activities such as holding money, holding bathroom doors, holding telephones, holding toys, and holding public objects (Raksanagara and Raksanagara, 2016). so that there is a transfer of germs from objects to the palm of the hand. Then germs grow and develop in the hands and cause a high number of germs in the hands (Pratami, Apriliana and Rukmono, 2013).

The number of germs decreases when washing hands with hand sanitizers, because hand sanitizers contain antiseptics in the form of alcohol that function to kill viruses and bacteria because alcohol works to destroy disease germs by breaking down proteins, splitting cells into several parts until finally lysis. Lysis is a state in which all matter in the germ's body undergoes diffusion out of cells (Retno A et al., 2020). The average number of the smallest germs at 30 seconds because the longer the contact time, the greater the decrease in the number of germs. This can be proved hammering the results in table 3.

In table 3 regarding the decrease in the number of germs, the results of the most decrease occurred in the variables before and after washing hands using hand sanitizer with a variation in contact time of 30 seconds (70.98%). This result is in line with the previous two studies, namely a time of 15 seconds is not inferior in reducing the number of germs but by 30 seconds it is able to reduce the number of germs more (Daniela P, et al., 2017). In the study of Harnoss, et al., 2020 showed results by washing hands for 15 seconds can reduce germs by 54.7% and for 30 seconds by 69.5%.

Data obtained by researchers regarding the bactericidal efficacy of several alcohol concentrations on bacterial species showed that 70% alcohol with a contact time of 15 seconds has not been able to kill bacteria such as *Escherichia coli* and *Mycobacterium tuberculosis*, while with a contact time of 30 seconds alcohol 70% has been able to kill these bacteria. This happens because in less than 30 seconds the active ingredient contained in the hand sanitizer is not enough time to penetrate the cell wall then denature the protein (WHO, 2009). Because *Escherichia coli* and other gram-negative bacteria have a relatively more complex and three-layered cell wall where the outer layer is lipoprotein, the middle layer is in the form of lipopolysaccharides and the inner layer is peptidoglycan so that the *E.coli* cell wall is more difficult to penetrate by antibacterial compounds (Maryadi, Yusuf and Farida, 2017). Data obtained by researchers regarding the bactericidal efficacy of several alcohol concentrations on bacterial species showed that 70% alcohol with a contact time of 15 seconds has not been able to kill bacteria such as *Escherichia coli* and *Mycobacterium tuberculosis*, while with a contact time of 30 seconds alcohol 70% has been able to kill these bacteria. This happens because in less than 30 seconds the active ingredient contained in the hand sanitizer is not enough time to penetrate the cell wall then denature the protein (WHO, 2009). Because *Escherichia coli* and other gram-negative bacteria have a relatively more complex and three-layered cell wall where the outer layer is lipoprotein, the middle layer is in the form of lipopolysaccharides and the inner layer is peptidoglycan so that the *E.coli* cell wall is more difficult to penetrate by antibacterial compounds (Maryadi, Yusuf and Farida, 2017).

There is a decrease in the number of germs after washing hands with hand sanitizer because the hand sanitizer product contains active ingredients in the form of alcohol and additional chlorhexidine which makes the ability to kill germs better than hand sanitizers that contain only one active ingredient (Yosi et al., 2013). Active ingredients such as chlorhexidine have a mechanism of action for denaturation of proteins and enzymes of microorganisms and inhibit cell respiration by means of which chlorhexidine will penetrate the cell wall then cause instability of the cell wall and protein denaturation occurs so that it ends with the lysis of the bacterial cell wall.

From the results of this study, hand washing treatment using hand sanitizers both with a time variation of 15 seconds and a time variation of 30 seconds descriptively are equally effective in killing germs, but when reviewed statistically, hand washing treatment using hand sanitizer with a time variation of 30 seconds is more effective because it has a higher average decrease in germs. But there

is still a risk of infection if there is an increase in the number of germs on the hands, this can happen when you often come into contact with contaminated objects, often hold public objects that have a high risk of contamination and lack of personal hygiene such as washing hands either using hand sanitizers or soap and running water so that germs will be easier to multiply.

CONCLUSION

The average number of germs in this study before washing hands was 2.3×10^2 CFU/cm². The average number of germs that wash their hands using a hands sanitizer with a contact time of 15 seconds is 1.3×10^2 CFU/cm² and a variation in contact time of 30 seconds is 0.7×10^2 CFU/cm². There is a significant difference (p value = 0.00) between the number of germs before and after washing hands using a gel hands sanitizer based on variations in contact time of 15 seconds and contact time of 30 seconds. Decrease in the number of germs washing hands using gel hand sanitizer with a variation in contact time of 15 seconds by 46.16% and variation in contact time of 30 seconds by 70.98%. The effective contact time for a decrease in the number of germs is 30 seconds.

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EFFECT OF VARIATIONS OF BLOOD CLOTTING TIME BEFORE CENTRIFUGATION ON CHOLESTEROL LEVELS WITH ENZIMATIC METHOD

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Abstract

Introduction: Pre-analytic is an important phase of laboratory examination. One of them is how to get serum. The stage of blood coagulation is important in obtaining a good serum. This phase is very influential on the quality of the serum. The reality in the field during clinical chemistry examinations is that there is a time difference in the clotting of blood samples to become serum. The purpose of this study was to determine the effect of variations in blood clotting time before centrifugation on cholesterol by the enzymatic method.

Method: The method used in this research is quantitative analysis with the type of experimental research. The treatment given to the object is the variation of blood clotting time before centrifugation with a time of 10, 20 and 30 minutes.

Results: The results of the average measurement of cholesterol levels with blood clotting time of 10, 20 and 30 minutes were 133.94 mg/dl, 148.23 mg/dl and 162.31 mg/dl. Cholesterol levels with clotting time of 10 and 30 minutes increased by 21% and clotting time of 20 and 30 minutes by 9%. The data obtained were statistically analyzed using the Oneway ANOVA test for clotting time 10 with 30, the results obtained sig 0.003 and coagulation time 20 with 30 obtained sig 0.214.

Conclusion: The results of this research indicate that between groups of 10 with 30 minutes of clotting time there is a significant effect on cholesterol levels and clotting time of 20 with 30 minutes has no significant effect on cholesterol levels.

Key words : CHOD-PAP, cholesterol, clotting time, oneway annova, serum

INTRODUCTION

Lipid is water insoluble molecules such as cholesterol and triglycerides. Lipids transported by proteins in the bloodstream are called lipoproteins. Lipids are produced in the liver and obtained from foods such as meat and dairy products. Lipid and lipoprotein profiles are generally used as a normal assessment of elevated blood cholesterol levels (hypercholesterolemia) and elevated blood levels of fatty substances (hypertriglycerides) associated with an increased risk of cardiovascular disease (Fristiody & Ruslin, 2020).

Measurement of cholesterol levels serves to determine the level of cholesterol in the blood. In the clinical chemistry measurement guidelines of the Ministry of Health Number 1792/MENKES/SK/XII/2010 regarding cholesterol assessment, the technique used is CHOD PAP (*Cholesterol Oxidase-Peroxidase Aminoantipyrin*). Cholesterol examination using serum or plasma specimens. Serum is a liquid blood containing clotting factors that does not use anticoagulants when collecting serum specimens. Complete coagulation takes 30 minutes, depending on the presence of clot activator. The sample was then centrifuged to separate blood cells and serum (Warekoi & Richard Robinson, 2017). The main difference between plasma and serum is that serum does not contain and some potassium is released from platelets (serum potassium is slightly higher in serum than plasma (Bishop et al., 2013).

Pre-analytic is an important part of laboratory examination. One example is how to obtain serum. The stage of blood coagulation is important in obtaining a good serum. The blood clotting phase before centrifugation has an influence on the quality of the serum. Serum is obtained from blood that is accommodated in a tube without anticoagulant and allowed to clot for 30 minutes then centrifuged using 3000 RPM for 15 minutes and will produce a clear yellow liquid (Nugraha, 2015). When in the field the fact in clinical chemistry examinations, there are differences and variations in the time of blood clotting before centrifugation to become serum. There is a difference in blood clotting time from the time specified to shorten the time, this method is actually not in accordance with the procedure. The difference in blood clotting time before

centrifugation in the manufacture of serum that is not according to the procedure then the serum can cause differences in the interpretation of the results.

Research conducted by Adi (2019) revealed that there was a significant difference in cholesterol levels from clot blood before centrifugation and direct centrifugation. Similarly, research conducted by Sari (2018) found that there was a significant difference in HDL cholesterol levels from blood that was directly centrifuged and clot before being centrifuged. On the basis of this background, the researchers wanted to conduct research on the effect of blood clotting time on cholesterol levels. Based on this problem, researchers are interested in knowing the effect of blood clotting time before centrifugation on cholesterol levels by the enzymatic method. This study provides variations in blood clotting time 10 minutes, 20 minutes and 30 minutes before centrifugation. Whereas in the previous study only treated blood that was clot and not clot before centrifugation.

METHOD

This type of research is an experimental quantitative research. The study was conducted in February-June 2022 at Clinical Chemistry Laboratory, East Bekasi. The population in this study were laboratory workers at Private Hospital East Bekasi, who were willing to have blood drawn with informed concern. The sampling technique in this study was purposive sampling. There were 17 respondents. The inclusion criteria in this study were laboratory workers at Private Hospital, East Bekasi, who were <30 years old. Exclusion criteria in this study were icteric, lipemic and lytic serum.

A. Tools and Materials

1. Tools

The tools used in this study were Semi Chemistry Analyzer (Ratyo RT-1904C), micropipette (Dragon lab) (10 and 1000 µL), tube rack, tip, bath, centrifugation, tourniquet, holder.

2. Materials

The materials used in this study were cholesterol reagent (Dumolabs), distilled water, handsocon, mask, plain vacutainer, needle, swab alcohol, plaster, yellow and blue tips.

B. Procedure

1. Pre Analytic

The pre analytical stage in the study begins with blood taking done and preparing tools and materials. Next, the patient is identified and then a tourniquet at a distance of 3-4 cm from the puncture site and palpated to determine which vein to use. Venous blood sampling can use the median cubital vein, saphalic vein and basilic vein. Clean the puncture site with 70% alcohol and let it dry for 30-60 seconds. Then the vein is pierced with a needle and the blood will enter the plain vacutainer tube. Then the blood of each tube was labeled 10 minutes, 20 minutes and 30 minutes.

2. Analytic

The analytical step was carried out by making serum with clott times before centrifugation, namely 10 minutes, 20 minutes and 30 minutes. After obtaining the serum, cholesterol was checked. Examination of cholesterol in serum is done by preparing 3 test tubes labeled blank, reagents and samples. The examination of cholesterol levels in this study was carried out in duplicate. In the blank tube was added 10 µl of distilled water and 1000 µl of cholesterol reagent. In the standard tube, 10 µl of standard reagent and 1000 µl of cholesterol reagent were added and 10 µl of serum and 1000 µl of cholesterol reagent were added to the sample tube. Then the blank, standard and sample tubes were incubated at 37°C for 10 minutes using an incubator/waterbath. Then the cholesterol level was measured using a semi chemistry analyzer with a wavelength of 500 nm. The post-analytic stage was carried out by entering cholesterol levels into the SPSS version 26 program.

RESULTS

This research was conducted at the clinical chemistry laboratory, East Bekasi. Blood sampling was carried out by 17 respondents consisting of women and man. The number of women amounted to 16 people or 94% and man amounted to 1 person or 6%. With respondents who fit the inclusion and exclusion criteria. The volume of blood taken by each respondent was 9 ml of blood. Examination of cholesterol levels using serum using blood clotting times of 10, 20 and 30 minutes. Based on the results of the examination of cholesterol levels of 17 respondents whose blood was given variations in clotting time, the cholesterol levels were obtained as follows:

Table 1. The result of the average level of cholesterol on the variation of blood clotting time

Times	N	Minimum (mg/dL)	Maksimum (mg/dL)	Mean (mg/dL)	Std.
10 minute	17	83,67	178,26	133,94	22,52
20 minute	17	89,22	189,20	148,23	27,23
30 minute	17	120,32	192,38	162,27	22,04

Based on table 1 shows that the lowest cholesterol level in the 10 minute clotting time was 83.67 mg/dL, the highest was 178.26 mg/dL, and the average was 133.94 mg/dL. Cholesterol levels during the clotting time of 20 minutes increased. The lowest was 89.22 mg/dL, the highest was 189.20 mg/dL, and the average was 148.23 mg/dL. At a clotting time of 30 minutes, the lowest level was 120.32 mg/dL, the highest was 192.38 mg/dL, and the average was 162.27 mg/dL.

Table 2. The Statistical Test Oneway Annova between time groups

Clotting Time	Clotting Time	Sig.	Interpretation
30 minute	10 minute	0,003	There was a significant effect between clotting times of 10 and 30 minutes on cholesterol level
	20 menit	0,214	There was no significant effect between clotting times of 20 to 30 minutes on cholesterol levels.

Statistical test in table 2 by comparing the time groups, it was found that the clotting time of 10 to 30 minutes obtained a sig value of 0.003, which means that there is a significant effect between the clotting time of 10 minutes and 30 minutes on cholesterol levels, while at the blood clotting time of 20 to 30 minutes, the sig value is 0.214. which indicates that there is no significant effect between clotting times of 20 minutes and 30 minutes on cholesterol levels. tes obtained a sig value of 0.003, which means that there is a significant effect between the clotting time of 10 minutes and 30 minutes on cholesterol levels, while at the blood clotting time of 20 to 30 minutes, the sig value is 0.214. which indicates that there is no significant effect between clotting times of 20 minutes and 30 minutes on cholesterol levels.

DISCUSSION

Cholesterol levels were tested using the colorimetric enzymatic method, namely CHOD-PAP. The principle of this method is that cholesterol in the presence of the enzyme cholesterol esterase will form fatty acids and free cholesterol. The free cholesterol formed is then converted into cholesterol-4-en-3-on and hydrogen peroxide (H₂O₂) by the enzyme *cholesterol oxidase* (CHO). Hydrogen peroxide with phenol and 4-aminophenazone by the enzyme *peroxidase* (POD) will be converted into colored compounds. The

absorbance of this color is proportional to the cholesterol in the sample (Nugraha & Badrawi, 2018).

Based on table 1, the percentage increase in blood cholesterol levels by comparing cholesterol levels with a clotting time of 10 to 20 minutes shows an increase in cholesterol levels by 10%. At the freezing time of 10 to 30 minutes, there was a significant increase of 21% and at the freezing time of 20 to 30 minutes, there was an increase in cholesterol of 9%. This result is in accordance with the theory that the specimen has not undergone complete coagulation, which will cause the fat content to not be completely released so that it affects the fat content (Nugraha, 2015).

Research conducted by Adi (2019) showed that there was a significant difference in cholesterol levels from frozen blood before centrifugation and direct centrifugation. The pre-analytic stage has a significant impact on the analysis of fat content, such as checking cholesterol and triglyceride levels. Blood that was first centrifuged directly without a coagulation process will produce less serum with blood that has undergone a complete coagulation process. This is because the coagulation process is not perfect and the fat content is still bound in the serum.

Sari (2018) revealed that in examining HDL cholesterol levels there were differences in HDL levels between blood that was directly centrifuged and frozen before centrifugation. According to the Decree of the Minister of Health of the Republic of Indonesia Number 1792/MENKES/SK/XII/2010 concerning guidelines for clinical chemistry examination in obtaining serum, the blood is allowed to coagulate for 30 minutes and centrifuged using a speed of 3000 RPM for 5-15 minutes. To avoid changes in dissolved substances due to hemolysis effects, serum examination is carried out in less than 2 hours.

CONCLUSION

Based on the *Oneway Anova* test between the 10 minute and 30 minute clotting time group sig 0.003 was obtained, it can be concluded that there is a significant effect between the 10 minute and 30 minute clotting time on cholesterol levels. At the clotting time of 20 minute and 30 minute, sig 0.214 was obtained, which indicated that there was no significant effect between the clotting time of 20 and 30 minute on cholesterol levels.

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ANALYSIS TOTAL PLATE COUNT (TPC) ON SNACKS

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Abstract

Introduction: Snacks are a type of food that is often eaten outside the time of the main meal and it is usually consumed to relieve hunger for a while, in the community are a necessity that cannot be separated in daily life. The composition contained in snacks varies which can provide benefits and can even cause various problems for the health of the body, for example microbial contamination found in snacks. Microbial contamination found in snacks can be related to the process of selecting raw materials, production processes, packaging processes, marketing processes, and food storage processes. The purpose of the study was to test microbiological samples of snacks based on the Total Plate Count (TPC).

Method: The methods used in this study are quantitative. The study was conducted in June 2022 at the Bacteriology Laboratory of STIKes Mitra Keluarga, Bekasi City. The sample of snacks that used in this study was collected by 17 people. The snacks are weighed as much as one gram and mashed first, then diluted to a dilution of 10^{-4} . In the 10^{-3} and 10^{-4} dilutions, the samples were incubated using the spread plate and pour plate methods at 37°C for 1x24 hours. After that, the colonies formed were counted using the Total Plate Count (TPC) formula and reported in units of CFU's/mL.

Results: The results of this study indicate that from 5 samples of snacks, there is 1 sample of snacks that have a Total Plate Count (TPC) value greater than 2.500, namely the egg drop biscuits sample with a Total Plate Count (TPC) value $1,3 \times 10^6$ CFU's/mL.

Conclusion: After conducting research on samples of snacks, it was found that one of the five samples used, egg drop biscuits had the highest Total Plate Count (TPC) numbers, which is $1,3 \times 10^6$ CFU's/mL. Then the sample of egg drop biscuits exceeds the standard from SNI 01-3553-2006 which has been set, namely with the maximum Total Plate Count (TPC) limit 1×10^5 colonies/ml.

Key words : Nutrient Agar, Pour Plate, Snacks, Spread Plate, Total Plate Count (TPC)

INTRODUCTION

Snacks are food that can relieve hunger someone temporarily very attracted by various groups, especially children and teenagers because of the different colors interesting and delicious taste. The rise Various types of food that are produced and consumed by various groups in this modern era are increasing from year to year. This development is due to the community's need for snacks and the public's desire to enjoy the different flavors offered by producers in food with an attractive appearance and affordable prices, so the right decision to enliven the industrial world is snack food. Snacks are found in many places such as in shops, mini markets and supermarkets (Oktavia, 2018). The presence of microorganisms that are certain pathogens or microorganisms that need to be careful in snacks can cause health problems consumer. This can be caused by snacks directly in into the consumer's body and digested by stomach which will indirectly circulated throughout the body (Oktavia, 2018).

Based on Food Law No. 7 years 1996 (Mukhtar et al., 2017), the safety of a food is condition and effort needed to prevent food from possible biological, chemical and other objects that can interfere, harm and endanger human health. Microbiological analysis is important in determine the safety and quality of a food (Stahlhut, Struve, Krogfelt, & Reisner, 2012). Safe products are products that are free of preservatives or chemicals that are not recommended. Regulations and SNI set two types of maximum limit for Total Plate Count (TPC), from start to end. The regulation does not explain the initial and final information, while SNI explains that the initial TPC is tested at the factory and the final TPC is tested on the market. The maximum limit of the initial TPC is 10^2 CFU/ml while the final TPC is 10^5 CFU/ml. For purpose of compliance with

microbiological criteria for the final product of a lot/batch, generally only one criteria is established for TPC (Makhabbah, 2022).

One of the parameters used in determining the feasibility and safety of food is to measure the microbiological content of food. Given the low price, the concern that arises is the use of raw materials that have the potential to enter food or beverage contaminants in the form of microorganisms that are harmful to the body. The presence of these microorganisms can cause digestive disorders. Foods whose safety quality is guaranteed are those that are free from biological hazards, chemical hazards, and physical hazards (BPOM RI, 2012). Biological hazards result from living things such as microbes that can produce toxins, which can cause disease (FAO, 2009). The results of research in 2012 by the Food and Drug Supervisory Agency (BPOM) showed that there were 141 extraordinary cases due to food poisoning. One of the causes is the addition of preservatives, dyes and harmful microbial contamination. not meeting food safety standards can cause health problems (Rizki, 2016).

Microbiological contamination testing on snacks includes quantitative and qualitative tests. The quantitative microbiological contamination test is the Total Plate Count (TPC) test, while the qualitative microbiological contamination test is the Most Probable Number (MPN) test (Oktavia, 2018). In this study, only quantitative tests were carried out by analyzing the total number of plates using the pour plate and spread plate methods. Microorganisms contained in snacks can potentially cause food infection and food intoxication so snacks consumed can cause diarrhea and even poisoning for consumers (Depkes RI, 2006). The aim of this study is to determine the amount of Total Plate Count (TPC) with the pour plate and spread plate methods on snacks, namely sugar crackers, grilled corn, roast beef sticks, cassava clanting, and egg drop biscuits.

METHOD

The methods used in this study are quantitative. The study was conducted in June 2022 at the Bacteriology Laboratory of STIKes Mitra Keluarga, Bekasi City. The sample of snacks that used in this study was collected by 17 people. The snacks are weighed as much as one gram and mashed first. In this study, two methods were used. which is spread plate and pour plate methods. For the spread plate method, the sample that has been weighed and mashed is then put into a test tube that contains 9 ml of sterile distilled water for the first dilution, then a gradual dilution is carried out to a dilution of 10^{-4} . The last 2 dilutions were planted as much as 0.1 ml onto NA (Nutrien Agar) media twice for each dilution (duplo). After that it is leveled with drugalsky rods. Incubate at 30° C for 1-2 x 24 hours. After growing, the colonies were counted.

Foe the pour plate methods, The samples that were weighed and crushed were then put into a test tube contains 9 ml of sterile distilled water for the first dilution, followed by a gradual dilution to a dilution of 10^{-4} . The last 2 dilutions were taken as much as 1 ml and inoculated into in an empty petri dish and then add the liquid NA (Nutrien Agar) medium, do it in duplicate for each dilution. Flatten the surface by shaking the petri dish to form a figure 8. Incubate at 30° C for 1-2 x 24 hours. After growing, the colonies were counted.

The colony requirements determined to be counted are as follows ; one colony is counted as 1 colony, two overlapping colonies are counted as 1 colony, several related colonies are counted as 1 colony, two colonies that are close together and can still be distinguished are counted as 2 colonies, colonies that are too large (larger than half the area of the cup) do not counted, colonies that are less than half the size of the cup are counted as 1 colony. Formula to calculate relative cells (CFU's/ml = number of colonies x dilution factor).

The formula for calculating the total plate number according to SNI 01-2332.3-2006. A plate containing less than 25 colonies or plates without colonies, if the two dilutions used, the colonies are less than 25, then record the colonies present, but express the calculation as less than 25 and multiplied by 1/d, where d is a factor the first dilution used and reported as an estimate TPC. If a plate containing 25-250 colonies the formula is,

$$N = \frac{\sum c}{[(1 \times n_1) + (0,1 \times n_2)] \times (d)}$$

N= Number of colonies (colonies/ml or colonies/g)

C = Number of colonies in all counted plate

n₁= Number of plates in the first calculated plate

n_2 = Number of plates in the second calculated plate
d = First dilution calculate

RESULTS

Based on the results of the Total Plate Count (TPC) analysis on snack samples that have been carried out by Medical laboratory technology students batch 2021 STIKes Mitra Keluarga below are obtained:

Table 1. Total Plate Count (TPC) result

No.	Sample	TPC (CFU's/mL)
1	Sugar crackers	$2,5 \times 10^4$ CFU's/mL
2	Grilled corn snack	$2,5 \times 10^4$ CFU's/mL
3	Roast beef stick	$2,5 \times 10^4$ CFU's/mL
4	Cassava klaning	$2,5 \times 10^4$ CFU's/mL
5	Egg drop biscuits	$1,3 \times 10^6$ CFU's/mL

The results of the Total Plate Count (TPC) analysis on five samples of snacks obtained the highest result, $1,3 \times 10^6$ CFU's/mL which is from the egg drop biscuits sample. Meanwhile, for the lowest result, $2,5 \times 10^4$ CFU's/mL. On nutrient agar media, the bacteria that grow from the snack samples looks white (Nonpigmented) with a convex surface.

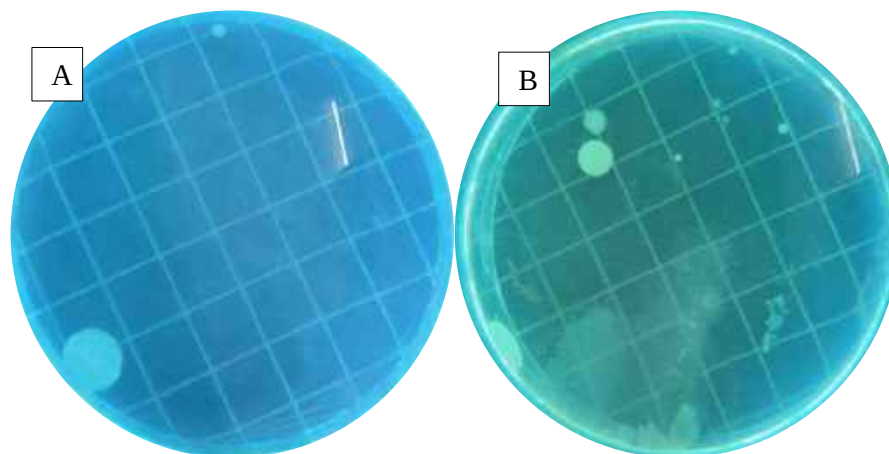


Figure 1. (A) Bacterial colonies from snack sample on nutrient agar media with pour plate method (B) Bacterial colonies from snack sample on nutrient agar media with pour plate method.

DISCUSSION

After observing, based on the results obtained of the Total Plate Count (TPC) analysis on five samples of snacks obtained the highest result, $1,3 \times 10^6$ CFU's/mL which is from the egg drop biscuits sample. Meanwhile, for the lowest result, $2,5 \times 10^4$ CFU's/mL. Based on guidelines contamination criteria from SNI 01-3553-2006 on snacks maximum Total Plate Count (TPC) limit 1×10^5 colonies/ml. So in this study, 1 out of 5 samples could be declared unfit for consumption because the number of bacteria had exceeded the threshold. In this case, there are several factors that can cause contamination of the snacks. Storage conditions after drying may also be of high humidity, thus leading to an increase in moisture levels hence creating conducive environments for growth and proliferation of microorganisms. Unhygienic conditions during production (for instance lack of protective clothing, lack of hand washing areas, drying on dirty surfaces), storage and slow sun-drying especially during the rainy seasons, often results in bacteria and mould contamination (Chiona et al., 2014). Generally, the bacteria that contaminate food are salmonella sp. and

Escherichia coli. If food contaminated with these bacteria is consumed and enters the body of a person who has a weakened immune system, it can cause bacterial poisoning or infection. Symptoms caused by infection begin to appear after 12-24 hours and are characterized by lower abdominal pain (abdominal pains), dizziness, diarrhea, vomiting, fever and headache (Ivani, 2019).

In this study, the method used was pour plate and spread plate. Based on the results obtained, the average bacteria that grew were bacteria on the plate using the spread plate method. Meanwhile, on plates that use the pour plate method, there are no bacteria that grow, the growth of bacteria is only on the surface of the media. The pour plate method aims to count the number of living cells in both aerobic and anaerobic conditions because in this method it will be seen that the bacteria growing on the surface of the media are aerobic and all of the agar media are anaerobic bacteria (Nataya, 2015). So it can be seen that the bacteria contained in the food sample are aerobic.

CONCLUSION

Based on the results of the study, it can be concluded that sugar crackers, grilled corn snacks, roasted beef sticks, and cassava klanting are safe for consumption because the amount of microbial contamination does not exceed the maximum limit of the Indonesian National Standard which is 10^5 CFU/ml. While the results on the egg drop biscuits sample showed the amount of microbial contamination that exceeded the maximum limit of the Indonesian National Standard, namely the TPC value of the egg drop biscuits sample is $1,3 \times 10^6$ CFU's/mL.

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RELATIONSHIP OF D-DIMER VALUE WITH CT VALUES IN ADULT AND ELDERLY COVID-19 PATIENTS

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Abstract

Introduction: COVID-19 is the name for the disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). COVID-19 enters host cells with the help of Angiotensin Converting Enzyme 2 (ACE2) receptors, these receptors are located in endothelial cells of blood vessels, heart, lungs, kidneys, liver and brain. This virus can cause cell or tissue damage, causing inflammation. Excessive inflammation will increase the value of D-Dimer. One of the tests that can detect the presence or absence of the virus in the body is RT-PCR. RT-PCR detects the presence of genetic material including viruses. The purpose of the study was to determine the relationship between the D-Dimer value in adults and the elderly who were confirmed positive for COVID-19 at private hospital in North Jakarta.

Method: The method used in this research is to use secondary data with a cross sectional research design. The data collection of this research was purposive sampling and used the Spearman Correlation test analysis method.

Results: The results of data collection obtained as many as 391 samples consisting of 147 samples of the adult group and 244 samples of the elderly group. Data processed by SPSS program with Spearman correlation test showed that there was a correlation between D-Dimer value and Ct value, the result obtained was sig 0,008 and correlation coefficient -0,135. The correlation coefficient value obtained is negative, so the relationship between the two variables is not undirectional.

Conclusion: The results of this study indicate that the value of D-Dimer with Ct values is correlated.

Key words : Adults, COVID-19, D-Dimer, Elderly and SARS-CoV-2.

INTRODUCTION

At the end of 2019, more precisely in December 2019, the phenomenon of pneumonia cases caused by a virus in Wuhan, Hubei Province, China. The virus that causes this outbreak is still included in the zoonotic Human-Coronavirus (HCoV) which can seriously threaten health. The International Committee on Taxonomy of Viruses (ICTV) has announced that this new coronavirus has been classified as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Coronavirus Disease 2019 (COVID-19) was officially named by the World Health Organization (WHO) for the disease caused by the virus (Li et al., 2020). This COVID-19 pathology is characterized by symptoms such as dry cough, fever, easy fatigue and can also affect the respiratory, digestive, musculoskeletal and nervous systems. These symptoms may vary depending on the organ system involved (Çalica Utku *et al.*, 2020).

SARS-CoV-2 belongs to the positive RNA group, has an envelope and is single-stranded. This virus has 4 genes, namely Alphacoronavirus (α), Betacoronavirus (β), Gammacoronavirus (γ) and Deltacoronavirus (δ) (Ge et al., 2020). The spike protein (S) is shaped like a crown on the outer surface of the virus, this protein is the hallmark of this virus. This virus is small with a diameter of 65-125 nm and has single-stranded RNA 26 to 32 ks (kilobase) long (Shereen *et al.*, 2020). COVID-19 enters host cells with the help of Angiotensin Converting Enzyme 2 (ACE2) receptors, these receptors are located in endothelial cells of blood vessels, heart, lungs, kidneys, liver and brain. Viruses can enter target organs and can cause cell or tissue damage, causing inflammation. The resulting inflammation causes hypercoagulability and increased fibrinolysis. Hypercoagulability and increased fibrinolysis can be caused by high levels of D-Dimer in serum. Excessive inflammation can increase D-Dimer (Jain, 2020).

In a study of 129 patients ranging in age from 19-91 years with an average age of 48 years with confirmed COVID-19, the results showed an increase in D-Dimer by 15% and significantly more in patients with severe COVID-19 infection requiring hospitalization compared to mild illness (Lehmann *et al.*, 2021). D-Dimer is the end product of fibrin degradation, which is a small protein

fragment that is in the blood after the blood clot is degraded by the process of fibrinolysis. Elevated D- Dimer values occur in the elderly due to increased rates of clot formation and activated fibrinolysis instates of systemic inflammation, lipid disorders and obesity (Tita-Nwa *et al.*, 2010).

To detect the SARS-CoV-2 virus in the body, the RT-PCR (reverse Transcription Polymerase Chain Reaction) method can be used. RT-PCR detects the presence of pathogen-specific genetic material including viruses, samples are treated with several chemical solutions that can remove substances such as proteins, fats and extract only RNA. The RNA is reverse transcribed to DNA using specific enzyme. Then, DNA fragments are added that will complete certain parts of the transcribed viral DNA. The added fragments serve to build DNA strands during amplification and add marker labels to the strands that are useful for detecting viruses. Inside the RT-PCR machine there is a cycle that creates a new copy of the target portion of the viral DNA. Each cycle doubles the previous number, then the DNA strands release a fluorescent dye. When a certain level of fluorescence is exceeded, it confirms that the virus is present. The cycle can also estimate the severity of the infection, the fewer cycles the more severe the viral infection (International Atomic Energy Agency, 2020).

The cycle threshold (Ct) can be defined as the thermal cycle number at which the fluorescent signal exceeds that of the background and thus passes the threshold for positivity. A typical RT-PCR assay will have a maximum of 40 thermal cycles. The lower the Ct value the higher the quantity of viral genetic material in the sample. Ct values obtained in this way are semi-quantitative and are able to distinguish between high and low viral load. A 3-point increase in Ct value is roughly equivalent to a 10-fold decrease in the decrease in the quantity of viral genetic material (Public Health England, 2020). Research conducted by Wijayanti (2022) revealed that the D-Dimer value and the Ct value were not related. Based on these problems, the researchers wanted to test whether increased levels of D-Dimer in COVID-19 patients had a relationship with Ct values. This study used a period of 1 year and the sample data obtained were 391. Whereas in the previous study, only 5 months of research were conducted.

METHOD

This study uses a comparative type with a cross sectional design. The data collection method used is purposive sampling. The data obtained were processed with IBM SPSS statistics 26 using the Spearman correlation test. The research site is in one of the North Jakarta Private Hospitals. The time of the study was carried out on June 6, 2022 with the data period taken from August 2020 to August 2021. Inclusion criteria were COVID-19 patients who were confirmed by RT-PCR examination and did a D-Dimer examination at the age of 26 – >65 years. The exclusion criteria were RT-PCR examination results with Ct values above 30.

RESULTS

Data on COVID-19 patients at a private hospital in North Jakarta collected during the period August 2020 to August 2021, obtained 391 patients. Data of 391 research subjects who complete the exclusion and inclusion criteria that have been set. Patients data obtained from the medical records of a private hospital in North Jakarta are as follows :

Table 1. Age group of confirmed COVID-19 patients

Age (Years)	Amount	Percentage (%)	Number of Groups	Group Percentage (%)
Adult				
26 – 35	64	16,37	147	37,6
36 – 45	83	21,23		
Elderly				
46 – 55	97	24,81	244	62,4
56 – 65	84	21,48		
>65	63	16,11		
Total	391	100	391	100

Based on table 1, the results of data on confirmed COVID-19 patients on 391 patients samples, it was found that there were 64 adolescents aged 26 – 35 years old (16,37%), age 36 – 45 years as many as 83

people (21,23%), the elderly age 46 – 55 years were 97 people (24,81%), aged 56 – 65 years were 84 people (21,48%), aged >65 years were 63 people (16,11%). The youngest age in this study was 26 yearsold and the oldest was 100 years old.

Table 2. Descriptive results

		<i>Statistic</i>	<i>Std Error</i>
D-Dimer	<i>Mean</i>	1,2187	0,06723
	<i>Median</i>	0,7500	
	<i>Variance</i>	1,767	
	<i>Std. Deviation</i>	1,32945	
	<i>Minimum</i>	0,51	
	<i>Maximum</i>	9,64	
	<i>Range</i>	9,13	
		<i>Statistic</i>	<i>Std Error</i>
Gen N	<i>Mean</i>	22,6958	0,21932
	<i>Median</i>	22,8800	
	<i>Variance</i>	18,808	
	<i>Std. Deviation</i>	4,336883	
	<i>Minimum</i>	10,87	
	<i>Maximum</i>	29,81	
	<i>Range</i>	18,94	

Based on table 2, the minimum value of D-Dimer examination is 0,51, the maximum value is 9,64 and the average value is 1,2187. For the Ct value of the N gene, the minimum value is 10,87, the maximum value is 29,81 and the average is 22,6958.

Table 3. Spearman correlation test results

			D-Dimer	Gen N
Spearman's rho	D-Dimer	Correlation Coefficient	1,000	- 0,135
		Sig. (2-tailed)	-	0,008
		N	391	391
	Gen N	Correlation Coefficient	- 0,135	1,000
		Sig. (2-tailed)	0,008	-
		N	391	391

The results of the Spearman correlation test obtained a P value 0,008 with a correlation coefficient of – 0,135.

DISCUSSION

The results of data processing on 391 samples revealed that the number of COVID-19 infections was more experienced by the elderly with a percentage of 62,4%. In the elderly the immune system is weakened, making it more difficult for the body to fight infection. The age factor is very influential on exposure to COVID-19, the elderly are very vulnerable to disease because their bodies have impaired organ and tissue function abilities (Ernawati, 2021).

The minimum value for D-Dimer examination is 0,51, the maximum value is 9,64 and the average value is 1,2187. Significant increases in D-Dimer values are common in severe COVID-19 patients. An increase in the value of D-Dimer in the blood is a sign of suspicion of thrombosis, thrombosis is the formation of a blood clot in a blood vessel (Wijayanti et al., 2022). The Ct value of Gen N obtained a minimum value of 10,87, a maximum value of 29,81 and an average value of 22,6958, it can be seen that the Ct value obtained varies. The Ct value is inversely proportional to the amount of target nucleic acid in the sample, meaning that the lower the Ct value, the amount of nucleic acid increases in the sample (PAMKI, 2020).

The results of the Spearman correlation test showed that the probability value of D-Dimer and Gen N

was 0,008, so there was a correlation between the two variables because the value was $<0,05$. Judging from the value of the correlation coefficient between D-Dimer and Gen N of $-0,135$, it means that the correlation is very weak between the results of D-Dimer and Gen N. The correlation coefficient value obtained is negative, so the relationship between the two variables is not unidirectional. The non-unidirectional relationship shows the greater the value of D-Dimer, the lower the value of Gen N, or vice versa.

CONCLUSION

Based on the Spearman correlation test between D-Dimer and Gen N, sig 0,008 was obtained, it can be concluded that there is a correlation between D-Dimer and Gen N.

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DESCRIPTION OF NAIL FUNGUS ON PLANTATION WORKERS IN BOJONG SARI VILLAGE, JATISARI WARD, JATIASIH DISTRICT, BEKASI CITY

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Abstract

Introduction: Onychomycosis is a fungal nail infection that causes various types of fungi such as *Candida sp.* and dermatophytes. Species that usually cause nail fungal infections in humans are *Epidermophyton floccosum*, *Microsporium canis*, *Trichophyton concentricum*, *Microsporium gypseum*, *Epidermophyton floccosum*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*. The prevalence of onychomycosis infection has increased quite high from 2% to 14% in the last 20 years. Factors that affect nail fungus infection are age, individual hygiene, and environmental hygiene. Therefore, it is necessary to conduct research on the description of nail fungus on plantation workers in Bojong Sari Village, Jatiasih District, Bekasi City.

Method: This type of research is descriptive with a cross sectional design. The sample in this study amounted to 40 plantation workers who live in Bojong Sari Village, Jatiasih District, Bekasi City. The sampling technique used was the 20% Potassium Hydroxide (KOH) method. Data analysis was carried out with a descriptive approach.

Results: The results of this study showed that 5 (12.5%) respondents were positively infected with nail fungus, while 35 (87.5%) respondents were negative for nail fungus infection.

Conclusion: In this study it can be concluded that the description of nail fungus infection in plantation workers in Bojong Sari Village, Jatiasih District, Bekasi City as many as 5 (12.5%) respondents were positive for nail fungus, and 35 (87.5%) negative people. The risk factors that have the potential to cause plantation workers to get nail fungus infection are not using footwear, not using gloves, not cutting nails regularly and not keeping the house clean. Suggestions for further research can be done by analyzing the relationship of risk factors for nail fungus infection to the incidence of nail fungus infection with the SDA culture method.

Key words : Age, Environmental Hygiene, Individual Hygiene Nail Fungus, Plantation Workers

INTRODUCTION

Onychomycosis is a fungal nail infection caused by various types of fungi such as *Candida sp.* and dermatophytes. Species that usually cause nail fungal infections in humans are *Epidermophyton floccosum*, *Microsporium canis*, *Trichophyton concentricum*, *Microsporium gypseum*, *Epidermophyton floccosum*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* (Sutanto, 2013). According to Science et al. (2012) one of the factors that influence nail fungus infection is age. Age is one of the risks that affect the incidence of onychomycosis, while according to Chandra (2007) the level of personal hygiene is very important such as hand and toe nail care because it has the potential to cause nail fungus infection. Each condition of the fingernails and toenails will affect the incidence of nail fungus infection in a person.

According to Bhatia (2015) the prevalence of onychomycosis infection has increased quite high from 2% to 14% in the last 20 years. Nail fungal infections can cause discoloration of the nails such as white, yellow, brownish, and the nails become cracked and the nail surface is uneven, in addition, onychomycosis infection can cause clinical disorders such as reducing the appearance of nails and nail fungus infection is difficult to treat. This has an impact on reducing the quality of life and comfort of people with nail fungus infection.

Considering the problems and impacts caused by nail fungus infection, it is necessary to do research on the description of nail fungus infection, especially in plantation workers. The selection of the subject of farmers and plantation workers is because they have a habit of not using footwear when working, so that when working, mud easily gets into the nails and has the potential to cause nail fungus infection (Widiati et al. 2016). According to Tabri (2016) infections that occur on the nails can be caused by someone who works or is in direct contact with a humid and dirty environment such as the work of farmers.

The difference between this study and previous research lies in the location, research subjects, and the number of research samples. In this study, located in Bojong Sari Village, Jatiasih District, Bekasi City, the subjects of this study were plantation workers totaling 40 respondents as research samples to be examined for the presence of nail fungus. The choice of subjects was due to the fact that some of the residents of Bojong Sari Village work on plantations and the workers' individual hygiene levels are still very low, such

as not using footwear, not using gloves when they work, and these workers are in direct contact with soil, water, or mud. This research has conducted a preliminary survey and it was found that many plantation workers experience symptoms of nail fungus infection with the characteristics of yellow nails, uneven nail surface, and nails that do not look shiny. This is the basis of interest in conducting research on the description of the incidence of nail fungus infection in plantation workers in Bojong Sari Village, Jatisari Village, Jatiasih District, Bekasi City.

METHOD

The population in this study are plantation workers who live in Bojong Sari Village, Jatisari Village, Jatiasih District, Bekasi City. In Bojong Sari Village, Jatisari Village, Jatiasih District, Bekasi City, there are 2 RW, namely Rw 01 and Rw 02. In this study, the population was taken from Rw 01 because the number of residents who worked as plantation workers was more in Rw 01 than Rw 02. The sample used In this study, there were 40 samples from Bojong Sari Village, Jatisari Village, Jatiasih District, Bekasi City.

A. Tools and Materials

1. Tools

Plastic bag clip, Tweezers, Microscope (Olympus), Object glass, Deck glass, Gloves, Mask, Nail clippers

2. Materials

KOH solution potassium hydroxide 20% nail scrapings of plantation workers in Bojong Sari Village, Jatisari Village, Jatiasih District, 70% alcohol cotton

B. Procedure

1. Pre Analytic

Select a subject with the characteristics of discolored nails, uneven surface, and the nail plate is not shiny Sutanto, (2013) Clean the nails with an alcohol swab on the surface area of the nail plate. Wait until it dries and take the nail scraping on the inside of the nail. Take a sample with a sterile razor and insert it the sample into a plastic bag clip with tweezers and give the identity according to the name and age, then the sample is ready to be brought to the STIKes Mitra Keluarga laboratory.

2. Analytic

The sample was taken from a plastic clip bag with tweezers, then took the sample and placed it on the object glass, then dripped it with 20% KOH solution, then heated it over low heat and did not let it evaporate, then covered it with a glass deck and examined it using a microscope with an objective magnification. 40x.

3. Analytical post

The sample was taken from a plastic clip bag with tweezers, then took the sample and placed it on the object glass, then dripped it with 20% KOH solution, then heated it over low heat and did not let it evaporate, then covered it with a glass deck and examined it using a microscope with an objective magnification. 40x.

RESULTS

This research was conducted in Bojong Sari Village, Jatisari Village, Jatiasih District, Bekasi City in February 2022. The sample of this study consisted of 40 plantation workers' nail scrapings. Samples were examined using the direct preparation method at the Mycology Laboratory of STIKes Mitra Keluarga Bekasi.

Table 1. Results Of Examination Of Direct Preparation Of KOH (20%)

Results		Total
Positive	Negative	
5 (12,5%)	35 (87,5%)	40 (100%)

This research has carried out a preliminary test of reagents between concentrations of 10% and 20%, at a concentration of 10% it is necessary to fixation with a Bunsen flame while at a concentration of 20% it is not necessary to fixation with a Bunsen flame. Based on table 4.1, the results of direct examination of the 40

samples examined were positive 5 (12.5%) and negative 35 (87.5). Sampling was based on the characteristics of nail fungus including changes in the color of the nails, this is in accordance with research conducted by Setianingsih., et al (2015) about the characteristics of nail fungal infections such as yellow, brown and white and nails can become cracked, uneven surface, and reduce the beauty of one's nails and can reduce the quality of life of a person affected by nail fungus infection. Based on Anggowarsito's research, (2018) Meanwhile, if healthy nails have the characteristics of healthy nails, the nails do not change color, the surface is smooth, there are no spots, no holes, and the surface is smooth.

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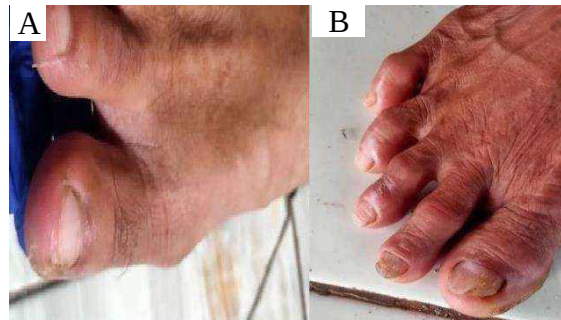


Figure A shows a nail that does not have the characteristics of a nail fungus. Figure B shows nails that have the characteristics of nail fungus

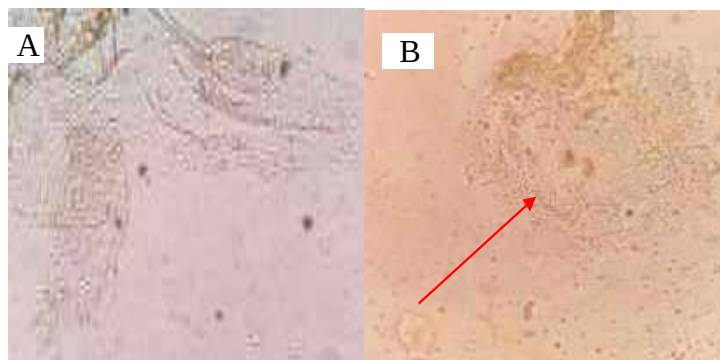


Figure A shows negative nail fungus. B is positive for nail fungus (red arrow). Hyphae in Nail Scraping Preparations Direct Examination of 20% KOH method. (40x magnification)

Positive samples obtained in this study amounted to 5 (12.5%) with hyphae found in the five samples. Hyphae have a non-insulated structure or are called aseptate. The characteristics of the spores that are commonly found in nail scraping preparations are groups of yeast cells or spores that are unicellular oval or buds form. The characteristics of hyphae appear branched and septal, hyphae can be shaped into arthrospores on nail preparations that look like spores that are densely arranged (Sutanto, 2013). The factors that cause nail fungus infection are individual hygiene and environmental hygiene, the table below is a percentage of the habits of individual plantation workers and environmental hygiene.

Table 2. Length Of Work On The Plantation In One Week

Choice	Results		Total
	Positive	Negative	
Every day	4 (10%)	10 (25%)	14 (35%)

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2x in one week	1 (2,5%)	15 (37,5)	16 (40%)
3x in one week	0 (0%)	10 (25%)	10 (25%)
Amount	5 (12,5%)	35 (87,5)	40 (100%)

There are 4 workers (10%) who are positive for nail fungus infection who work every day for one week, while 1 person (2.5%) only works 2x a week.

Table 3. Use Of Footwear At Work

Choice	Results		Total
	Positive	Negative	
Yes	1 (2,5%)	19 (47,5%)	20 (50%)
Not	4 (10%)	16 (40%)	20 (50%)
Amount	5 (12,5%)	35 (87,5%)	40 (100%)

Plantation workers who use footwear at work, footwear that can cover up to the ankles. Based on plantation workers who were positive for nail fungus infection, 4 (10%) did not use footwear at work, while 1 (2.5%).

Table 4. Frequency Of Clipping Nails In Two Weeks

Choice	Results		Total
	Positive	Negative	
1x	5 (12,5%)	13 (32,5%)	18 (45%)
2x	0 (0,0%)	14 (35%)	14 (35%)
3x	0 (0,0%)	8 (20%)	8 (20%)
Amount	5 (12,5%)	35 (87,5)	40 (100%)

The percentage of plantation workers who cut their nails 2-3 times within two weeks, none of them were infected with nail fungus. while the plantation workers who only cut their nails once in two weeks experienced a nail fungus infection.

Table 5. Frequency Of Bathing In One Day

Choice	Results		Total
	Positive	Negative	
1x bath in one day	4 (10%)	11 (27,5%)	15 (37,5%)
2x bath in one day	1 (2,5%)	16 (40%)	17 (42,5%)
>2x bath in one day	0 (0,0%)	8 (20%)	8 (20%)
Amount	5 (12,5%)	35 (87,5)	40 (100%)

Percentage of plantation workers how many times to bathe in one day. In plantation workers who were positive for nail fungus infection who only took 1 bath in one day as many as 4 (10%) people, for plantation workers positive for nail fungus infection who took 2 baths in one day as many as 1 (2.5%) people. This shows that plantation workers who only take 1 bath in a day have a high risk of nail fungus infection because people who bathe only 1x the body of the worker is dirty because of work that produces sweat and then becomes damp so that fungus can grow.

The percentage of 5 (12.5%) people infected with nail fungus. 2x plantation workers are not positive. Meanwhile, less than 2 plantation workers clean the floor in one. the plantation workers how many times to clean the floor of the house in one day. Plantation workers who are positive for nail fungus infection who do not clean the floor of the house are 4 (10%), for plantation workers who are positive for nail fungus infection who clean the floor of the house once in one day are 1 (2.5%), while workers who are positive for nail fungus infection who cleaned the floor of the house 2x there was no 0 (%).

Table. 7. The Frequency Of Workers After Finishing Work Cleans Up

Choice	Results		Total
	Positive	Negative	
Yes	1 (2,5%)	23 (57,5%)	24 (60%)
Not	4 (10%)	12 (30%)	16 (40%)
Amount	5 (12,5)	35 (87,5%)	40 (100%)

Percentage of plantation workers how many times to bathe in one day. In plantation workers who were positive for nail fungus infection who only took 1 bath in one day as many as 4 (10%) people, for plantation workers positive for nail fungus infection who took 2 baths in one day as many as 1 (2.5%) people. This shows that plantation workers who only take 1 bath in a day have a high risk of nail fungus infection because people who bathe only 1x the body of the worker is dirty because of work that produces sweat and then becomes damp so that fungus can grow.

Table 8. Use Of Gloves On Plantation Workers

Choice	Results		Total
	Positive	Negative	
Yes	0 (0%)	22 (55%)	22 (55%)
Not	5 (12,5%)	13 (32,5%)	18 (45%)
Amount	5 (12,5%)	35 (87,5%)	40 (100%)

percentage of plantation workers whether or not they use gloves at work. In plantation workers who were positive for nail fungus infection who did not use gloves at work, there were 5 (12.5%) for workers who were positive for nail fungus infection using gloves, there were 0 (0%) people.

Table 9. Age Data Of Respondents Infected With Nail Fungus

Sample Code	Age
S – 220222 – 01	45 Tahun
S – 220222 – 02	50 Tahun
S – 220222 – 06	68 Tahun
S – 240222 – 15	52 Tahun
S – 250222 – 18	67 Tahun

DISCUSSION

According to the Ministry of Health. RI is the age of 45-65 is the age of the elderly, while the age of 68-67 years is the elderly. Based on table 9, the age of the nail fungus infection ranges from 45 - 67 years, this age category means that people infected with nail fungus have entered a young age, so it can be interpreted that age is one of the risk factors for nail fungus infection because they are getting older. A person's age, his health will decrease

The prevalence of onychomycosis is lower in children compared to adults this is due to reduced exposure to infected environments such as: shared baths, public changing rooms and saprophytic fungi, smaller nail surface area (Mpharmsc et al., 2010). Research conducted by Widasmar, (2018) said that elderly people tend to have circulation disorders that are associated with decreased nail growth rates. This increases the risk of colonization by dermatophytes and other organisms. Preliminary studies suggest that there is a correlation between age and onychomycosis. Approximately 20% of the population >60 years of age and up to 50% of subjects >70 years of age are reported to have onychomycosis (5,20). Another study says that age is correlated with nail fungal infection is Mpharmsc et al., (2010) correlation between increasing age and onychomycosis can be attributed to reduced peripheral circulation, inactivity, suboptimal immune status, diabetes, larger and distorted nail surface, slower growing nails. Rubrum infection that does not appear to be associated with interfamilial transmission.

This research was conducted on 40 respondents of plantation workers in Bojong Sari village, Jatisih sub-district, Bekasi city. Positive results are 5 (12.5%), this is because plantation workers are in direct contact with soil, water, mud. This is in accordance with research conducted by Widiati et al., (2016) who said that residents who work as farmers and plantation workers have an activity that is not using footwear, even they do not use footwear and direct contact with the mud becomes a problem. risk of fungal nail infection.

Limitations in this study were not using LPCB (Lactophenol Cotton Blue) reagent so it could not determine the genus of nail fungus from samples of plantation workers' nail scrapings, mushroom culture method had not been carried out using SDA (Sabouraud Dextrose Agar) media so that it could not be determined to the genus level, and did not perform a correlation analysis on the relationship between nail fungus infection and individual and environmental hygiene factors. The advantages of this study are that it has complete subject data, including data on the frequency of working in one week, the use of footwear at work, the frequency of cutting nails in two weeks, the frequency of taking a bath in one day, the frequency of cleaning the floor in one day, workers after working to clean themselves or not, we3ggw use of gloves after work, and data on the age of respondents infected with nail fungus.

CONCLUSION

This study can be concluded that the description of nail fungus infection in plantation workers in Bojong Sari Village, Jatisari Village, Jatisih District, Bekasi City as many as 5 people (12.5%) positive toenail fungus, and 35 people (87.5%) negative nail fungus. . Risk factors that have the potential to cause plantation workers to get nail fungus infection are not using footwear, not using gloves, not cutting nails regularly and not keeping the house clean.

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THE EFFECTIVENESS OF IMMERSION TIME IN LEMONGRASS POWDER IN REDUCING FORMALIN LEVELS IN SALTED FISH

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Abstract

Introduction: Salted fish is one of the foodstuffs that are widely consumed by the community. The rate of food poisoning due to chemicals in 2019 based on BPOM data was 7% and increased in 2020 to 16%. This increase was due to the Covid-19 pandemic so that traders experienced a decline in sales. Many traders try to extend the shelf life of their products by adding prohibited preservatives, one of which is formalin, which causes an increase in poisoning rates. Saponins are one of the bioactive compounds in plants that can reduce formaldehyde. Lemongrass is a plant that has been studied to contain saponins. The use of lemongrass is also processed in powder form to make it more practical and extend the shelf life of lemongrass. Lemongrass powder is used as an alternative ingredient in reducing formalin levels in foodstuffs, namely salted fish. The purpose of this study was to determine the effect of soaking salted fish with lemongrass powder in reducing formalin levels based on variations in soaking time.

Method: This research was conducted at the STIKes Mitra Keluarga laboratory. The type of research used is experimental. with 3 experimental groups. The control group (KK) is a negative and positive control. The treatment group (X1) was salted fish before the soaking treatment with lemongrass powder was carried out. The treatment group (X2) was salted fish after soaking treatment with lemongrass powder based on 6 time variations, namely, 5 minutes, 15 minutes, 25 minutes, 35 minutes, 45 minutes, and 55 minutes.

Result: Positive samples were determined using a UV-VIS spectrophotometer and Nash reagent. The lowest increase in formalin reduction was 9,299 ppm by soaking lemongrass powder for 5 minutes. The highest level was 0.553 ppm by soaking lemongrass powder for 55 minutes.

Keywords : Salted Fish, Formalin, Saponin, KMnO₄, UV-Vis Spectrophotometry.

INTRODUCTION

Fish is a marine product that has a source of protein and is in great demand by the public. Marine products are easily damaged, so it is necessary to add additives. In the food processing business, preservatives are often applied to maintain product durability and freshness. Many producers still use dangerous food additives (BTP) such as formalin in food, one of which is salted (Widayanti & Laksmita W., 2017). Salted fish products are widely consumed because of their affordable price, longer shelf life, and easy availability (Lestari *et al.*, 2022)

Formalin or formaldehyde (H₂CO) is a colorless solution with a pungent odor. Formalin sold in the market generally has a content of 37% in water with the addition of methanol up to 15% as a preservative stabilizer (Modeong *et al.*, 2022) Formalin is added by some manufacturers to maintain product shape, extend shelf life, improve visualization of food products and manipulate various foods that are no longer fit for consumption. Economic motives are the reason traders add harmful BTP in their products to reduce production costs (Wijaya *et al.*, 2012)

Foods containing formaldehyde have negative effects that will appear several years later. Short-term effects that will occur are irritation, stomach pain, and dizziness. Long-term effects that cause damage to the respiratory tract, liver, kidneys, pancreas, heart, brain, central nervous system and cancer. Formalin is prohibited as a food additive based on the Regulation of the Minister of Health No. 33 of 2012 (Fatma *et al.*, 2021)

Food poisoning due to chemicals in 2018 reached 11% (BPOM, 2018). Food poisoning due to chemicals in 2019 reached 7.01% (BPOM, 2019). Food poisoning due to chemicals in 2020 reached 16% (BPOM, 2020). The increase in the number of food poisoning due to chemicals in 2019-2020 was due to the Covid-19 pandemic. Merchant turnover experienced a decline in sales. Many traders work around so that their products last longer by adding preservatives. Preservatives used include those that are prohibited,

causing an increase in poisoning rates.

Saponins are one of the bioactive ingredients in plants studied that can reduce formalin levels. Saponins are widely contained in several tribes of flowering plants, namely Liliaceae, Amaryllidaceae, Poaceae and Dioscoreaceae (Darma & Marpaung, 2020). Lemongrass is one of the plants belonging to the Poaceae family which contains saponins and is easy to obtain and widely used in everyday life. The use of lemongrass is also widely processed in powder form to make it more practical and extend the shelf life of lemongrass. Based on the problems and impacts regarding the formalin contained in salted fish, it is necessary to conduct research on reducing formalin levels in salted fish using natural ingredients as an alternative solution, one of which is lemongrass powder.

Research conducted by Sugiarti (2020), showed a maximum decrease in the formalin content of salted squid by 72.11% from soaking in salt water for 90 minutes. The formalin level reduction test was also carried out by Sarwindah & Wardoyo (2019), which showed the level of formalin in white tofu with a solution of miana leaves (*coleus benth*) containing saponins, the highest decrease was 95.51% for 90 minutes. Cases of using formalin in salted fish still occur, one of which is in the city of Bandung. The results showed that from 25 samples sampled from 5 different traders, it was stated that 6 samples of salted fish in the Bandung City Simple Market were positive for formalin (Noorrela & Munggaran, 2021).

Based on the above case, there are still irresponsible traders who use formalin as a food preservative. The lack of research that proves the decrease in formalin levels with saponin compounds in lemongrass powder is the reason why researchers conduct research on the title "Effectiveness of Soaking Lemongrass Powder in Reducing Formalin Levels in Salted Fish". Salted fish samples were soaked in 4% formalin for 60 minutes and tested for levels using UV-Vis spectrophotometry, then soaked with lemongrass powder for 5 minutes, 15 minutes, 25 minutes, 35 minutes, 45 minutes, and 55 minutes again by UV-Vis spectrophotometry.

METHOD

This research was conducted from February to June 2022 at the Mitra Keluarga College of Health Sciences Laboratory. The type of research to be conducted is experimental with the pretest-posttest control group design. In this study, the treatment carried out was soaking salted fish containing formalin using lemongrass powder based on time variations of 5 minutes, 15 minutes, 25 minutes, 35 minutes, 45 minutes, and 55 minutes.

Qualitative test phytochemical test of saponins on lemongrass powder was carried out by dissolving 1 gram of lemongrass powder with 1 mL of distilled water (1:1). The solution is shaken for 1 minute, if there is foam then 0.1 N HCl is added. Stable foam for 10 minutes with a height of 1-3 cm indicates the presence of saponins (Selfiana *et al.*, 2022). Qualitative test of KMnO₄ on salted fish was carried out by making positive control by soaking salted fish in 4% formalin for 60 minutes and negative control made from salted fish which was not soaked in 4% formalin. the negative control was soaked in distilled water for 60 minutes. A sample of 10 grams was ground with a mortar and pestle. The sample powder was dissolved in 30 ml of distilled water, then filtered. 2 ml of the sample filtrate was pipetted and 2 drops of 0.1N KMnO₄ were added. The presence of formalin is indicated by the loss of the pink color of KMnO₄ (Rambe *et al.*, 2022)

Quantitative test of UV-VIS Spectrophotometry was carried out by making Nash reagent according to SNI ISO 14184-2:2015. Nash reagent was prepared by dissolving 37.5 g of ammonium acetate and dissolved in distilled water. The solution was added 0.75 mL of glacial acetate and 0.5 mL of acetyl acetone. The sea was transferred to a 250 mL volumetric flask and diluted using aquadest to the right mark. The reagents were stored in a dark bottle for 12 hours before use (Yulianti & Safira, 2020).

Preparation of 1000 ppm formalin mother liquor was carried out by dissolving 2.70 mL of 37% formalin (V/V) or equivalent to 370,000 ppm. The solution was put into a 1000 ml volumetric flask. The solution was added with distilled water up to the mark and homogenized. Standard solutions of 0 - 2 ppm were made with concentrations of 0 ppm, 0.05 ppm, 0.1 ppm, 0.5 ppm, 0.75 ppm, 1 ppm, 1.5 ppm and 2 ppm. The 1000 ppm mother standard solution was pipetted 0 ml, 0.005 ml, 0.01 ml, 0.05 ml, 0.075 ml, 0.1 ml, 0.15 ml and 0.2 ml, respectively. The solution was added with distilled water up to a volume of 100 ml at each concentration. The sea was pipetted as much as 2 ml of each concentration and added Nash reagent up to 3 ml into the volumetric flask. The solution was heated in a water bath at 40±2°C for 30 minutes and cooled for 10 minutes. The absorption was observed using a UV-Vis spectrophotometer (Suseno, 2021).

The maximum wavelength with nash reagent was determined by pipetting 2 mL of a standard solution of 0.05 ppm formalin and put into a closed test tube, then 3 mL of nash reagent was added. The test tube was then homogenized and the solution was heated in a water bath at a temperature of $40\pm 2^{\circ}\text{C}$ for 30 minutes after which it was cooled. The absorption was observed using a UV-Vis spectrophotometer (Wardani *et al.*, 2020)

Quantitative testing of the sample was carried out by taking 2 ml of salted fish solution that had been soaked in 4% formalin. Then 3 ml of nash reagent was added to the solution and heated with a water bath at $40\pm 2^{\circ}\text{C}$ for 30 minutes and cooled for 10 minutes. The absorption was observed using a UV-Vis spectrophotometer. After that prepared measuring cups that have been labeled with names 5 minutes, 15 minutes, 25 minutes, 35 minutes, 45 minutes, and 55 minutes, adding 4 grams of lemongrass powder to each measuring cup. Add 100 ml of distilled water until dissolved. Furthermore, salted fish that has been soaked in 4% formalin is put into a measuring cup as much as 10 grams. The solution was allowed to stand for an appropriate period of time in each measuring cup, then the solution was filtered and tested for levels with a UV-Vis spectrophotometer.

RESULTS

The qualitative test of saponin compounds tested on lemongrass powder showed a stable foam for 10 minutes with a height of 1-3 cm indicating the presence of positive saponin compounds.



Figure 1. Saponin foam qualitative phytochemical test

Qualitative test with KMnO_4 was carried out on positive controls made by soaking salted fish in 4% formalin solution for 60 minutes, while negative controls used salted fish without formalin. The results of the examination showed that the positive sample contained formalin where the pink color of the KMnO_4 reagent was missing.



Figure 2. KMnO_4 test results positive samples contain formalin

Determination of the maximum wavelength using a 1000 ppm formalin solution whose absorption was observed at a wavelength of 400-600. Based on these measurements, the maximum wavelength was found at 535 nm.

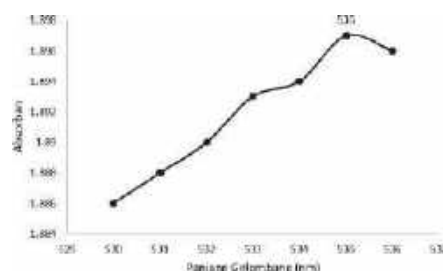


Figure 3. Maximum wavelength graph

Determination of the calibration curve using the concentration of standard solutions obtained absorption results at five concentrations, namely 0 ppm, 0.05 ppm, 0.1 ppm, 0.5 ppm, 0.75 ppm, 1 ppm, 1.5 ppm and 2 ppm showing the absorbance value. of 0, 0.219, 0.297, 0.356, 0.450, 0.577, 0.699 and 0.797. The calibration curve equation is used to show the relationship between the x and y axes. The linear regression equation obtained is $y = 0.1061x - 0.0531$ with a correlation coefficient of $R^2 = 0.9823$.

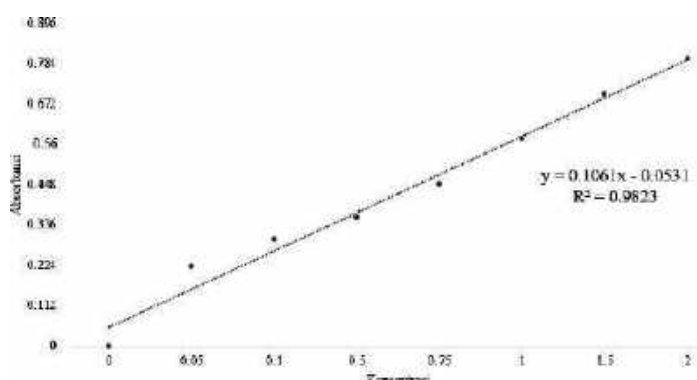


Figure 4. Linear regression curve of absorbance relationship with formalin concentration

Quantitative test using UV-Vis spectrophotometer at a wavelength of 535 nm to increase the reduction of formalin levels in salted fish with lemongrass powder is obtained in Table 1.

Table 1. Formalin Levels in Salted Fish Samples After Soaking in Lemongrass Powder Based on Time Variations

Repetition	Formalin Levels (ppm) After Soaking Lemongrass Powder Based on Time Variations (minutes)						
	0	5	15	25	35	45	55
1	12.932	9.388	7.447	5.637	2.470	1.273	0.576
2	12.781	9.219	7.437	4.959	2.433	1.377	0.557
3	13.262	9.313	7.334	5.109	2.414	1.217	0.538
4	12.781	9.275	7.305	4.911	2.414	1.330	0.538
Mean	12.939	9.299	7.381	5.154	2.433	1.299	0.553
Standard deviation	0.227	0.071	0.072	0.333	0.027	0.069	0.018
Reduced formalin	0%	28.13%	42.9%	60.7%	81.2%	89.9%	95.7%

The statistic test was carried out with the ANOVA test using SPSS version 26. The data obtained $<\alpha 0.05$, the data were normal and homogeneous. The result of the ANOVA $<\alpha$ test is 0.05 which proves that there is an effect of the effectiveness of reducing formalin levels by soaking lemongrass powder.

Table 2. One way anova statistical test

	Soaking time (minutes)					
	0 and 5	0 and 15	0 and 25	0 and 35	0 and 45	0 and 55
	Significance					
Normality test	0.143	0.143	0.143	0.143	0.596	0.596
Homogeneity Test	0.154	0.173	0.500	0.070	0.106	0.105
One way anova test	0.000	0.000	0.000	0.000	0.022	0.022

DISCUSSION

The qualitative test of saponin compounds tested on lemongrass powder showed foam stability. Stable foam for 10 minutes with a height of 1-3 cm indicates the presence of positive saponin compounds (Selfiana *et al.*, 2022). Foaming occurs due to the nature of saponins which can lower the surface tension of water when shaken. The decrease in surface tension caused by the presence of hydrophilic compounds that can damage hydrogen in the air.

The addition of HCl serves to increase the polarity so that the hydrophilic group will bind more stable and form foam (Ngginak *et al.*, 2021). Research conducted by Purbowati (2021) where the foam is stable as high as 1-3 cm which indicates a positive result containing saponins in lemongrass. Another study on the saponin foam test conducted by Sapitri (2022) on lemongrass leaves produced a stable foam which indicated the presence of saponins.

Samples of salted fish that have been soaked in 4% formalin in a qualitative test with KMnO₄. Positive control was made by soaking salted fish in 4% formalin solution for 60 minutes, while negative control used salted fish without formalin. The results of the examination showed that the positive sample contained formalin where the pink color of the KMnO₄ reagent was missing. This is in accordance with Rosita's research (2022) where the identification of formalin in tofu samples reacted with 0.1 N KMnO₄ will still produce a pink-purple color if the sample is negative for formalin. A positive sample containing formalin will not change color, so the sample will remain clear when KMnO₄ is added.

In Rambe's study (2022) a solution containing formalin compounds when KMnO₄ was dripped changed its color, namely the loss of the pink-purple color of the KMnO₄ reagent. The color change is due to the functional group containing an aldehyde, and a ketone is a carbonyl, which makes the presence of a carbonyl group more active than a ketone. The aldehyde group is rapidly oxidized to a carbonyl group or formic acid with an oxidizing agent such as KMnO₄. Samples that does not contain formalin, it does not have the antioxidant KMnO₄ which causes no color change because it does not have a substrate that can be oxidized by KMnO₄.

The determination of the maximum wavelength is observed at wavelength 400-600 and the maximum wavelength is found at 535 nm. Based on the research of Suseno (2021) obtained a maximum wavelength of 412.5 nm, when compared with this study, there is a change in the maximum wavelength. According to Krisnawati (2018) formalin with Nash reagent has an optimum absorption at 400-600 nm. The wavelength of 535 nm in this study still entered the criteria for the optimum measurement of formalin levels. The difference in wavelength occurs because the scope of research is different from one researcher to another, causing a shift in wavelength by several factors such as equipment conditions, differences in reagents, differences in standard standard solutions and differences in tools used (Sari *et al.*, 2021)

The calibration curve is used to show the relationship between analyte concentration (x-axis) and calibration (y-axis). To make calibration curve, the standard is prepared with the analyte concentration is known. The standard absorbance is determined at the wavelength and the results are depicted in a graph (Seidman *et al.*, 2021). Determination of the calibration curve using the concentration of the standard solution, that is, the absorption results at the five concentrations are 0 ppm, 0.05 ppm, 0.1 ppm, 0.5 ppm, 0.75 ppm, 1 ppm, 1.5 ppm and 2 ppm showing the value absorbance of 0, 0.219, 0.297, 0.356, 0.450, 0.577, 0.699 and 0.797.

The calibration curve equation is used to show the relationship between the x and y axes. The linear regression equation obtained is $y = 0.1061x - 0.0531$ with a correlation coefficient of $R^2 = 0.9823$. According to Yulianti & Safira (2020) the linearity of the calibration curve can be seen by calculating the value of the correlation coefficient (r), the curve can be said to be linear if the value of r 0.98. Thus, the

test curve satisfies the test requirements.

At a wavelength of 400-600 the ocular color in the solution is green-yellow and the absorbent color will be absorbed into purple (Seidman *et al.*, 2021). Formalin is basically colorless. Nash reagent is used to add color to the test solution. Acetylacetone and ammonium in Nash reagent will react with formalin, where condensation will occur to form a compound 3,5-diacetyl-1,4-dihydro-2,6-lutidine (DDL). The heating treatment will produce a fixed yellow color, then the color on the instrument will produce a purple color (Hayun *et al.*, 2017)

Reducing formalin levels in salted fish using lemongrass powder based on time variations, showing that all samples experienced decrease in any given time variation. Formalin salted fish without immersion (0%) the formalin content was calculated using visible spectrophotometer. Average levels of formalin in fish samples salty which is not treated using lemongrass powder as much as 12,939 ppm. Formalin levels in salted fish samples soaked with lemongrass powder experienced an increase in reduction and the highest increase was 95.7% at the longest immersion time variation of 55 minutes.

Formalin is reduced by saponin compounds in lemongrass powder. The decrease in levels was caused by the saponification reaction (soap formation process), where soap is included in the surfactant class. Surfactants in saponins are amphipathic, i.e. they contain hydrophobic (non-polar) and hydrophilic (polar) groups, where the surface active mechanism binds to formaldehyde molecules, reduce the surface tension to a very low level, making a soap solution (surfactant) this makes the ability of saponins to reduce formalin better than soaking with plain water. After formalin binds to saponins, saponins dissolve and form micelles. The round and oval parts of the micelles are heads that point and interact with water, formalin (polar) and formalin that react with micelles can dissolve in water (Mayasari *et al.*, 2022).

According to the International Program on Chemical Safety (IPCS), the safe limit level of formalin that can be tolerated in the body is 1 mg/L (Donal Nababan *et al.*, 2019). while according to the European Food Safety Authority (EFSA) in 2014 the limit for formalin exposure through food consumption is 100 milligrams per day (BPOM, 2019). So the results of reducing formalin levels in salted fish with lemongrass powder carried out in this study showed the most effective immersion for the longest time, namely 0.553 ppm at 55 minutes, where the level of formalin at that time was already below the recommended threshold.

The results of statistical tests carried out by normality test, data $<\alpha$ that is 0.05 which proves that the data is normally distributed. The homogeneity test of data $<\alpha$ is 0.05 which proves that the data is homogeneously distributed. One Way Anova statistical test data $<\alpha$ is 0.05 which proves that there is an effect of the effectiveness of reducing formalin levels by soaking lemongrass powder for 0 minutes and 55 minutes. If the probability (Sig) $<$ then H_0 is rejected and H_a is accepted which indicates a significant effect of variations in the soaking time of lemongrass in the form of formalin in salted fish.

CONCLUSION

The effectiveness of formalin reduction using lemongrass powder based on time variations with UV-VIS spectrophotometry obtained the longest time of 55 minutes with a concentration of 0.553 ppm and reducing formalin by 95.7%.

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DIFFERENCES IN THE NUMBER OF BACTERIA AND CHARACTERISTICS IN THE ENDO AGAR MEDIUM IN DISHWASHING SPONGES WITH LONG USE

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Abstract

Introduction: Sponge is one of the common scrubbing tools found in Indonesia as a dishwasher. Soaking a dishwashing sponge in a wet container can make the sponge moist and become an ideal place for microorganisms to grow such as *Escherichia coli* bacteria that can grow on a wet surface. Some factors that still often occur in the community are in users of dishwashing sponges that exceed 3 days of use and are not replaced, this can make *Escherichia coli* breed in the sponge. Food can be contaminated caused by dishwashing sponges, which are transmitted through cutlery. A case study in the United States, after 1,000 kitchen washcloths and washing sponges that had been collected, obtained 10% where the objects contained *Salmonella* and *Escherichia coli*

Method: Bacterial identification is carried out on 10 dishwashing sponges used for 1 week and 2 weeks

Results: The characteristics of colonies obtained in BHIA media in sponge samples of 1 and 2 weeks are circular, with a margin of entire, opaque, white-gray or milky-white with a smooth texture, in Endo Agar media grow colonies of red to metallic green color, Simmon citrat agar negative, glucose and lactose positive tests.

Conclusion: Based on the results of the ALT calculation study, it was found that the dishwashing sponge used 2 weeks more than the dishwashing sponge used for 1 week. ALT calculation with a duration of use of 2 weeks obtained results Sp1 = 6.0×10^7 , Sp2 = 4.7×10^6 , Sp3 = 7.2×10^7 , Sp4 = 6.0×10^5 , Sp5 = 6.6×10^7 . While the calculation results of ALT 1 week are Sp1 = 4.6×10^7 , Sp2 = 1.6×10^6 , Sp3 = 4.8×10^7 , Sp4 = 5.4×10^5 , Sp5 = 4.5×10^7 . The characteristic of bacteria that are suspected to grow on dishwashing sponges is *Escherichia coli* bacteria

Key words : Brain Heart Infusion Agar , Dishwashing sponge, Endo Agar, Food borne disease, Total Plate Count (TPC).

INTRODUCTION

Sponge is one of the common scrubbing tools found in Indonesia as a dishwasher. Soaking a dishwashing sponge in a wet container can make the sponge moist and become an ideal place for microorganisms to grow such as *Escherichia coli* bacteria that can grow on a wet surface. If the sponge is not dry or damp because the sponge is soaked in a container, it can be an ideal place for bacteria to grow (Gaffar *et al.*, 2014). Storing a good dishwasher is something that really needs to be considered. Soaking a dishwashing sponge in a wet container can make the sponge will be damp and become a nest for breeding microorganisms. According to Kalem (2019) some factors that still often occur in the community are in users of dishwashing sponges that exceed 3 days of use and are not replaced, this can make *Escherichia coli* multiply in the sponge.

Food can be contaminated caused by dishwashing sponges, which are transmitted through cutlery (Rossi *et al.*, 2012). Eating or drinking utensils that have been contaminated by bacteria can cause food borne disease (Fadhila *et al.*, 2017). Food borne disease is a disease that causes poisoning of the body and is caused by consuming food that has been contaminated with various microorganisms. Some microorganisms that can cause Food borne disease are *Escherichia coli* bacteria (Motarjemi *et al.*, 2006). Generally, tableware has been regulated by the Regulation of the Minister of Health of the Republic of Indonesia No. 715 / MENKES / SK / V / 2003 which states about, that in eating or drinking utensils must not contain bacteria more than 100 colonies / cm² on the surface and there are no *Escherichia coli* bacteria. According to the Regulation of the Minister of Health of the Republic of Indonesia No. 1096/MENKES/PER/VI/2011 said that regarding sanitary hygiene, eating and drinking utensils must not have *Escherichia coli* germs or other germs. According to the Decree of the Minister of Health No. 715,

said food sanitation hygiene is an important thing that can control equipment, food, and factors of people that can cause disease or health. Gaffar *et al* (2014) in identifying bacterial growth in dishwashing sponges by soaking the sponge overnight, *E.coli* bacterial growth was obtained. Based on previous research by Andini *et al* (2021) obtained *E. coli* bacteria in dishwashing sponges used for 6 weeks. The study conducted previously by researchers came from Arizona, United States, after 1,000 kitchen washcloths and washing sponges that had been collected, obtained 10% where the objects contained *Salmonella* and *Escherichia coli* (Riboldi G., 2002).

METHOD

The research used in this study is an experiment using 2 variables, namely Independent and Dependent. The intervention or treatment in this study was the duration of use of dishwashing sponges, namely 1 week and 2 weeks. This study conducted an examination on the dishwashing sponge to see the comparison of the number and characteristics of bacteria. This research was conducted from February to March 2022. The research was conducted at the Bacteriology Laboratory of the Tingi School of Health Sciences, Mitra Keluarga. The samples used in this study were 5 dishwashing sponges with a duration of use of 1 week and 5 dishwashing sponges with a duration of use of 2 weeks.

Samples that have been used for 1 week and 2 weeks are taken using a sterile cotton swab by means of, The whole cotton swab is dipped first into the sterile aquadest. Sampling by rubbing the cotton swab rotates so that the entire surface of the cotton is in contact with the surface of the sample. (Ramadhani and Wahyuni., 2020). Then carry out the titular dilution by means of samples containing bacteria are put into the first dilution tube ($1/10$ or 10^{-1}) aseptically. After the sample enters, it is then homogenized, then take 1 ml from retailers 10^{-1} using a measuring pipette then transfer it to dilution 10^{-2} aseptically and then dihomogenized. The transfer is continued until the last dilution tube, which is 10^{-5} using a sterile pipette tip (Ramadhani and Wahyuni., 2020).

Then planting colonies on BHI-A media by pipette 100 μ l at a dilution of 10^{-2} to 10^{-5} , then incubated in an incubator with a temperature of 37°C for 24 hours. After planting on BHI-A media, as many as 10 different colonies using ose then take and plant on Endo Agar media and then incubated in an incubator with a temperature of 37°C for 24 hours. Colonies of bacteria growing on BHI-A media after 24 hours are calculated manually using a coloni counter tool. Calculating the number of bacterial colonies by means, the cup is placed on a bright flash or LED. After that, do gram coloring by the way, the violet kistal is picketed and then color the preparation evenly, then wait up to 1 minute. After waiting for 1 minute rinse the preparation under running water. Next, pipette iodine then drip on the preparation until evenly distributed, wait up to 1 minute. After that washing with running water and then removing the color with alcohol. Recoloring with safranin then waited for 1 minute, after 1 minute rinse the preparation with running water then dried (Sujaya, 2016).

RESULTS

On a sample of dishwashing sponges with a duration of use of 1 week. The characteristics of colonies growing in BHI-A media are dominated by colonies with a circular shape, with an entire margin, the colony cannot be translucent (opaque), white-gray or milky-white with a smooth texture, as in the table. According to Indrayati and Fatimah (2018) colony characteristics in table 4.1 below, it is suspected to be a colony characteristic of the bacteria *Escherichia coli*.

Sample dishwashing sponge with a duration of use of 2 weeks. The characteristics of colonies growing in BHI-A media are dominated by colonies with a circular shape, with a margin of entire, the colony cannot be translucent (opaque), gray-white or milky-white with a smooth texture. According to Indrayati and Fatimah (2018) colony characteristics are suspected to be colonies of *Escherichia coli* bacteria.



The result of the number of colonies on dishwashing sponges used for 1 week is less than that used for 2 weeks and can be seen in figure 1. After obtaining a culture colony from BHI-A media, then inoculation is carried out on Endo Agar media to find out the nature of the colony growing in BHI-A media, whether the colony can ferment lactose or not.

Table 1. Characteristics of colonies in Endo Agar media

No	Sample code	Colony color
1	3	White
2	4	does not grow
3	5	Red
4	6	metallic red

Colonies in scratches coded 5 and 6 with culture colonies obtained from BHI-A media are then streaked in Endo Agar media, which is thought to be a characteristic colony of *Escherichia coli* bacteria, because colonies are round, convex, entire and gray-white or milky-white in color. In the results of inoculation in Endo Agar media, there are growing colonies of red color until there is metallic green. *Escherichia coli* bacteria can grow on Endo Agar media with the characteristics of colonies of red to metallic dark red color (Indrayati and Fatimah, 2018).

Bacterial colonies that grow with the characteristics of colonies of metallic red to green color in Endo Agar media are taken and inoculated on SCA media to test whether colonies in Endo Agar media can grow on SCA media.

Table 2. Characteristics of bacteria on biochemical tests

No	Sample code	Result		
		Simon Citrat	Glucose	Lactose
1	5	-	+	+
2	6	-	+	+

The results on the citrate test obtained negative results, and in glucose and lactose tests, positive results were obtained. Then in the gram staining test results, the results of red-colored bacteria or gram-negative bacteria were obtained. Different colony numbers can be caused by discharging factors with a longer period of time. According to Kalem (2019) several factors that cause the growth of *Escherichia coli* bacteria in sponges are caused by use that exceeds 3 days of use and are not replaced with new ones. According to Ermia (2021) the high number of bacteria is due to poor washing stages, poor storage of hygiene tools.

DISCUSSION

The result of the number of colonies on dishwashing sponges used for 1 week is less than that used for 2 weeks and can be seen in figure 1. In table 1 scratches with code 4 are seen colonies do not grow on the medium, on strokes with code 3 are white, 5 and 6 are seen the growth of red to metallic red-colored bacteria. Colonies in scratches coded 5 and 6 with culture colonies obtained from BHI-A media are then streaked in Endo Agar media, which is thought to be a characteristic colony of *Escherichia coli* bacteria, because colonies are round, convex, entire and gray-white or milky-white in color. In the results of inoculation in Endo Agar media, there are growing colonies of red color until there is metallic green. *Escherichia coli* bacteria can grow on Endo Agar media with the characteristics of colonies of red to metallic dark red color (Indrayati and Fatimah, 2018).

The observation results obtained in the citrate test were negative in colonies in Endo Agar media which had a characteristic red to metallic green color which was suspected to be *Escherichia coli* showed negative results by not experiencing discoloration in the sirat test media. *Escherichia coli* bacteria do not use citrate as a source of carbon which is indicated by the absence of discoloration in the citrate test media. Simmon Citrate In order to function as a testing medium to determine the properties of bacteria, the bacteria can ferment citrate or not as a source of carbon (Rahayu and Gumilar, 2017).

The results of the bacterial fermentation test on glucose and lactose media obtained positive results with the marked gas formation in the durham tube. According to Harley (2012) the occurrence of discoloration from red to yellow is caused by the presence of phenol red indicators that blind the occurrence of acid formation in this carbohydrate fermentation test medium. According to Jorgensen (2015) *Escherichia coli* bacteria can ferment glucose and lactose which is in accordance with the results in this study. According to Mahon (2015) bacteria can be able to ferment carbohydrates, so there is a glycolysis process that can produce the final product, namely in the form of pyruvate and converted so that acid formation occurs. Then the acid is converted into H₂ and CO₂ through an enzyme mechanism, namely hydrogen lyase, so that it can produce gases that are formed in the durham tube.

Based on the results above on the staining of Gram bacteria A shows a red color, indicating that the yaang tumbu bacteria are Gram negative. This *Echerichia coli* bacteria is a Gram-negative bacterium, when given violet crystal staining then decolorized with alcohol will fade or disappear in gram-negative bacteria and when dripped the counter-coloring agent, namely safranin, will retain its color, which is red, this is because the cell wall of the bacteria has a different chemical structure. Gram-negative bacteria have only one layer of peptidoglycan on the cell wall bound to the outer membrane lipoprotein. Gram-negative bacteria contain teicoic acid because they only contain a small amount of peptidoglycan, so the cell wall is more susceptible to mechanical damage.

CONCLUSION

Based on the results of the ALT calculation study, it was found that 2-week dishwashing sponges were more than dishes-washed sponges used for 1 week with a sig value of 0.002. ALT calculation with a duration of use of 2 weeks obtained results Sp1 = 6.0 ×10⁷, Sp2 = 4.7×10⁶, Sp3 = 7.2×10⁷, Sp4 = 6.0 ×10⁵, Sp5 = 6.6×10⁷. While the calculation results of ALT 1 week are Sp1 = 4.6 ×10⁷, Sp2 = 1.6×10⁶, Sp3 = 4.8×10⁷, Sp4 = 5.4 ×10⁵, Sp5 = 4.5×10⁷. The characteristic of bacteria that are suspected to grow on dishwashing sponges is *Escherichia coli* bacteria

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ISOLATION AND ANTIBIOTIC SUSCEPTIBILITY OF BACTERIA FROM POTENTIAL NOSOCOMIAL SURFACE IN STIKES MITRA KELUARGA, INDONESIA

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Abstract

Introduction: Nosocomial is a common infection in hospitals, health care facilities or environments where pathogens grow. Transmission of nosocomial infections occurs due to the presence of pathogenic bacteria that attack the officers, visitors or someone with susceptible immunity. The causes of nosocomial infections are bacteria, viruses, fungi and parasites. Transmission that occurs by microorganisms has the potential to contaminate food that enters the human body. Generally, this transmission can be through contact with organisms either directly or indirectly. Antibiotic sensitivity test aims to test the sensitivity of a bacterium to antibiotics. Bacteria that are sensitive to antibiotics will show an inhibition zone with a large diameter, the larger the inhibition zone formed, the more inhibited bacterial growth will be.

Method: This study aims to isolate bacteria that have the potential to be a source of pathogens in STIKes and hospitals. This research is a qualitative descriptive research. The samples used in this study were security guards' phones, parking attendants' cellphones, women's closets, motorcycle handlebars, hospital canteen sauce bottles, hospital cafeteria windows, hospital parking attendants' keyboards, hospital parking attendants' mice and the cross button. In addition, researchers also used 2 media, namely general media or Mueller-Hillon (Oxoid, UK) and *Blood Agar* (BA).

Results: The results of this study are that from all samples found pathogenic bacteria that can hemolyze blood and from the 14 samples of which 1 sample came from Ladies Toilet1 that had the most resistance to antibiotics, as many as 4 antibiotics were gentamicin, rifampicin, tetracycline, chloramphenicol, and cefixim.

Conclusion: This study concluded that the potential for pathogenic bacteria from STIKes and hospitals could hemolyze BA media and 1 of 14 samples was resistant to 4 antibiotics.

Key words : Antibiotic, *Blood Agar*, Nosocomial, Resisten, *Staphylococcus aureus*.

INTRODUCTION

In everyday life, there are many bacteria that cause contaminations. Most people believe the high potential for bacterial contaminants only occurs in hospital and dirty environments. Infections that occur in hospitals, health care facilities or the surrounding environment where pathogenic bacteria grow are called nosocomial infections. Transmission of nosocomial infections occurs due to the presence of pathogenic bacteria that attack officers, visitors or someone with susceptible immunity. According to (Al-Ghamdi, et al., 2011), sources of pathogenic bacteria can be found in the air, water, soil, food and surrounding objects (such as mobile phones, computer equipment, motorcycle handlebars).

Nosocomial infections can be caused by Gram-positive cocci (*Staphylococcus* spp., *Micrococcus* spp.), but also spore-forming rods (*Bacillus* spp.) or Gram-negative bacteria, which can be transmitted through devices such as mobile phones or computer devices. Mobile phones, computer equipment, and motorcycle handlebars have a high potential for nosocomial infections, this is because these objects can act as cellular reservoirs for pathogenic microbes. Mobile phones, computer equipment, and motorcycle handlebars that are often used and are rarely disinfected routinely are the triggers for microbial transmission ((J, 2012); (Koroglou, et al., 2015); (Siegmund, et al., 2010)). Transmission that occurs by

microorganisms has the potential to contaminate food that enters the human body. Generally, this transmission can be through contact with organisms either directly or indirectly. In addition, droplet transmission can occur through the respiratory tract by large droplets (Ferioli, et al., 2020)

Antibiotic sensitivity test aims to test the sensitivity of a bacterium to antibiotics. Bacteria that are sensitive to antibiotics will show an inhibition zone with a large diameter, the larger the inhibition zone formed, the more inhibited bacterial growth will be. If an inhibition zone with a small diameter is found, it can be interpreted as bacteria experiencing resistance or intermediate to antibiotics (Khusuma, Safitri, Yuniarni, & Rizki, 2019)

The purpose of this study was to determine the presence or absence of pathogenic bacteria on surrounding objects, namely mobile phones, computer equipment, motorcycle handlebars, toilets, canteen sauce boxes, canteen windows and parking bars located at the hospital and STIKes Mitra Keluarga.

METHOD

This study was conducted with descriptive qualitative. This study was conducted on June 2022 at Microbiology Lab STIKES Mitra Keluarga. The sample that used in this research was 14 samples including the security and parking attendants' cellphones, toilets, motorcycle handlebars, bottle's sauce in the canteen, storefronts in the canteen, parking attendant's keyboard in school and in hospitals, mouse parking attendants on school, parking doorstop buttons. Media that used are Blood agar and Mueller-Hillon (Oxoid, UK).

*/-For isolation bacteria, it's necessary to do a swab on the surface of the object using a cotton bud that has been moistened with 0.8% NaCl and streak on the blood agar media. After that, incubation (Memmert, Germany) media at 37°C for 24 hour.

Furthermore, bacteria that grow on agar media are inoculated on Mueller-Hillon agar (Oxoid, UK) using a cottonbud that has been moistened with 0.5% Mac Farland and taking the bacterial isolates with the cotton buds. Then, streak on Mueller-Hillon (Oxoid, UK) media and place the antibiotics on the surface of the media Mueller-Hillon agar media and antibiotics are placed on the surface of the media. Antibiotics that used in this study are Ampicillin, Imipenem, Gentamicin, Chloramphenicol, Rifampicin, Amoxycillin clavulanic acid, Tetracycline, Cefixime (Oxoid, UK). Then incubated (Memmert, Germany) at 37°C for 24 hour. In this study, identification of the results was carried out by measuring the diameter of the clear zone on nutrient agar media. Interpretation result was compared with CLSI 2016 for resistance criteria.

RESULTS

Observations regarding nosocomial examinations carried out in the area of Mitra Keluarga Hospital and STIKes Bekasi in 2022 are shown in the table below:

Tabel 1 Observation Result

Sample	Diameter BA (mm)	AMP 10	IPM 10	Clear Zone (mm)					CFM 5
				C 30	AMC 30	CN 10	TE 30	RD 30	
Securty's phone	10	20(S)	45 (S)	0 (R)	29 (S)	10 (R)	10 (R)	30 (S)	0 (R)
Parking attendant phone	7	30 (S)	30 (S)	44 (S)	42 (S)	44 (S)	30 (S)	30 (S)	40 (S)
Ladies toilet 1	1	0 (R)	17 (R)	16 (I)	16 (I)	12 (R)	8 (R)	15 (-)	20 (S)
Ladies toilet 2	7	16 (I)	32 (S)	26 (S)	12 (R)	27 (S)	33 (S)	35 (S)	0 (R)
Motorcycle handlebar revo 1	6	36 (S)	50 (S)	30 (S)	38 (S)	26 (S)	30 (S)	44 (-)	8 (S)
Motorcycle handlebar revo 2	6	35 (S)	40 (S)	24 (S)	40 (S)	27 (S)	30 (S)	40 (S)	8 (S)
Sauce bottle kantin 1	8	25 (S)	52 (S)	34 (S)	34 (S)	25 (S)	30 (S)	40 (S)	26 (S)
Sauce bottle kantin 2	8	27 (S)	50 (S)	30 (S)	32 (S)	25 (S)	33 (S)	40 (S)	24 (S)
Canteen storefront right	6	23 (S)	56 (S)	23 (S)	36 (S)	26 (S)	31 (S)	58 (-)	26 (S)
Canteen storefront left	6	23 (S)	0 (R)	32 (S)	34 (S)	30 (S)	31 (S)	0 (R)	17 (I)
Hospital parking keyboard 1	11	23 (S)	50 (S)	25 (S)	34 (S)	28 (S)	30 (S)	44 (-)	20 (S)
Hospital parking keyboard 2	11	22 (S)	42 (S)	24 (S)	32 (S)	28 (S)	34 (S)	30 (-)	0 (R)
STIKES parking mouse	7	15 (S)	32 (S)	30 (S)	26 (S)	22 (S)	23 (S)	0 (R)	0 (R)
Parking cross button	5	18 (S)	47 (S)	26 (S)	23 (S)	23 (S)	20 (S)	28 (S)	0 (R)

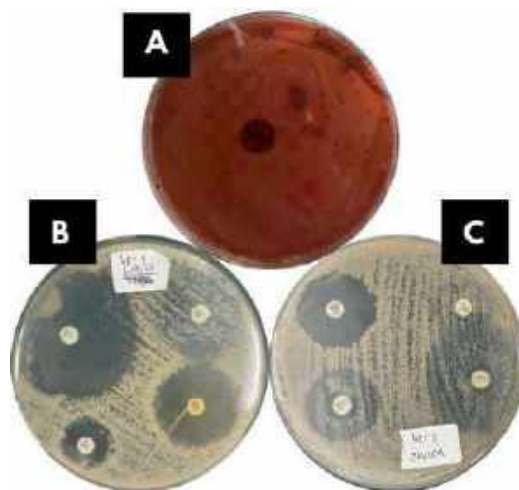


Figure 1 Ladies Toilet 1

The results of the identification of nosocomial bacteria in the Hospital area and the Mitra Keluarga STIKes area, namely Enterobacteriaceae. On BA media, from all samples there were colonies of bacteria capable of hemolyzing blood with the diameters in the table above. In the antibiotic test, of the 14 samples there was 1 sample that was resistant to ampicillin antibiotics, 2 samples were resistant to imipenem, 1 sample was resistant to chloramphenicol, 1 sample was resistant to amoxycillin clavulanic acid, 2 samples were resistant to gentamicin, 2 samples which were resistant to tetracycline, 2 samples were resistant to rifampicin and 5 samples were resistant to cefixime. The female toilet sample 1 can hemolyze BA media with a diameter of 10 mm as shown in Figure 1. In addition, this sample is resistant to 4 antibiotics.

DISCUSSION

From the results of the study on 14 samples obtained 3 samples that have resistance to antibiotics. In this study, Blood agar media and Nutrient agar media were used for bacterial growth. In the Blood agar media, 3 different samples were used by swab using a *cotton bud* on the surface of the closet, cell phone, and computer mouse. After cultured in the Blood agar media, bacteria was able to hemolyze the Blood agar media so that it was marked by the presence of a clear zone area adjacent to the growth of bacteria. Blood agar media is a differential medium used to determine which bacteria are capable of catabolizing red blood cells or known as the hemolysis process. There are 3 ways for bacteria to hemolyze Blood agar media. If bacteria can destroy red blood cells and there are areas that appear greenish near the area of bacterial growth then it is called α -hemolysis. However, if bacterial enzymes can destroy all red blood cells and there is a clean zone in the adjacent area of bacterial growth it is called β -hemolysis. Whereas γ -hemolysis occurs if there is no bacterial enzyme that can damage red blood cells, then the area adjacent to bacterial growth will remain red. In the Nutrient agar media, 3 different samples were used by swab using a *cotton bud* on the surface of the closet, cell phone, and computer mouse. In this study, cotton bud was dipped into NaCl 0.8% for moistening the cotton bud. After cultured on Blood agar media, the bacteria were tested on Nutrient agar media to test for antibiotic resistance against bacteria. Media Nutrient agar is a standard medium for the growth of various types of bacteria. This media is also a complex medium that has a high nutritional content such as meat extract, yeast extract, and protein. Some bacteria that can grow well on Nutrient agar media such as *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* (Safitri et al, 2010).

All of samples contained hemolytic bacteria. One representative hemolytic bacteria from all samples were tested for antibiotic susceptibility. In this study used 8 types of antibiotics that can be used to fight bacteria isolated from various places, the results of the 14 isolated samples there were 3 samples that showed resistance to antibiotics. The first resistant sample was obtained from a cell phone at a security officer of STIKes Mitra Keluarga which showed bacteria resistant to the antibiotic *Gentamicin* with a clear zone diameter of 10mm, *Tetracycline* 10mm, and *Cefixime* which did not show any clear zones. This is consistent with previous study which states that cell phones have antibiotic-resistant because cell phones are usually stored in bags or even placed in contaminated places. This study is in line with Debnath's study which states that the batteries from cell phone samples are usually resistant to *Tetracycline* and *Chloramphenicol* antibiotics. are usually resistant to *Tetracycline* There are 5 types of bacteria that *S. aureus*, *S. epidermis*, *Pseudomonas aureginosa*, *E. coli*, and *Salmonella typhi*. Meanwhile, bacteria that are resistant to *Chloramphenicol* are *S. aureus*, *E. coli*, and *Salmonella typhi* (Debnath, Bhowik, Islam, & Chowhury, 2019). The second resistant sample was obtained from the results of the female toilet swab number 1 which is a sanitation facility for defecation and urination. The toilet is also a very risky place for transmitting a disease, such as through faecal-oral and others, because the toilet can be used by many people. Enteropathogens or pathogens that can be found on human skin, such as *Staphylococcus aureus*, can be very easily transmitted between individuals by touching public restrooms (Maryanti & Amir, 2019). The results showed that the swab sample in the female toilet no. 1 was resistant to *Ampicillin* with no clear zone forming, *Imipenem* with a clear zone diameter of 17mm, *Gentamicin* with a clear zone diameter of 12mm, and *Tetracycline* with a clear zone of 8mm. One of the studies conducted in Nigeria by isolating bacteria from the toilet surface found the results of *Staphylococcus*, *E.coli*, *Bacillus*, and *Klebsiella* but in this study no resistance test was carried out (Sampson, Esheyigba, & Baridam, 2019).

The results of the third resistant sample were obtained from a computer device, namely in the form of a security guard mouse. The computer is a tool that is very much needed because of its many uses in helping work and being a tool that is needed in services such as processing and storing data. In caring for the computer, the thing that must be considered is cleanliness. One of them is the cleanliness of the mouse which is very rarely done, the mouse sometimes looks very clean due to the contact between the hands and the surface of the mouse so that it looks clean and does not need periodic cleaning. In the study, bacteria were resistant to *Rifampicin* and *Cefixime* with no clear zone formation. The bacteria that usually contaminate computer devices such as keyboards and mice are *Enterobacter sp.*, *Staphylococcus epidermis*, *Staphylococcus aureus*, *Escheria coli*, and *Pseudomonas sp.* According to study conducted by Olu-Taiwo in 2021 stated that all these bacteria were resistant to antibiotics but *Enterobacter sp.* have a low level of resistance (Olu-Taiwo, Laryea, Kweku Mykels, & Forson, 2021)

CONCLUSION

The results of this study indicate that from the 14 samples used, there are 3 samples that have a level of resistance to antibiotics, including the female toilet sample 1, the security officer's cellphone, and the parking attendant's mouse. The results of the study of resistance to antibiotics were mostly found in the female toilet sample 1 because it was resistant to 4 antibiotics.

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TEST OF TOTAL PLATE COUNT AND BACTERIA CHARACTERISTICS IN ENDO AGAR IN REFILLABLE DRINKING WATER AT DEPOT, CILEUNGSI DISTRICT, BOGOR

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Abstract

Introduction: Refill Drinking Water Depot (DAMIU) is a business entity that processes raw water into drinking water. The quality requirements for drinking water quality are regulated in the Minister of Health Regulation Number 492/MENKES/PER/IV/2010 based on the bacteriological requirements of drinking water, namely 0/100 ml of water and the quality standards for demineralized water or refillable drinking water which are regulated in the Indonesian National Standard (SNI 6241:2015) which is $1,0 \times 10^2$ CFU.

Method: The design of this research is descriptive. Samples were planted using the spread plate method after starified dilution. The object of the research is refill drinking water taken from refill drinking water depot along Cileungsi District, Bogor Regency with a population of 10 samples and taken using a non-probability method with purposive sampling technique. Data analysis using SPSS V.25 software with One Way ANOVA (Analysis of Variance) statistical test.

Results: The result of the One Way ANOVA statistical test obtained a p value of 0.01 (<0.05), which indicates that there is a significant difference in the amount of growth between samples of refill drinking water at depot.

Conclusion: Based on SNI 6241:2015, the results of the TPC calculation showed that 3/10 DAMIU (30%) be found the bacteriological requirements of drinking water, namely in DAM 3 of 0.5×10^2 CFU/ml, DAM 5 of 0.4×10^2 CFU/ml, and DAM 6 of 0.4×10^2 CFU/ml. Pathogenic bacteria suspected of contaminating refilled drinking water based on the characterization of Endo Agar and biochemical test media in the form of Simmon Citrate Agar, D- Glucose Broth and Lactose Broth tests are *Escherichia coli*, *Klebsiella sp*, and *Pseudomonas aeruginosa*.

Key words : Brain Heart Infusion Agar (BHI-A), Coliform, Endo Agar, *Escherichia coli*, One Way ANOVA Test, Refill drinking water, Total Plate Count Method (TPC)

INTRODUCTION

The high demand for drinking water encourages consumers to use find an alternative to found the needs of water in the body. Water Packaged Drinking (AMDK) is the first alternative to fulfill drinking water needs, for reasons that are more practical and hygienic. But over time the price of bottled water with various brands available to more expensive. Therefore, consumers turn to the second alternative, which is by consume a drinking water produced by Refill Drinking Water Depot (DAMIU). DAMIU is included in the business that processes treatment of raw water for drinking water. When compared in terms of price between AMDK and DAMIU of course refill water is cheaper. But Although the price is relatively affordable, not all drinking water depots are filled has a hygienic quality that is well maintained (Mila, Nabilah, and Puspikawati, 2020).

According to Permenkes No. 492/MENKES/PER/IV/2010 based on the bacteriological requirements of drinking water, which is not allowed to contain disease bacteria that exceeds the predetermined threshold, which is 0/100 ml of water, while the quality standards for demineralized water or refilled drinking water are regulated in the Standard National Indonesian (SNI 6241:2015) which is 1.0×10^2 CFU. According to data from the Bogor City Health Office, in 2017 bacteriological tests were carried out on 61 DAMIU. The test results from the 61 depots, 31 depots contained pathogenic bacteria that is *Escherichia coli*.

The presence of pathogenic bacteria such as *E. coli* that exceeds the threshold can cause health impacts for humans, one of which is diarrhea. As for the prevalence of diarrhea, according to the Profile of the Health Office of the City of Bogor, there has been an increase in cases over the last 2 years. In 2019 in Bogor City there were 18,492 cases of diarrhea, while cases of diarrhea that occurred in 2020 increased by

18,751. Diarrhea is a common condition that results from reduced absorption of water by the intestines or usually an increase in water secretion which is described as loose, watery stools that occur three or more times a day. Diarrhea is grouped into three categories; watery, fatty (malabsorption), and infectious. In the case of watery diarrhea is usually caused by bacteria. One of the pathogenic bacteria that attacks is *E. coli* which causes injury to the intestinal epithelium so that it is unable to absorb water and causes the feces to become watery (Valerie and Nicholas, 2017).

Based on the impacts and problems that occur in the community, a study related to counting the number was carried out to see the characteristics of pathogenic bacteria in drinking water depots. Previous research by Rahayu and Kusmawati (2018) regarding *E.coli* contamination in complementary drinking water from drinking water reservoirs in Malang City *E.coli* contamination was found in 18 of 20 samples collected from 20 samples in Malang City. Another study by Monikayani et al (2020) on Banjarmasin brand additional gallons of water and the most likely amount of additional water found that 5 out of 15 samples had the highest probability of having 2/100 ml coliform. Other researchers by Sudiana and Sudirgayasa (2020) regarding the Contamination Test of Coliform Bacteria and *E. coli* at the Refill Drinking Water Depot (DAMIU) in Tabanan District, Bali at 2 DAMIU with the 9 series tube method, both of which obtained MPN results of *E. coli* contamination of 93/100 ml.

Previous research by Mas and Pradina (2021) regarding Bacteriological Drinking Water Quality Tests in the Bukit Jimbaran Area at 3 DAMIU obtained positive results of *E. coli* indicating that the sample was not suitable for consumption. Research conducted by Atnafu et al (2021) regarding Microbial Community Structure and Diversity in Drinking Water Supply, Distribution System and Household Use Places in Addis Ababa City, Ethiopia on 38 samples collected from several different locations obtained dominant results from the genus *Pseudomonas*, *Legiolla*, *Klebsiella*, *Escherichia*, and *Actinobacteria*.

Based on previous research, researchers will conduct research to identify and calculate the number of pathogenic bacterial colonies using the Total Plate Count (TPC) method on refill drinking water in Cileungsi District, Bogor because until now there has been no Research on the content of pathogenic bacteria in refill drinking water which is sold along the road in Cileungsi District, Bogor.

METHOD

This research was conducted on February 14, 2022 to March 4, 2022 at the Bacteriology Laboratory of STIKes Mitra Keluarga. The sampling site for this research was conducted along the road in Cileungsi District, Bogor. The type of research used is descriptive research. The sampling method used is nonprobability with purposive sampling technique. In this study, bacteria were identified on endo agar media and counted the number of bacteria using the TPC method on refill drinking water.

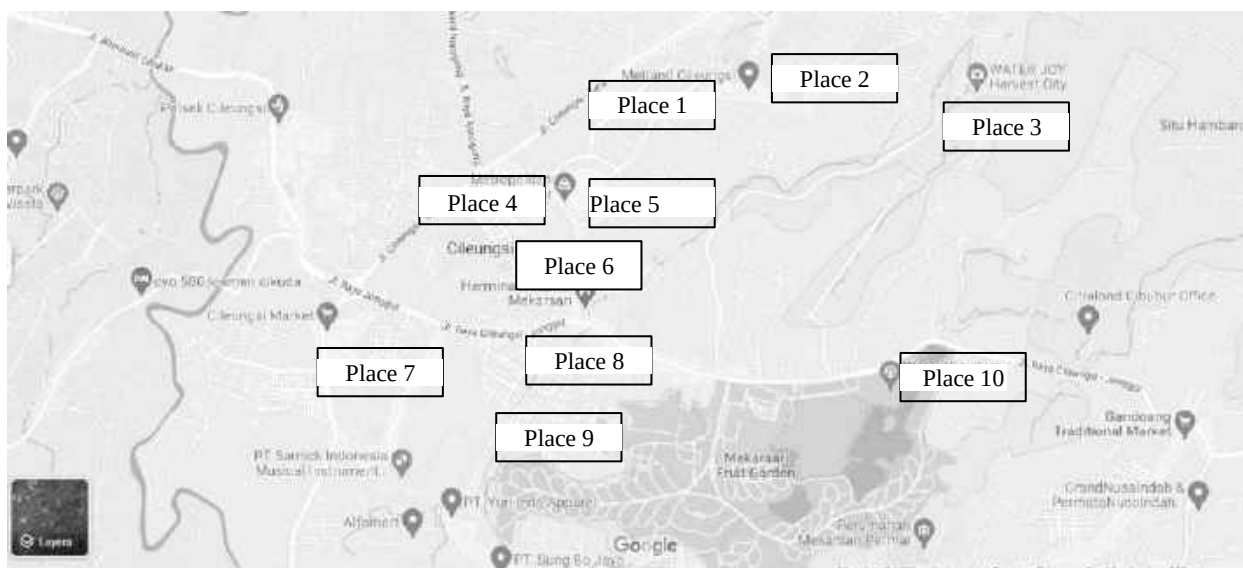


Figure 1. Locations along the road in Cileungsi District, Bogor indicate where to take refill drinking water Samples

Sterilize glass bottles to be used by wrapping them with clean paper tightly. The wrapped glass bottles were sterilized in an autoclave for 15 minutes at 121°C. The glass bottles then dry sterilized in an oven at 108°C for 2 hours (Hadijah, 2017). Eppendorf tubes were prepared as many as 3 tubes containing 900 µl NaCl in each tube. Then aseptically put the sample into the Eppendorf tube. The ratio of the sample weight to the volume of NaCl is 1:9. Then homogenize the sample and NaCl in the first dilution eppendorf tube. Pipette 100 µl from the first tube (10^{-1}) and transfer it to the second tube (10^{-2}) aseptically with the same ratio as before, which is 1:9. After that the sample was homogenized with NaCl in the second dilution eppendorf tube. The dilution was continued until the last dilution tube (10^{-3}) was followed by a similar method (Yusmaniar et al, 2017).

Planting on BHI-A Media, pipette 100 µl from each dilution tube (10^0 to 10^3) in 2 petri dishes containing BHI-A and then flatten using drugalsky, the work is carried out in the BSC. Cover the edges of the petri dish tightly using plastic wrap in a circular motion. Then incubated in an incubator at 37°C for 1 × 24 hours. After calculating with the TPC formula, then take the colonies separated from the BHI-A media using a ose needle. Then do the streak plate technique with the quadrant stroke method. Then cover the edges of the petri dish using plastic wrap until there are no air voids (Aryal, 2022).

Colonies that were separated from the Endo Agar medium were taken using a ose needle for further biochemical tests. The colonies taken were colonies that grew in metallic green, pink, and white colors. Then do the streak plate technique with the continuous stroke method. Then cover it with a tube stopper and cover it with plastic wrap. For liquid biochemical test media, enter the durham tube aseptically and make sure that no air enters (Aryal, 2022).

Colonies were growing on Endo Agar media were taken and applied to slide preparations. Drops of crystal violet dye, let stand for 5 minutes, and rinse with running water and then dry. Add 12 drops of Iodine/Lugol (mordant) solution, let stand for 45-60 seconds, then rinse with alcohol until no longer purple, then rinse with water. Add the second dye, namely Safranin, let stand for 1 minute, rinsed with running water and dry. Add immersion oil and examine under a microscope with 40x magnification and 100x objective lens (Yusmaniar et al, 2017).

RESULTS

The characteristics of bacteria growing on BHI-A media were dominated by circular, white colonies with entire margins, colonies that were opaque and markedly elevated, but flat on the entire surface (raised). Classification of colonies from BHI-A media was carried out to make it easier for each colony to be isolated on Endo Agar media. This classification is based on almost the same characteristics in each DAM sample that grows and advances to ALT calculations.

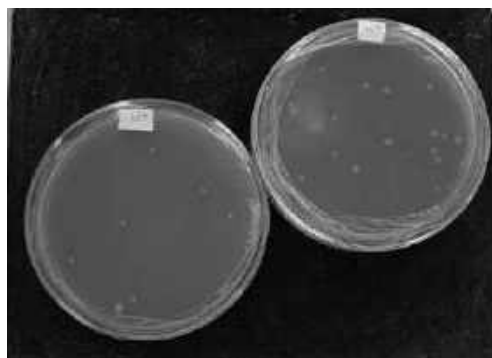


Figure 2. Colonies form of cultured bacteria from refill drinking water samples on BHI-A media

Table 1. Classification of Bacterial Colonies on BHI-A media

Kode Sampel	Karakteristik Koloni						
	Bentuk	Margin	Elevasi	Ukuran	Tekstur	Optik	Warna
A	<i>Circular</i>	<i>Entire</i>	<i>Flat</i>	<i>Large</i>	<i>Smooth</i>	<i>Opaque</i>	Putih
	<i>Circular</i>	<i>Entire</i>	<i>Flat</i>	<i>Large</i>	<i>Smooth</i>	<i>Opaque</i>	Putih
	<i>Circular</i>	<i>Entire</i>	<i>Flat</i>	<i>Moderate</i>	<i>Smooth</i>	<i>Opaque</i>	Putih
	<i>Circular</i>	<i>Entire</i>	<i>Flat</i>	<i>Moderate</i>	<i>Smooth</i>	<i>Opaque</i>	Putih
	<i>Circular</i>	<i>Entire</i>	<i>Flat</i>	<i>Small</i>	<i>Smooth</i>	<i>Opaque</i>	Putih
B	<i>Irregular</i>	<i>Curied</i>	<i>Flat</i>	<i>Large</i>	<i>Smooth</i>	<i>Opaque</i>	Putih
	<i>Irregular</i>	<i>Curied</i>	<i>Flat</i>	<i>Moderate</i>	<i>Smooth</i>	<i>Opaque</i>	Putih
	<i>Irregular</i>	<i>Curied</i>	<i>Flat</i>	<i>Moderate</i>	<i>Smooth</i>	<i>Opaque</i>	Putih
	<i>Irregular</i>	<i>Curied</i>	<i>Flat</i>	<i>Moderate</i>	<i>Smooth</i>	<i>Opaque</i>	Putih
	<i>Irregular</i>	<i>Curied</i>	<i>Flat</i>	<i>Small</i>	<i>Smooth</i>	<i>Opaque</i>	Putih
C	<i>Irregular</i>	<i>Undulate</i>	<i>Flat</i>	<i>Large</i>	<i>Smooth</i>	<i>Clear</i>	Putih
	<i>Irregular</i>	<i>Undulate</i>	<i>Flat</i>	<i>Large</i>	<i>Smooth</i>	<i>Clear</i>	Putih
	<i>Irregular</i>	<i>Undulate</i>	<i>Flat</i>	<i>Large</i>	<i>Smooth</i>	<i>Clear</i>	Putih
	<i>Irregular</i>	<i>Undulate</i>	<i>Flat</i>	<i>Moderate</i>	<i>Smooth</i>	<i>Clear</i>	Putih
	<i>Irregular</i>	<i>Undulate</i>	<i>Flat</i>	<i>Small</i>	<i>Smooth</i>	<i>Clear</i>	Putih
D	<i>Circular</i>	<i>Entire</i>	<i>Flat</i>	<i>Large</i>	<i>Smooth</i>	<i>Clear</i>	Putih
	<i>Circular</i>	<i>Entire</i>	<i>Flat</i>	<i>Large</i>	<i>Smooth</i>	<i>Clear</i>	Putih
	<i>Circular</i>	<i>Entire</i>	<i>Flat</i>	<i>Large</i>	<i>Smooth</i>	<i>Clear</i>	Putih
	<i>Circular</i>	<i>Entire</i>	<i>Flat</i>	<i>Moderate</i>	<i>Smooth</i>	<i>Clear</i>	Putih
	<i>Circular</i>	<i>Entire</i>	<i>Flat</i>	<i>Small</i>	<i>Smooth</i>	<i>Clear</i>	Putih

Classification of colonies from BHI-A media was carried out to make it easier for each colony to be isolated on Endo Agar media. This classification is based on almost the same characteristics in each DAM sample that grows and advances to ALT calculations.

Table 2. Calculation of the number of bacterial colonies using TPC method

Sampel Code	Bacteriological Quality of Drinking Water TPC (CFU/ml)	
	Result	Criteria
DAM 1	$5,5 \times 10^2$	TMS
DAM 2	$1,3 \times 10^2$	TMS
DAM 3	$0,5 \times 10^2$	MS
DAM 4	$1,2 \times 10^2$	TMS
DAM 5	$0,4 \times 10^2$	MS
DAM 6	$0,4 \times 10^2$	MS
DAM 7	$0,9 \times 10^1$	TMS
DAM 8	$1,05 \times 10^2$	TMS
DAM 9	$1,1 \times 10^2$	TMS
DAM 10	2×10^1	TMS
MS : Memenuhi Syarat		
TMS : Tidak Memenuhi Syarat		

Table 1 can be explained that MS (Qualify) and TMS (Not Qualify) of the 10 DAMIU in the Cileungsi sub-district, Bogor, all of which did not meet the bacteriological requirements in accordance with Permenkes No. 492/MENKES/PER/IV/2010 based on the bacteriological requirements of drinking

water, which is not allowed to contain bacteria that exceed the predetermined threshold, which is 0/100 ml of water. According to SNI 6241:2015 regarding demineralized water obtained through the process of distillation, deionization, reverse osmosis, and purification by other processes equivalent to or without the addition of oxygen (O₂) or carbon dioxide (CO₂), the bacteriological requirement is 1.0×10^2 , then DAMIUs that meet the standards are DAM 3 of 0.5×10^2 , DAM 5 of 0.4×10^2 , and DAM 6 of 0.4×10^2 .

Table 3. One Way ANOVA test

ANOVA					
ALT (CFU/ml)	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	151363182000.000	9	16818131333.333	4.589	.013
Within Groups	36651340000.000	10	3665134000.000		
Total	188014522000.000	19			

The result of the One Way ANOVA statistical test obtained a p value of 0.01 (<0.05), which indicates that there is a significant difference in the amount of growth between samples of refill drinking water at depot.

Table 4. Bacterial colonies on Endo Agar media

Sample Code	Color	Result
A	Metallic green	<i>Escherichia coli</i>
B	Pink	Koliform
C	Pink	Koliform
D	Colorless	Non-koliform

Table 4 shows the coliform group is able to produce acid and gas from lactose fermentation. On Endo Agar, coliforms appear as red colonies with a metallic green luster. This unique color is caused by the production of aldehydes from lactose fermentation, the aldehydes can liberate fuchsin from the colorless Schiff reagent (fuchsin-sodium sulfite) making colonies appear red. In the case of *E. coli*, the reaction is so strong that fuchsin crystallizes giving colonies a metallic green sheen. Selective agents contained in the media, sodium desoxycholate and sodium lauryl sulfate help to inhibit non-coliform (Indrayati and Akma, 2018).

Table 5. Bacterial colonies on biochemical media

No	Media	Result			
		A	B	C	D
1	Simmon Citrate	—	+	+	+
2	Glucose	+	+	+	+
3	Lactose	+	+	+	+

Table 5 shows biochemical tests are used to determine whether refill drinking water samples are contaminated with pathogenic bacteria, especially *Escherichia coli* or not. In the biochemical reaction, there are 2 media used, namely Simmon Citrate Agar and Sugar-sugar media. In the media the sugars used are glucose and lactose.

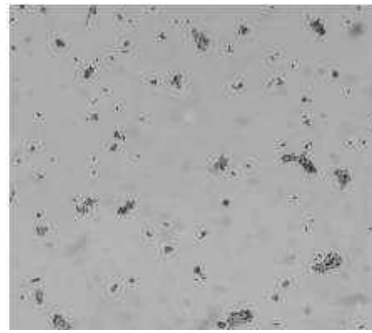


Figure 3. Gram stain test result

Colonies suspected of *Escherichia coli* were inoculated using ose with the characteristics of large colonies, convex elevation, smooth, pink in color with a metallic green luster (Indrayati and Akma, 2018).

DISCUSSION

Based on macroscopic observations from BHI-A media, it can be explained that of the 10 DAMIU in the Cileungsi sub-district, Bogor, all of which did not meet the bacteriological requirements in accordance with Permenkes No. 492/MENKES/PER/IV/2010 based on the bacteriological requirements of drinking water, which is not allowed to contain bacteria that exceed the predetermined threshold, which is 0/100 ml of water. According to SNI 6241:2015 concerning demineralized water obtained through the process of distillation, deionization, reverse osmosis, and purification by other processes equivalent to or without the addition of oxygen (O₂) or carbon dioxide (CO₂), the bacteriological requirement is 1.0×10^2 , then DAMIUs that meet the standards are DAM 3 of 0.5×10^2 , DAM 5 of 0.4×10^2 , and DAM 6 of 0.4×10^2 .

Statistical data testing was carried out with the One Way Anova test. The results of the statistical test showed that there was a difference in the average number of TPC calculation results on the 10 DAMIU used as samples with a One Way ANOVA test significance of 0.01. If the probability ($\text{Sig} < \alpha$) then H_0 is rejected and H_a is accepted which indicates the difference between each depot. The result of the One Way ANOVA statistical test obtained a p value of 0.01 (< 0.05), which indicates that there is a significant difference in the amount of growth between samples of refill drinking water at depot.

Characteristics of bacterial colonies based on morphology in BHI-A media in refill drinking water samples. Sample codes were ordered from DAM 1 to DAM 10. Bacterial colonies were then classified according to dominant characteristics. The characteristics of bacteria growing on BHI-A media were dominated by circular, white colonies with entire margins, colonies that were opaque and markedly elevated, but flat on the entire surface (raised). Classification of colonies from BHI-A media was carried out to make it easier for each colony to be isolated on Endo Agar media. This classification is based on almost the same characteristics in each DAM sample that grows and advances to the TPC calculation and then is coded for samples from A to D according to almost similar characteristics.

According to (Indrayati and Akma, 2018) the coliform group is able to produce acid and gas from lactose fermentation. On Endo Agar, coliforms appear as red colonies with a metallic green luster. This unique color is caused by the production of aldehydes from lactose fermentation, the aldehydes can liberate fuchsin from the colorless Schiff reagent (fuchsin-sodium sulfite) making colonies appear red. In the case of *E. coli*, the reaction is so strong that fuchsin crystallizes giving colonies a metallic green sheen. Selective agents contained in the media, sodium desoxycholate and sodium lauryl sulfate help inhibit non- coliform.

This study also identified using biochemical test are used to determine whether refill drinking water samples are contaminated with pathogenic bacteria, especially *Escherichia coli* or not. In the biochemical reaction, there are 2 media used, there are Simmon Citrate Agar and Sugars media. In the media the sugars used are glucose and lactose. Based on table 5, the results are (A) negative, (B) positive, (C) positive, and (D) positive. According to (Dewi et al, 2021) Simmon Citrate Agar is used to test the ability of organisms to utilize citrate as an energy source. Ammonium Dihydrogen Phosphate is the only nitrogen source. Dipotassium Phosphate acts as a buffer. Sodium Chloride maintains the osmotic balance of the medium. Sodium Citrate is the only carbon source in this medium. Magnesium Sulfate is a cofactor for various

metabolic reactions. Bacteria that can grow on this medium produce citrate permease enzymes that are able to convert citrate into pyruvate. Pyruvate can then enter the metabolic cycle of the organism for energy production. When bacteria metabolize citrate, the ammonium salt is broken down into ammonia, which increases alkalinity. The shift in pH changes the bromthymol blue indicator in the medium from green to blue above pH 7.6.

D-Glucose Broth media is a differential media. Tested an organism's ability to ferment glucose as well as its ability to convert the end product of glycolysis, pyruvic acid into a gaseous by-product. This is the test commonly used when identifying Gram-negative bacteria, all of which are glucose fermenters but only a few that produce gas. If an organism is able to ferment glucose, an acidic byproduct is formed and the pH indicator changes color. The end product of glycolysis is pyruvate, an organism capable of converting pyruvate to formic acid and formic acid to H₂ (g) and CO₂ (g), through the action of the enzyme hydrogen lyase formate. This gas is trapped in the Durham tube and appears as a bubble at the top of the tube. *Escherichia coli* can ferment glucose and produce gas.

Lactose broth gives excellent results for gas production at 45°C, which is characteristic of *Escherichia coli*. When preparing this medium, it is important to avoid overheating and distribute it into tubes prior to sterilization. Peptone and HM Peptone B in the medium are a source of nitrogen and carbon compounds, long chain amino acids, and other essential nutrients to organisms. Lactose is a fermentable carbohydrate for coliforms. Members of the coliform group are defined as gram-negative aerobic and facultative anaerobes, which ferment lactose with gas formation.

Gram stain of *Escherichia coli* bacteria showed that bacteria with short trunks and red color after the staining process. This matter proved that *Escherichia coli* bacteria are Gram negative bacteria. According to Afrianti Rahayu and Muhammad Hidayat Gumilar (2017) this is due to the concentration of lipids and the thickness of the peptidoglycan layer on the bacterial cell wall. In Gram-negative cells, alcohol increases the porosity of the cell wall by dissolving the lipids of the outer layer. Thus, the first dye i.e. Crystal Violet can be more easily removed from the peptidoglycan layer which is not tightly bound. Therefore, the leaching effect of alcohol results in the release of the unattached Crystal Violet complex, which renders the cells discolored or colorless. Only Gram-negative bacteria lose color, so their cells absorb Safranin as a red counter-dye.

CONCLUSION

Based on the results of the study, it can be concluded that the refill drinking water sold by the Refill Drinking Water Depot (DAMIU) in Cileungsi District is not in accordance with the Regulation of the Minister of Health Number 492/MENKES/PER/IV/2010 concerning Drinking Water Quality Requirements because it exceeds the threshold limit set by the Ministry of Health. has been set at 0/100ml. However, according to SNI 6241:2015 regarding the bacteriological requirements of demineralized water of 1.0×10^2 , the DAMIU that meets the standards are DAM 3 of 0.5×10^2 , DAM 5 of 0.4×10^2 , and DAM 6 of 0.4×10^2 . Pathogenic bacteria suspected of contaminating refilled drinking water are *Escherichia coli*, *Klebsiella sp*, and *Pseudomonas aeruginosa*. Allegedly due to contamination when refilling drinking water into gallons or containers used.

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RESISTANT TO EIGHT DIFFERENT ANTIBIOTICS BACTERIA WAS ISOLATED FROM NON-UTI FEMALE

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Abstract

Introduction : Urine is a liquid excreted by the kidneys which is then excreted through the urinary tract in the process of urination. In the urine there are various kinds of microorganisms that can cause various diseases, such as UTI. UTI can be caused by various bacteria including *Escherichia coli*, *Klebsiella sp*, *Proteus sp*, *Citrobacter*, *Pseudomonas aeruginosa*, and *Staphylococcus saprophyticus*. However, *Enterobacteriaceae* (including *Escherichia coli*) and *Enterococcus faecalis* are common causative bacteria, accounting for 95% of all bacteria that cause UTI. The purpose of this study was to count the number of bacteria that grew on NA and MCA media, and to determine the resistance of one of these bacteria.

Method : The method used in this study with descriptive qualitative. This study was carried out in May 2022 at the Bacteriology Laboratory of STIKes Mitra Keluarga, Bekasi City. Urine samples used in this study found 15 people. The urine used comes from midstream urine. Urine samples taken were immediately examined macroscopically.

Result : The results of this study showed that in the urine sample there were bacteria that grew on 13 NA media and 2 MCA media out of 15 total.

Conclusion : After doing research on bacteria, it was found that the antibiotics used in the study could not inhibit the growth of bacteria, because bacteria produce enzymes that can damage or inactivate antibiotics, genetic mutations of bacteria, changing proteins and the binding sites of antibiotics, and bacteria change their metabolism, so they are not affected by antibiotics.

Key word : Antibiotic, *Escherichia coli*, Urinary Tract Infections (UTI), Nutrient Agar, Mac Conkey Agar

INTRODUCTION

Urine is a fluid that is excreted by the kidneys and then excreted through the urinary tract in the process of urination. In the urine, there are various kinds of microorganisms that can cause disease, such as urinary tract infections (UTIs). According to the Indonesian Ministry of Health (2014), that in Indonesia, the prevalence of UTI is quite high, reaching 180,000 patients/year. Urinary tract infection is an infection caused by the growth of bacteria in human urine (Sari, 2018). Urinary tract infections can affect various types of patient age, but women experience UTI more than men, this happens because women have a shorter urethra than men (Purnomo., 2003). Urinary tract infections can be caused by various kinds of bacteria including *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella sp*, and *Proteus sp*.. However, *Enterobacteriaceae* (including *Escherichia coli*) and *Enterococcus faecalis* are common causative bacteria accounting for 95% of all bacteria causing UTIs (Sumolang et al., 2013). According to Sorlozano et al (2014), *Escherichia coli* from UTIs showed high susceptibility to imipenem, amikacin, fosfomycin and nitrofurantoin, with annual resistance rates < 5%, whereas resistance to cefuroxime, ciprofloxacin, and cotrimoxazole, key antibiotics in the treatment of community acquired UTIs, was recorded in > 20%-30% of isolates in most years.

Escherichia coli is an opportunistic normal flora in the digestive tract, these bacteria can be beneficial if the number of bacteria is normal, but if there is an increase in the number of bacteria, the bacteria become pathogens. These bacteria have virulence factors that can increase the colonization and invasion of bacteria into the urinary tract to cause infection (Harvey, 2007). The growth of bacteria can be prevented or inhibited by using antibiotics. Antibiotics are compounds produced by microorganisms such as bacteria, fungi, and *ancinomycetes* that suppress the growth of other microorganisms (Arivo, 2019). Excessive use of antibiotics in cases of UTIs causes the bacteria to become resistant. Prabowo (2012) stated that *E. coli* isolated from the urine of UTIs patients could be resistant to the antibiotics *ciprofloxacin*, *gentamicin*, *trimethoprim*, *amoxicillin* and *cefixime*.

Diagnosing a urinary tract infection can be performed by isolating bacteria in a urine culture. This method is the standard standard for determining bacteria in urine to determine the presence of infection in the urinary tract. Person has an infection in the urinary tract if pathogenic bacteria are found as much as 10^5 /mL middle portion of urine (Samirah, et al, 2006). The purpose of this study was to count the number of bacteria that grew on Nutrient Agar (NA) and Mac Conkey Agar (MCA) media, and to determine the resistance of one of these bacteria.

METHOD

The method used in this study with descriptive qualitative. This study was carried out in May 2022 at the Bacteriology Laboratory of STIKes Mitra Keluarga, Bekasi City. Urine samples used in this study found 15 people. The urine used comes from midstream urine. Urine samples taken were immediately examined macroscopically. urine was centrifuged (PLC-03 gemmy, taiwan) at 3000 rpm for 15 minutes, then the supernatant was discarded and some of the sediment was taken and dripped onto a slide and covered with a lid followed by microscopic observations. Then the urine was examined for urine culture on McConkey Agar (MCA) (Merck,Germany) and Nutrient Agar (NA) (Himedia,India) media. Bacterial isolates were purified using the scratch method and then incubated (Memmert,Gemany) at 37°C for 24 hours.

RESULTS

Based on the results of observations of urinary tract infection that have been carried out by medical laboratory technology students class 2020 STIKes Mitra Keluarga the following are obtained :

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Macroscopic					Microscopic (Bacteria, Epithelium, Leukocytes, Erythrocytes, Fungi, etc.)	Number of bacteria on NA . media	Different number of colonies in MCA
Name	Age	Gender	Colour	Clarity			
Sample 1	19	Female	Yellow	Clear	Epithelial Cell	33 x 10 ² CFU/mL	Not Found
Sample 2	18	Female	Yellow	Clear	Epithelial Cell	1 x 10 ² CFU/ mL	Not Found
Sample 3	19	Male	Yellow	Clear	Epithelial Cell, Crystal	2 x 10 ² CFU/ mL	Not Found
Sample 4	19	Female	Yellow	Clear	Epithelial Cell, Bactery, Leukocytes 1-2/ Fv	2 x 10 ² CFU/ mL	1 colony
Sample 5	21	Female	Yellow	Clear	Epithelial Cell, Leukocytes 1-2/ Fv	4 x 10 ² CFU/ mL	Not Found
Sample 6	21	Female	Yellow	Clear	Epithelial Cell	6 x 10 ² CFU/ mL	Not Found
Sample 7	19	Female	Yellow	Clear	Epithelial Cell, Bactery, Erythrocytes	49 x 10 ² CFU/ mL	Not Found
Sample 8	19	Male	Yellow	Clear	Epithelial Cell, Leukocytes 1-2/ Fv	Not Found	Not Found
Sample 9	19	Female	Yellow	Cloudy	Epithelial Cell	1 x 10 ² CFU/ mL	Not Found
Sample 10	20	Female	Yellow	Clear	Epithelial Cell, Leukocytes 1-2/ Fv, Erythrocytes 0-1/ Fv	Not Found	Not Found
Sample 11	19	Female	Yellow	Cloudy	Epithelial Cell	9 x 10 ² CFU/ mL	Not Found
Sample 12	19	Female	Yellow	Cloudy	Epithelial Cell, Leukocytes 1-2/ Fv, Erythrocytes 1-2/Fv	5 x 10 ² CFU/ mL	2 colonies (Clear dan white)
Sample 13	20	Female	Yellow	Cloudy	Epithelial Cell, Crystal (Gout)	5 x 10 ² CFU/ mL	Not Found
Sample 14	19	Female	Yellow	Clear	Epithelial Cell, Bactery, Leukocytes 0-1/Fv	5 x 10 ² CFU/ mL	Not Found
Sample 15	31	Male	Yellow	Clear	Fiber	2 x 10 ² CFU/ mL	Not Found

*Fv: Field of View

The results of the macroscopic examination of the urine showed that the urine was clear yellow in color and some of them were slightly cloudy in samples 9, 11, 12, and 13. Meanwhile, microscopic examination of the urine found some cells, such as epithelial cells, leukocytes in 1-2/Fv and Erythrocytes in 0-1 and 1-2/Fv. in sample no. 15 found the presence of fiber. Bacterial isolation on NA showed 2 samples that did not find any bacterial colonies on the NA media, namely samples 8 and 10. Then on Mac Conkey Agar (MCA) media only 2 clear and white colonies were found in sample 12 and 1 colony in sample 4.

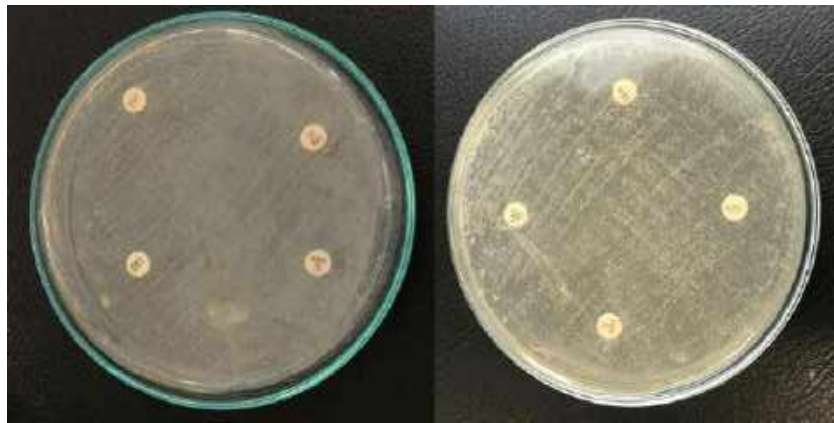


Figure. 1 samples of bacteria resistant to 8 antibiotics include Ampicillin, Imipenem, Gentamicin, Chloramphenicol, Rifampicin, Amoxycillin Clavulanic acid, Tetracycline, Cefixime. The inhibition zone wasn't shown.

DISCUSSION

Urine is the result of blood filtration by the kidneys which is then excreted by the body through the urinary tract, which is one part of the urinary system. Urine is excreted to get rid of metabolic waste, such as toxins from the body. Urinary tract infection is infections that occur along the urinary tract, including the kidneys itself due to the proliferation of microorganisms. Urinary tract infection often occurs in women, one of the causes is the female urethra is shorter so that bacteria contaminants are easier to pass through to the bladder (Mukarramah, 2018).

Routine urine examinations carried out include macroscopic examination (color and cloudiness), microscopic examination, and urine culture. Of the 15 urine samples, 4 urine samples were found to be slightly cloudy yellow, but this is still in the normal category and cannot cause urogenital infections, one of which is Urinary Tract Infection (UTI). According to Timothy (2020) turbidity in the urine is usually caused by the presence of particles in the urine such as increased blood pressure, the amount of protein, glucose, and uric acid can cause cloudiness in the urine caused by disruption of the glomerular filtration process so that many particles which escapes and passes into the urine.

The assessment of bacteriuria in this study was based on microscopic findings of bacteria and urine culture. The results of the examination of the urine sediment showed that 3 of the 15 samples were positive for bacteria. According to the central TQA (2015) in Sulistiani et al (2021), the results of urine sediment examination do not necessarily indicate a urinary tract infection. Because the presence of bacteria in the urine is usually due to storage of urine for too long or physiological bacteria.

Urine culture examination is one of the examinations to confirm the presence of Urinary Tract Infection (UTI). Urine culture is the culture of bacteria from urine material. Of all samples that have been tested for culture on *Nutrient Agar* (NA) media, bacterial counts were carried out and the highest results were found in Sample No.7 which was found to be 49×10^2 CFU/mL. The standard limit for a positive urine culture for UTI is $>100,000$ CFU/mL (Kwon *et al*, 2012). However, there were also samples that did not grow on the media, this was due to the skills of the researchers who did not master the technique when etching on the media. According to Rahmawati (2021) NA media is used because it contains high nutrients such as carbohydrates and proteins found in meat extracts and peptone which are needed by bacteria to grow and develop.

The bacteria that most often causes Urinary Tract Infection (UTI) is *Escherichia coli*, which is as much as 60-90%, *E. coli* is a normal flora in the vagina and rectum. Other bacteria that cause UTI are the most common, such as *Enterococcus spp.*, *Klebsiella-Enterobacter spp.*, *Proteus spp.*, and *Pseudomonas sp.*, in addition, group B *Streptococcus*, *Neisseria gonorrhoeae*

and *Chlamydia sp.* are found which are transmitted through sexual contact (Carreno, 2002). In a study by Giancarlo (2007) and Normaliska, et al. (2019), it was stated that *E.coli* are bacteria that are resistant to amoxicillin, tetracycline, chloramphenicol, and ampicillin.

In this study, we found urine bacteria from non-urinary tract infections female that was grown in MCA medium. Interestingly, this bacteria was resistant to eight different antibiotics including ampicillin, imipenem, gentamicin, chloramphenicol, rifampicin, amoxicillin, tetracycline, and cefixime. Resistance can occur because: 1) bacteria produce enzymes that can damage or inactivate antibiotics, 2) genetic mutations of bacteria, changing proteins and the binding sites of antibiotics, 3) bacteria changing their metabolism, so they are not affected by antibiotics, 4) bacteria change the permeability of cell membranes, making it difficult for antibiotics to penetrate. The results of the sensitivity of bacteria to antibiotics are determined by the diameter of the clear zone formed, the larger the diameter of the clear zone formed, the more sensitive the bacteria to an antibiotic (Parut, 2015). In study by Tangdogdu (2016) antibiotics commonly recommended for treatment of UTI's include co-trimoxazole (trimethoprim/sulfamethoxazole), nitrofurantoin, ciprofloxacin and ampicillin.

CONCLUSION

Based on the results of the eight antibiotic resistance test against one of the non-UTI women's urine, it can be concluded that the urine sample contained bacteria that were resistant to the eight antibiotics tested so that antibiotics could not inhibit bacterial growth on NA media.

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DIFFERENCES IN THE NUMBER AND CHARACTERISTICS OF BACTERIA IN ENDO AGAR MEDIA ON COCONUT DRINK USING ICE CUBES AND NOT IN KLAPANUNGGAL, BOGOR

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Abstract

Introduction: Coconut ice is a traditional processed drink that is widely sold on the roadside and is in great demand by consumers because of its affordable price and easy to find raw materials. Because the price is relatively affordable, many sellers do not pay attention to the cleanliness of their merchandise, there by allowing contamination of *Escherichia coli* bacteria which can cause diarrhea. The water used in making ice cubes must meet the drinking water quality requirements as stipulated in the Regulation of the Minister of Health of the Republic of Indonesia Number 492/MENKES/PER/IV/2010 concerning the requirements for drinking water quality based on microbiological parameters, namely 0 CFU/100 ml of water.

Method: The design of this research is descriptive. Samples were planted using the spread plate method after stratified dilution. The object of the research is coconut drink taken from young coconut ice sellers along Jalan Klapanunggal, Bogor Regency with a population of 10 samples and taken using a non-probability method with purposive sampling technique as many as 5 samples with 2 types of groups. Group 1 did not use ice cubes and group2 used ice cubes. Data analysis using SPSS V.25 software with Wilcoxon statistical test.

Results: The results of the Wilcoxon statistical test obtained a p value of 0.0025 (<0.05), which indicates that there is a significant difference in the amount of growth between samples of coconut drinks that do not use ice cubes and those who use ice cubes

Conclusion: Based on ALT calculations on samples that did not use ice cubes, the results obtained were AKM 1 (0.7×10^6 CFU/ml), AKM 2 (5.6×10^3 CFU/ml), AKM 3 (2.4×10^4 CFU/ml), AKM 4 (2.7×10^5 CFU/ml), AKM 5 (3.2×10^5 CFU/ml). As for the samples using ice cubes, the results obtained were AKM 1 (1.0×10^7 CFU/ml), AKM 2 (14.2×10^3 CFU/ml), AKM 3 (1.8×10^8 CFU/ml), AKM 4 (6.8×10^7 CFU/ml), AKM 5 (2.2×10^6 CFU/ml). Based on the Wilcoxon test, it was found that 0.0025 (<0.05: Ho rejected) concluded that there was a significant difference in the amount of growth between samples of coconut drink that did not use ice cubes and those who used ice cubes. The bacteria found from the endo agar medium were *Escherichia coli*, *Klebsiella* sp., and non-coliform bacteria.

Key words : Brain Heart Infusion Agar (BHIA), Coconut Drink, Coliform, Endo Agar, *Escherichia coli*, Ice Cubes, Klapanunggal, Total Plate Count Method (TPC)

INTRODUCTION

Coconut ice is a drink that is very popular with the people of Indonesia. Based on the results of a survey on the amount of daily drink consumption in the city of Bogor, the most selected category of adolescent respondents was coconut ice with a percentage (54%) (Rahayu *et al.*, 2019). The serving of coconut drinks is generally added with ice cubes. Ice cubes in the drink consumed must be hygienic, but currently the level of cleanliness of the water used in making ice cubes shows surprising results. There are ice cubes made from potentially polluted Ciliwung River water which is then given a bleaching agent so that it changes the color of the water from cloudy to clearer. This is known because the main ingredient for making ice cubes comes from water, which indicates the presence of pathogenic bacteria in the water (Elfidasari *et al.*, 2011).

Based on data from the monitoring results of microbiological parameters from the Regional Environmental Management Performance Information Document (DIKPLDH) of DKI Jakarta Province in 2019, high results were obtained, namely exceeding the quality standard value of 5000 jml/100 ml for both total coliforms. and faecal coliform parameters. High microbiological parameters can indicate the presence

of pollution in the Ciliwung River originating from domestic waste (Anonim, 2019).

The water used in making ice cubes must meet the drinking water quality requirements as stipulated in the Regulation of the Minister of Health of the Republic of Indonesia Number 492/MENKES/PER/IV/2010 concerning the requirements for drinking water quality which includes microbiological, physical, chemical and radioactive parameters contained in mandatory and additional parameters. Mandatory parameters are parameters that must be checked periodically, while additional parameters will be checked if geohydrological conditions indicate pollution. Microbiological parameters that must be examined include total coliform with colony forming units in 100 ml of water sample with a maximum limit of total coliform and *E. coli* of 0 cfu/100 ml (Kementerian kesehatan republik Indonesia, 2010).

The presence of pathogenic bacteria is caused by hygiene factors and lack of knowledge. The number of traders 46 people and only 1 person who participated in food and beverage hygiene and sanitation training. The application of this low hygiene can affect the presence of pathogenic bacteria in ice cubes (Rifta et al., 2016). In this study it was found that the main factor for the presence of pathogenic bacteria was poor hygiene, both the tools used and the environment for the coconut ice making process. (Setiawan et al., 2019).

In a study conducted by Sousa et al (2021), it was found that from 5 samples of coconut water sold in the City of Fortaleza, Brazil, 1 positive sample contained *E. coli* and 4 other samples were negative for *E. coli* or in accordance with the requirements according to the law. In another study in North Banjarmasin conducted by Hidriya et al (2021) from 9 samples, 6 samples (67%) were positive for *E. coli* and 3 samples (34%) were negative for *E. coli*.

Previous studies only identified pathogenic bacteria in ice cubes. Therefore, researchers will conduct research to identify and calculate the number of pathogenic bacterial colonies using the Total Plate Number (ALT) method on coconut ice that does not use ice cubes and uses ice cubes that are sold along the road in Klapanunggal District, Bogor because until now there has been no Research on the content of pathogenic bacteria in ice cubes used in making coconut ice which is sold along the road in Klapanunggal District, Bogor.

METHOD

This research was conducted on February 14, 2022 to March 4, 2022 at the Bacteriology Laboratory of STIKes Mitra Keluarga. The sampling site for this research was conducted along the road in Klapanunggal District, Bogor. The type of research used is descriptive research. The sampling method used is non-probability with purposive sampling technique. In this study, bacteria were identified on endo agar media and counted the number of bacteria using the ALT method on young coconut water samples using ice cubes and not using ice cubes.

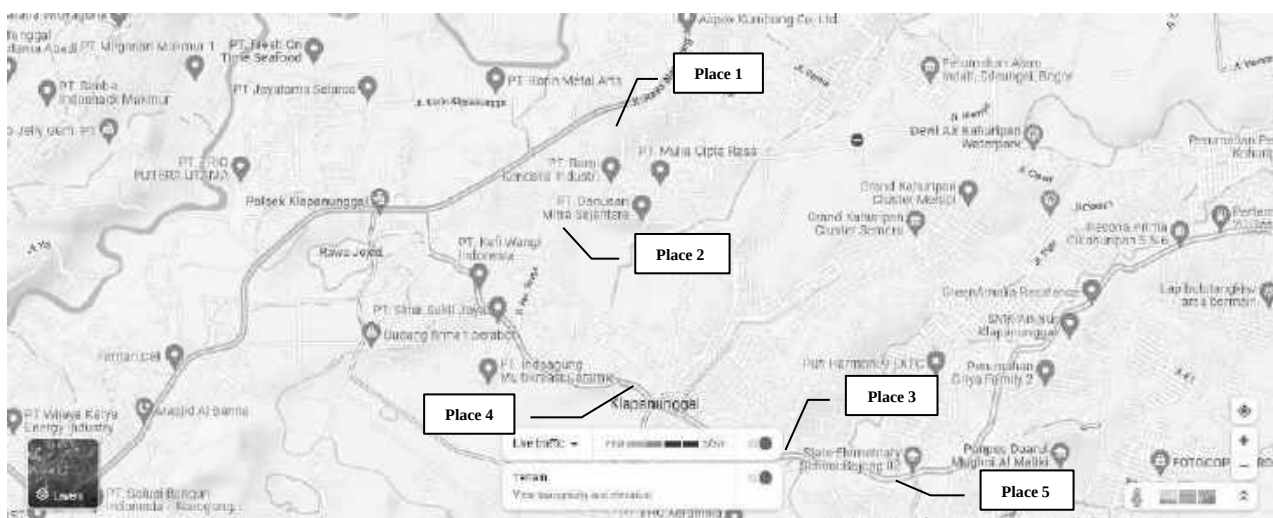


Figure 1. Locations along the road in Klapanunggal, Bogor indicate where to take coconut water samples

Prepare 5 dilution tubes containing 900 l of 0.8% NaCl in each tube. Samples using ice cubes and not using ice cubes were inserted into the first dilution eppendorf tube ($1/10$ or 10^{-1}) aseptically. The ratio of the weight of the sample to the volume of 0.8% NaCl is 1:9. After that the sample and 0.8% NaCl were homogenized in the first dilution eppendorf tube. 100 was taken from tube 10^{-1} with a micropipette and then transferred to the second tube (10^{-2}) aseptically. After that, the sample and 0.8% NaCl were homogenized in the second dilution test tube. Transfer is continued to the last dilution tube (10^{-5}) in the same manner. The tips used need to be changed at each level of dilution unless they are used from the same source (Yusmaniar *et al.*, 2017).

Take 100 in the last three dilutions and put the sample into sterile BHIA media using the spread plate technique by pouring the sample on the surface of the BHIA media and leveling it using drugalsky. Then cover with plastic wrap. Incubate the media in an incubator upside down at 37°C for 24 hours. After the incubation process is complete, observe and count the growing bacterial colonies using a colony counter (Sholehah, 2019). Count the total plate number on a dish containing 30 – 300 colonies and record the results.

After counting the colonies with the ALT formula, then take separate colonies and different colonies from BHIA media using a ose needle. Next, do the streak plate technique with the quadrant scratch method and cover the petri dish with plastic wrap. After that, incubation at 37°C for 24 hours. Endo Agar culture was taken using an ose needle, then planted into biochemical media. Cover with a tube cover and cover with plastic wrap. After that, incubation at 37°C for 24 hours (Saridewi *et al.*, 2017).

Then do the gram staining by taking the bacterial colonies separated from the Endo Agar media and placing the colonies on a sterile glass object and fixation. After that, drop the crystal violet and wait for 5 minutes. Then wash with running water. Next, drop Lugol's iodine, then wait 45-60 seconds. Then wash with running water. Then add 95% alcohol drop by drop until the purple color does not flow. Then wash with running water. Then drop fuchsin and wait for 1-2 minutes. Then wash with running water. After that, dry the preparation. The final step is to observe under a microscope with a magnification of $100\times$ (Yusmaniar *et al.*, 2017).

RESULTS

Coconut water samples using ice cubes obtained the growth of bacterial colonies that have macroscopic characteristics such as bacterial colonies on BHIA media in circular shape, moderate in size, yellow in color with entire margins, colonies impermeable to light (opaque) and flat on the entire surface (raised). While the samples that did not use ice cubes obtained characteristics such as bacterial colonies in circular shape, moderate in size, milky white in color with entire margins, opaque colonies and flat on the entire surface (raised).

According to research by Rachmawati *et al* (2016), the results of *Streptococcus mutans* colonies on BHIA media showed that the bacterial colonies were round, whitish yellow in color, the colonies were transparent, had a smooth, slippery texture, sometimes the colonies were piled up and the surface was slightly convex.

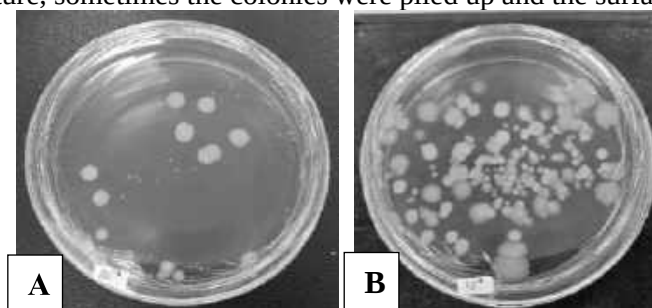


Figure 2. Bacterial colonies on BHIA media (A) bacterial colonies on samples that did not use ice stone (AKM 3 10^2) (B) bacterial colonies of bacteria in samples using ice cubes (AKM 2 10^2)

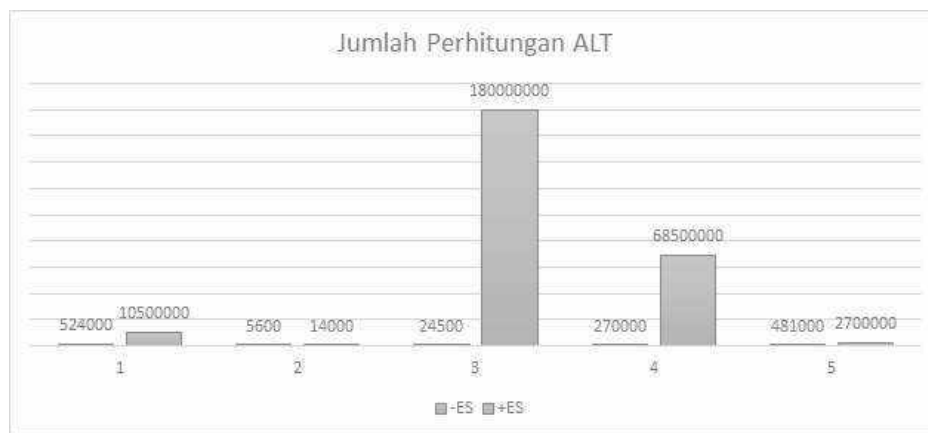


Figure 3. Calculation of the number of bacterial colonies using ALT. Method

Figure 3 shows the number of bacterial colonies obtained from the calculation of the number of colonies on BHIA media from samples that did not use ice cubes and those that used ice cubes, the results were varied, with an average total colony ranging from 5.6×10^3 to $1,8 \times 10^8$.

Based on Figure 3 shows the number of bacterial colonies obtained from the results of calculations using a colony counter, which will then be carried out statistical tests with SPSS, namely the dependent T test. If the results obtained from the normality test are not normally distributed, then use the Wilcoxon test, on the contrary if the results obtained from the normality test are normally distributed, use the Paired T test. The data in this study is less than 50 so using Shapiro-Wilk data. The results of the normality test are listed in table 1.

Table 1. Normality test

	Shapiro-Wilk		
	Statistic	Df	Sig.
Coconut Drinks That Don't Use Ice	.808	10	.018
Coconut Drink Using Ice	.728	10	.002

Table 1 shows the normality test data with Saphiro-Wilk, the p-value for the sample that does not use ice is 0.018, while the p-value of the sample that uses ice is 0.002. it is concluded that the p-value < 0.05 so that the data is not normally distributed.

Table 2. Wilcoxon test

	Sig. (2-tailed)
Coconut Drinks That Don't Use Ice-Coconut Drinks That Use Ice	.005

Table 2 shows the Wilcoxon test results obtained by sig. of the Wilcoxon test is 0.005. Because the test hypothesis of this study is 1-tailed, so the value of $p = 0.005/2 = 0.0025$.

Based on the identification of the characteristics of bacterial colonies on BHIA media, several different colonies were obtained in each sample from all the bacterial colonies obtained, different colonies were selected. Then the colonies that have been selected are inoculated on the endo agar medium. The results obtained are listed in figure 4.

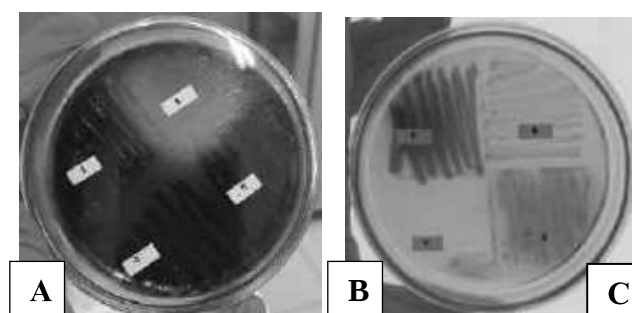


Figure 4. Bacterial colonies on Endo Agar media; A. metallic green color, B. colorless, C. pink color

Table 3 . Bacterial colonies on Endo Agar media

Sample Code		Color
1	AKM 1	Metallic green
2	AKM 3	Pink
3	AKM 3	Pink
4	AKM 4	Colorless

Table 3 shows the growth of bacteria on the endo agar medium, in the endo agar medium it can be seen that the bacterial colonies that grew showed metallic green, pink and colorless colors. The results of the study in table 3 sample (1) showed that the colonies that grew were metallic green, samples (2) and (3) showed that the colonies that grew were pink and sample (4) showed that the colonies were colorless.

This study also identified using biochemical tests with SCA media and sugar media, especially lactose and glucose media. After obtaining the difference in 3 color characteristics on the endo agar, then isolation was carried out on SCA media, lactose and glucose media. After incubation for 24 hours at 37°C the results are shown in table 4.

Table 4. Bacterial colonies on biochemical media

Sample Code		Biochemical Test Results		
		SCA	Glucose	Lactose
1	AKM 1 (1, 3, 4)	-	+	+
2	AKM 3 (5, 8)	+	+	+
3	AKM 3, 4, 5 (11-20)	+	+	+
4	AKM 1, 3, 4 (2, 6, 7, 9, 10)	-	+	-

Based on table 4, the results are (1) negative, (2) positive, (3) positive, (4) negative. According to Puspita *et al.*, (2020) the negative SCA test results indicated that there was no color change in the SCA media which initially turned green to blue. On the other hand, in a positive SCA test result, there is a color change, which means that bacteria have the ability to break down/use citrate as the only carbon source. The results of the glucose test on D-glucose media were positive. As for lactose media, the results of (1), (2), (3) were positive, while the results of (4) were negative.

Based on the results of biochemical tests on the SCA test, glucose test and lactose test, it is suspected

that the type of bacteria *E. coli* was obtained. Furthermore, the gram staining test used a metallic green colored colony sample from endo agar media. The results of the gram staining show that the gram staining test obtained red coccus-shaped bacteria which are included in the gram negative.

DISCUSSION

Based on macroscopic observations from BHIA media, the characteristics of bacterial colonies that grew were circular in shape, moderate in size, milky white in color with entire margins, colonies were opaque and flat on the entire surface (raised). According to research by Rachmawati *et al* (2016), the results of *Streptococcus mutans* colonies on BHIA media showed that the bacterial colonies were round, whitish yellow in color, the colonies were transparent, had a smooth, slippery texture, sometimes the colonies were piled up and the surface was slightly convex.

The results of the calculation of the number of colonies on BHIA media from samples that did not use ice cubes and those that used ice cubes, obtained varied results, with the average total colonies ranging from 4.6×10^3 to 2.3×10^8 . All samples showed similar results. exceeds the maximum limit of total coliform in water that is allowed by Permenkes No. 492/MenKes/PER/IV/2010 which is 0 CFU/100 ml of water. According to Ariyani & Anwar (2006) in general, snacks and drinks contain relatively high bacteria, with an average of 10^5 CFU/ml. The high content of contamination of the snacks and drinks indicates that there is no preheating and the use of unclean water. From the ALT calculation data, then the data will be analyzed using SPSS which obtained the sig value. of the Wilcoxon test is 0.005. Because the test hypothesis of this research is 1-tailed, so the value of $p = 0.005/2 = 0.0025$ (<0.05 : H_0 is rejected) meaning that there is a difference in the amount of growth in the coconut drink samples that do not use ice cubes and those who use ice cubes.

Based on the identification of the characteristics of bacterial colonies on BHIA media, several different colonies were obtained in each sample, from all the bacterial colonies obtained, different colonies were selected. Then the colonies that have been selected are inoculated on the endo agar medium. The results obtained showed the presence of bacterial growth in the endo agar medium, in the endo agar medium it was seen that the bacterial colonies that grew showed metallic green, pink and colorless colors. The results of the study in table 3 (1) showed that the colonies that grew were metallic green which were coliform bacteria that could ferment lactose. Tables 3 (2) and (3) show that the colonies that grow are pink which are still included in the coliform bacteria that can ferment lactose. Table 3 (4) shows that the colorless colonies are included in non-coliform bacteria that cannot ferment lactose.

According to (Kubizniaková *et al.*, 2020) endo agar is usually used to detect coliform bacteria. The selectivity of the endo agar medium was determined by the presence of fuchsin and sulfite as well as the content of carbon substrate in the form of lactose. Bacterial colonies that grow can be distinguished based on lactose positive bacteria (pink or red colonies with a greenish metallic luster) and lactose negative bacteria (cream or colorless colonies). According to (Megahati, 2011) endo agar is a selective medium for gram positive and a good growth medium for gram negative bacteria. Colonies that produced a red or shiny metallic color included *E. coli*, whereas *Proteus*, *Salmonella*, *Shigella* produced a faint pink color and *Pseudomonas* did not.

This study also identified using biochemical tests with SCA media and sugar media, especially lactose and glucose media. Based on table 4, the results are (1) negative, (2) positive, (3) positive, (4) negative. According to Puspita *et al.*, (2020) the negative SCA test results indicated that there was no color change in the SCA media which initially turned green to blue. On the other hand, positive SCA test results have a color change, which means that bacteria have the ability to break down/use citrate as the only carbon source. *E.coli* bacteria are not able to utilize citrate as the only carbon source. From the results of the SCA test, the negative results of SCA were in tubes (1) and (4) which were originally on the endo agar medium, which was suspected of being *E.coli* in tubes (1), (2), and (3). including *E.coli* is just a tube (1). In tubes (2) and (3) coliform bacteria are suspected and in tubes (4) non-coliform bacteria are suspected.

Based on table 4, the results of the glucose test on D-glucose media obtained positive results with the discovery of gas in an inverted durham tube. As for lactose media, the results of (1), (2), (3) were positive which was indicated by the presence of gas in the inverted durham tube. According to Putri & Kurnia (2018), the presence of carbon gas in the Durham tube chamber is caused by the tightly closed test tube, the longer the tube is tightly closed it can cause a lot of gas spaces to form and indicate a positive result. In

addition, (Kamaliah, 2017) added that the gas formed could also come from lactose fermentation which will form CO₂ gas. According to research (Wandriavel *et al.*, 2012) the presence of gas in the media indicates that there are colonies of coliform bacteria in the media. In tubes (2) and (3) showed the presence of turbidity. According to (Kamaliah, 2017) there was turbidity formed because there were bacteria that produce lactic acid from lactose fermentation. Turbidity can be caused by an increase in the amount of lactic acid, so that the lactose will clot. The clumps formed from lactose are what cause turbidity. A negative (4) result does not indicate the presence of gas or turbidity.

Table 4 shows that the sample is suspected to be contaminated with *E. coli*, because the lactose and glucose test obtained positive results and the SCA test obtained negative results. Tables 4 (2) and (3) indicate that the suspected sample belongs to *Klebsiella sp.* bacteria, because the SCA test obtained positive results and the glucose and lactose tests obtained positive results. As for table 4 (4) shows that the suspected sample belongs to non coliform bacteria, because the glucose test obtained positive results and the lactose test obtained negative results and the SCA test obtained negative results. According to Tauran *et al* (2013) the results of the biochemical test of gram negative bacteria using conventional methods on *E. coli* bacteria obtained negative SCA test results and positive lactose glucose tests. *Klebsiella sp.* the results of the SCA test were positive and the glucose-lactose test was positive.

In the gram staining test, the results obtained are red coccus-shaped bacteria which are classified as gram negative. *E. coli* is a gram negative bacteria. According to Prasetya *et al* (2019), the gram negative bacteria layer has a special characteristic, namely having a thin peptidoglycan layer, while gram positive bacteria have a thick peptidoglycan layer.

CONCLUSION

Based on ALT calculations on samples that did not use ice cubes, the results obtained were AKM 1 (0.7×10^6 CFU/ml), AKM 2 (5.6×10^3 CFU/ml), AKM 3 (2.4×10^4 CFU/ml), AKM 4 (2.7×10^5 CFU/ml), AKM 5 (3.2×10^5 CFU/ml). As for the samples using ice cubes, the results obtained were AKM 1 (1.0×10^7 CFU/ml), AKM 2 (14.2×10^3 CFU/ml), AKM 3 (1.8×10^8 CFU/ml), AKM 4 (6.8×10^7 CFU/ml), AKM 5 (2.2×10^6 CFU/ml). Based on the Wilcoxon test, it was found that 0.0025 (<0.05: Ho rejected) concluded that there was a significant difference in the amount of growth between samples of coconut drink that did not use ice cubes and those who used ice cubes. The bacteria found from the endo agar medium were *Escherichia coli*, *Klebsiella sp.*, and non-coliform bacteria.

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OVERVIEW OF BLOOD LEAD LEVELS IN TRAFFIC OFFICERS ON JL. IR. H. JUANDA EAST BEKASI USING THE ICP-MS METHOD

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Abstract

Introduction: The contribution of air pollution to vehicles that use fuel oil shows a contribution of 49% worldwide. About 70% of the lead contained in fuel by burning gasoline will be released into the air. This has an impact on increasing carbon emissions and waste production that will pollute the city of Bekasi. Alkyl lead is used as a gasoline fuel mixture. Alkyl lead also functions as an anti-knock additive in fuel, namely to reduce the impact of engine work so as to reduce noise when combustion occurs in motorized vehicle engines. This source currently accounts for the most lead levels in the air. Lead can cause air pollution in two forms, namely gases and particulates. Lead is one of the main pollutants released when burning fuel oil in vehicles. Lead that enters the human body even in small amounts can be dangerous, because it accumulates in the body and eventually causes toxic effects on various organ functions. One of the people who can experience lead poisoning from vehicle emissions on the highway is a traffic officer. Lead compounds can enter the human body through digestion, respiration, and skin. The mouth and respiratory tract are the most common routes by which lead enters the human body. The purpose of this study was to measure and determine blood lead levels in traffic officers using the ICP-MS method.

Method: The method used in this study is to use a blood sample of EDTA which is then injected into the ICP-MS device.

Results: The results obtained are $< 2.28 \mu\text{g/L}$ or equivalent to $< 22.8 \text{ g/dL}$.

Conclusion: Based on the research that has been done, it can be concluded that the blood lead levels of 10 respondents showed no detectable lead in the blood, namely $< 2.28 \mu\text{g/L}$.

Key words : Blood, lead, ICP-MS, purposive sampling, traffic officers.

INTRODUCTION

Air pollution results from increased vehicle emissions and traffic congestion. This pollution usually occurs in big cities. The contribution of air pollution to vehicles that use fuel oil shows a contribution of 49% worldwide. Indonesia itself is still dominated by the use of lead-type fuel. About 70% of the lead contained in fuel by burning gasoline will be released into the air. The city of Bekasi is one of the buffers for the metropolitan city of Jakarta. This causes the population and mobility of residents in this city to continue to increase. The population in Bekasi City reaches 2,402,465 people. The large population is also directly proportional to energy needs, reduced vegetation cover and increased daily waste. This has an impact on increasing carbon emissions and production waste that will pollute the city of Bekasi. Air pollution is still the biggest problem in big cities where the pollutants in the air are harmful to human health and the environment. Based on the estimated quality index data (AQI) in 2022, the air pollution level in the city of Bekasi is 152 AQI US. This indicates an unhealthy level of pollution. The ingredients in air pollution include $\text{PM}_{2.5}$ 28.7 g/m^3 , PM_{10} 42.4 g/m^3 , O_3 3.9 g/m^3 , NO_2 21.5 g/m^3 (Apriani & Wibowo, 2019).

Alkyl lead (TEL and TML) is used as a gasoline fuel mixture. Alkyl lead serves to increase lubrication and increase combustion efficiency. Alkyl lead also functions as an anti-knock additive in fuel, which is to reduce the impact of the engine working so that it can reduce noise when combustion occurs in a motorized vehicle engine. This source currently contributes the most lead levels in the air (Gusnita, 2012). Lead can cause air pollution in two forms, namely gas and particulate matter. Lead gas that comes from the use of fuel from burning vehicle gasoline is usually in the form of TEL or TML. Commonly used additives in vehicle fuels typically include a combination of 62% tetraethyl lead, 18% ethylene dichloride, 18% ethylene dibromide, and nearly 2% other compounds (Koh, 2021). Lead is one of the main pollutants released when burning fuel oil in vehicles. Vehicles are the main cause of air pollution. Air pollution is caused by exhaust fumes such as gasoline. Dust particles containing heavy metals such as CO as a by-product, hydrocarbons (HC) and lead. Lead that enters the human body even in small amounts can be

dangerous, because it accumulates in the body and eventually causes toxic effects on various organ functions. The first effect of chronic lead poisoning before reaching the target organs in humans is a disturbance in hemoglobin biosynthesis (Ardillah, 2016).

One of the people who can experience lead poisoning from vehicle emissions on the highway is one of the traffic officers. A traffic officer is someone who regulates traffic rules on the highway. Traffic officers have an important role, namely making traffic engineering to minimize congestion that occurs on the highway. Staff at work always wear masks. These tools are used to protect themselves from dust contamination that is inhaled while on the road. The use of personal protective equipment can reduce the risk of disease associated with lead exposure, and is expected to pose little risk associated with their work. Lead compounds can enter the human body through digestion, respiration, and skin. The mouth and respiratory tract are the most common routes by which lead enters the human body. Many human activities are caused by residues of lead-containing products that are burned and inhaled directly, such as transportation (Maskinah, 2017). In the body, as much as 95% of blood lead binds to erythrocytes and can inhibit aminolevulinic acid dehydratase (ALAD), thereby inhibiting hemoglobin synthesis. Lead is excreted in several organs of the human body, especially in the kidneys and in the digestive tract. Lead excretion through urine is 75% - 80%, 15% in feces and the rest is excreted through bile, sweat, hair and nails (Rosita & Lidiawidiarti, 2018).

METHOD

A. Tools and Materials

1. Tools :

The tools used in this study were the ICP-MS Agilent series 7800, measuring flask, micropipette, vacutainer tube (EDTA), waterbath, handsocon, syringe, tourniquet, alcohol swab, cooler, water bath and fume hood.

2. Material:

The material used are whole blood, HNO_3 (Merck), Aqua milli (Millipore), and H_2O_2 (Merck).

B. Procedure

1. Blood Specimen Collection

The specimen used was 3 ml of blood taken using an EDTA tube. The blood specimen taken is venous blood. The venous blood sampling is done by preparing tools and materials before sampling. Selection of veins by palpating the patient's arm at the median cubital location after insertion of a 3- finger tourniquet above the puncture site. Clean the puncture site with 70% alcohol and allow the alcohol to dry. Next, the vein is punctured with a needle and withdraw the syringe suction until the syringe is completely filled. Then remove the tourniquet, pull the needle and the site of the puncture mark is covered with tape. The blood in the syringe is transferred into the EDTA vacutainer tube and then inverted 8-10 times (Gandasoebrata, 2016).

2. Blood Sample Preparation

0.5 ml of blood is pipetted and put into a test tube. Blood was added with 1 ml of concentrated HNO_3 . The mixture was heated in a water bath with a temperature of 90°C . The mixture was added with 0.5 ml of H_2O_2 . The solution was reheated in a water bath at 90°C for 2 hours. Chill. Add 10 ml of Aqua milli, then filtered. The sample is injected into the ICP-MS Agilent series 7800.

3. Preparation of lead main liquor 1000 ppm

$\text{Pb}(\text{NO}_3)_2$ weighed as much as 1.59897 gram then added concentrated HNO_3 solution to the mark of the 1000 mL volumetric flask (Fajriah, 2017).

4. Preparation of lead standard solution

- Preparation of standard solution of Pb 100 ppm

5 ml of 1000 ppm Pb main liquor was pipetted, then 5 ml of HNO_3 was added. The solution homogenized until evenly distributed. Add aqua milli to the limit of the 50 ml volumetric flask (Fajriah, 2017).

RESULTS

A. Respondents overview

Respondents in this study were traffic officers. This traffic officer serves as a traffic controller on the highway. Traffic officers aged 23-45 years with a total of 3 women and 7 men who meet the inclusion criteria who have worked 1-25 years. Traffic officers work 1-8 hours a day. During work, traffic officers always wear masks as personal protective equipment. The subjects of this study were traffic officers on Jl. Ir. H. Juanda, East Bekasi. The sample was taken by purposive sampling technique, the number of samples obtained was 10 respondents with data collection techniques carried out by filling out questionnaires. The results obtained were < 2.28 g/L or equivalent to < 22.8 g/dL. These results are still within the normal limits for lead levels in the blood based on the Decree of the Minister of Health of the Republic of Indonesia Number 1406/MENKES/IX/2002, which is 10-25 g/dL (Theory, 2002).

B. Sample preparation

Sample preparation is the process of eliminating components other than the analyte that will interfere with the analysis process. Preparation in this study using the method of destruction. The purpose of destruction is to separate organic compounds and metals contained in the sample so that only lead metal is present in the sample (Asmorowati et al., 2020). Sample preparation was carried out using the wet digestion method, because in the process the sample was able to decompose organic matter in the sample with the help of concentrated oxidizing acid and heat (Kristianingrum, 2012).

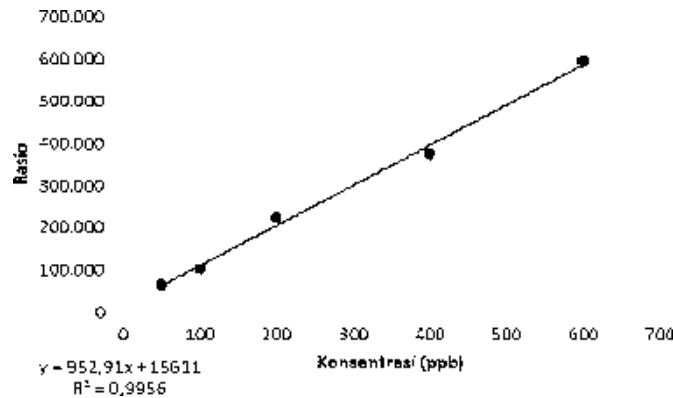
Lead standard solution calibration curve

Concentration (ppb)	Standard volume created (μ l)	Standard solution (ppm)
50	25	100
100	50	100
200	100	100
400	200	100
600	300	100

Lead calibration curve data

No	Solution	Concentration (ppb)	Ratio
1.	Standart 1	50	6.4991
2.	Standart 2	100	10.2883
3.	Standart 3	200	22.3713
4.	Standart 4	400	37.7018
5.	Standart 5	600	59.5883

The measurement of the intensity ratio of the lead standard solution after measuring the absorbance of the lead standard series solution, and data from each solution were then included in the calibration curve. The regression equation obtained for the standard blood lead is $y = 952.91x + 15611$ with the relation coefficient (R^2) is 0.9956.



Picture 1. Lead standard solution curve

The lead standard solution curve was made by making standard concentrations of 50, 100, 200, 400, 600 ppb from 100 ppm stock solution. Based on the line equation data obtained from the standard solution curve of lead, namely $y = 952.91x + 15611$ with a relation coefficient (R^2) so that the lead concentration is obtained as a reference in determining the lead content in the sample. The curve above shows a linear relationship between concentration and absorbance, the relation coefficient (R^2) of lead is 0.9956 $r > 0.95$, which means this value shows a high correlation with the relationship between concentration and ratio (Lusiana, 2012).

Table 1. The results of lead levels in the blood of traffic officers

No	Code sample	The results $\mu\text{g/L}$	The results $\mu\text{g/dL}$	Description
1.	1	< 2,28 $\mu\text{g/L}$	< 22,8 $\mu\text{g/dL}$	Not detected
2.	2	< 2,28 $\mu\text{g/L}$	< 22,8 $\mu\text{g/dL}$	Not detected
3.	3	< 2,28 $\mu\text{g/L}$	< 22,8 $\mu\text{g/dL}$	Not detected
4.	4	< 2,28 $\mu\text{g/L}$	< 22,8 $\mu\text{g/dL}$	Not detected
5.	5	< 2,28 $\mu\text{g/L}$	< 22,8 $\mu\text{g/dL}$	Not detected
6.	6	< 2,28 $\mu\text{g/L}$	< 22,8 $\mu\text{g/dL}$	Not detected
7.	7	< 2,28 $\mu\text{g/L}$	< 22,8 $\mu\text{g/dL}$	Not detected
8.	8	< 2,28 $\mu\text{g/L}$	< 22,8 $\mu\text{g/dL}$	Not detected
9.	9	< 2,28 $\mu\text{g/L}$	< 22,8 $\mu\text{g/dL}$	Not detected
10.	10	< 2,28 $\mu\text{g/L}$	< 22,8 $\mu\text{g/dL}$	Not detected

Information :

In accordance with Threshold Limit Value For Chemical Substance And Physical Agents And Biological Exposure Indices 2017.

DISCUSSION

In the study of W A Saud & Purwati (2020) the description of blood lead levels in gas station operators at the Kliwon market in Surakarta showed that 10 respondents from gas station operators obtained normal lead levels according to the CDC standard, which is < 10 g/dL. In general, the older a person is exposed to lead will damage the liver and kidneys, therefore it is necessary to encourage workers who are at risk of being exposed to lead to pay more attention to work safety by using personal protective equipment that can reduce lead exposure. Lead is also found in motor vehicle exhaust. The operation of motorized vehicles will release air pollutants that have a negative impact, both on the environment and human health. Lead emissions as exhaust from motor vehicle fumes enter the air in the form of gas. Lead emission is a side effect of combustion that occurs in vehicle engines from TEL and TML compounds added to fuel (Purnama, 2015).

Lead that enters the human body even in small amounts can be dangerous, because it accumulates in the body and eventually causes toxic effects on various organ functions. The first effect of chronic lead poisoning before reaching the target organ is a disturbance in hemoglobin biosynthesis (Ardillah, 2016). If this is not immediately addressed, the toxic effects of lead metal will continue, including the human organs, especially the nervous system, the human blood formation system, the kidneys, and the heart system. Lead can also cause high blood pressure and anemia. The relatively high accumulation of lead in the blood will cause digestive tract syndromes, consciousness, and central nervous system damage and behavioral changes (Palar, 2008).

If the body is exposed to lead every day with a long enough working period, it can cause lead to enter the body, then it will stick to red blood cells and dissolve red blood cells before the regeneration stage. The nature of lead damage depends on the intensity of exposure and the time it takes red blood cells to regenerate, but continued exposure to lead will cause blood to circulate in the body and then continue into the bloodstream to the bone marrow (Maskinah, 2017). In old age the level of sensitivity is higher than the average adult, usually because the activity of the biotransformase enzyme decreases with age and the resistance of certain organs to the effects of lead decreases. The older a person is, the higher the concentration of lead that accumulates in body tissues (Mahendra & Ardiani, 2015).

CONCLUSION

Based on the research that has been done, it can be concluded that the blood lead levels of 10 respondents showed no detectable lead in the blood, namely < 2.28 g/L. Based on the results of the research that has been done, it is recommended that further research be more varied in determining the criteria for selecting respondents, research locations and examinations related to lead, namely hemoglobin.

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