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PROCEEDING

The 3rd International Allied Health Students Conference 2023

"Innovation and Future Prospect in Health for Well-being"

September 13-14, 2023
virtually by Zoom Cloud Meetings

Publisher :
Research and Community Engagement
Sekolah Tinggi Ilmu Kesehatan (STIKES)
Mitra Keluarga

TABLE OF CONTENTS

COVER

TITLE PAGE

FOREWORD

THE BOARD OF COMITEE

TABLE OF CONTENTS

ISSN 3031-0911

PROCEEDINGS

THE 3rd INTERNATIONAL ALLIED HEALTH STUDENTS CONFERENCE (IAHSC) 2023

"Innovation and Future Prospect in Health for Well-being"

Bekasi, West Java, Indonesia, 13-14th September 2023

Organized by:

Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga

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2023

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Website : https://stikesmitrakeluarga.ac.id/
Email : info@stikesmitrakeluarga.ac.id

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WELCOMING SPEECH - HEAD OF STIKES MITRA KELUARGA

Assalammu'alaikum wr.wb, Salam Sejahtera, Om Swastyastu, Namo Buddhaya, Salam Kebajikan, Rahayu.

First and foremost, our grateful to God Almighty, Allah SWT who provides us with health and productivity. May God's peace be upon us.

On behalf of Mitra Keluarga School of Health Sciences (STIKes Mitra Keluarga) and the committee, I am pleased to welcome you to the third (3rd) International Allied Health Students Conference (IAHSC) 2023.

We believe that innovation is one of the keys to sustainable development in health and well-being. There is no such achievement in human development without great innovation and great leadership, and there is no future prospect in health without great transforming science, knowledge, and technology among researchers and professionals.

Participation and collaboration by health students, academics, and professionals or what is known as 'Penta helix approach', might contribute to the growth and development of health innovation for well-being. STIKes Mitra Keluarga provides a vision: "Become an Innovative Health Education Institution at the national level by 2035". Therefore, the theme of the 3rd IAHSC 2023 "Innovation and Future Prospect in Health for Well-being", may accommodate our strategy to support the achievement of our vision.

We also believe that efforts to do this conference annually could be the way to empower the learning process both in scientific-based knowledge and communication and management skills.

Based on the above considerations, our purpose is to increase the participation of health students, academics, and professionals in scientific development based on health innovation for well-being.

During the course of two-day (September 13-14, 2023), IAHSC 2023 offers a full scientific program featuring keynote speakers' session, 16 plenary lectures representing each study program in STIKes Mitra Keluarga (Nursing, Nutrition, Medical Laboratory Technology, and Pharmacy), and 55 oral presentation sessions.

STIKes Mitra Keluarga, the organizing committee, and partners are delighted to welcome you all to the IAHSC 2023 virtually. Thank you to the Government of Indonesia (BRIN and LLDIKTI III), the Corporate of Mitra Keluarga, health related industries, and partners for supporting our event. Thank you for all participants, and please enjoy the conference.

Head of STIKes Mitra Keluarga

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

WELCOMING MESSAGE - HEAD OF RESEARCH & COMMUNITY ENGAGEMENT STIKES MITRA KELUARGA

Dear Colleagues
On behalf of Research and Community Engagement STIkes Mitra Keluarga, it is our great pleasure to welcome you to 3rd International Allied Health Student Conference (IAHSC) 2023 that will be held in virtual meeting from 13-14 September 2023. The 3rd IAHSC theme this time is “Innovation and Future Prospect in Health For Well Being”. Based on this theme, we want to raise the issue of innovation, especially in the health sector, which is urgently needed at this time. Climate change, weather and types of disease in the world force the world of health to be able to find solution to every health problem in a fairly short time.

Innovation can support the acceleration of finding solutions to these health problems. Innovations can be made by all groups involved in the world of health, one of which is health students. With this conference, we want to initiate health students to provide ideas that are the result of research related to innovation findings in the health sector covering the fields of nursing, nutrition, pharmacy, and medical laboratory technology. We are also bring various speakers from various countries to gain knowledge about innovations in the health sector that are applied in various countries. At The end of my message, I would like to say thank you to the keynote speakers and all speakers, STIKes Mitra Keluarga, Student and Lecture Committee, and also all participants and presenters.

Kind Regards

Afrinia Eka Sari, S.TP., M.Si
Head of Research and Community STIKes Mitra Keluarga

Welcoming Message and Chairperson Report of the 3rd IAHSC 2023

Assalammu'alaikum wr.wb, Salam Sejahtera, Om Swastyastu, Namo Buddhaya, Salam Kebajikan.

We are thankful to God Almighty, Allah SWT who provides us with health and productivity. May God's peace be upon us.

On behalf of the Organizing Committee of the 3rd International Allied Health Students Conference 2023, it is an honor to welcome you to the 3rd International Allied Health Students Conference (IAHSC) 2023, virtually hosted by STIKes Mitra Keluarga.

As the chairperson of this event, let me provide you with our report. The IAHSC this year in 2023 has a theme: "Innovation and Future Prospect in Health for Well-being". Our purpose is to increase the participation of health students, academics, and professionals in scientific development based on health innovation for well-being. Specifically, our objectives are:

- Increasing awareness of the health students, academics, and professionals towards multidisciplinary health sciences to collaborate and promote future prospects in health for well-being.
- Increasing networking and challenges in the innovation of health through an update in global health innovation.

The program event of the 3rd IAHSC 2023 will provide you with a multiple board of scientific material delivered by our keynote speakers, 7 plenary speakers who are expert representatives from academic and professional fields on Day 1 (September 13, 2023), and continue to the next day, on Day 2 (September 14, 2023), our program will be provided by 8 plenary speakers, followed by oral presentation session parallelly located at 4 breakout room. Our last agenda item will be the sharing of knowledge and experiences by Health Student Association, representative from West Java Province, Indonesia.

The total number of participants who have registered in our event was 55 oral presenters and more than 400 students from Nursing, Nutrition, Medical Laboratory Technology, and Pharmacy Study Program, among others. We are very pleased to welcome all undergraduate, postgraduate students, lecturers, and Professors from:
1. STIKes Mitra Keluarga, Indonesia
2. Universiti of Sultan Zainal Abidin, Malaysia
3. Taipei Medical University, Taiwan
4. Uttara Adhunik Medical College, Dhaka, Bangladesh
5. Universitas Pendidikan Indonesia, Bandung, Indonesia
6. Sebelas Maret University, Surakarta, Indonesia
7. Institut Ilmu Kesehatan Nahdlatul Ulama, Tuban, Indonesia
8. STIKes Pekanbaru Medical Center, Indonesia
9. Politeknik Katolik Mangunwijaya, Semarang, Indonesia

We believe that our success has resulted from all team members great performances. For this reason, I am sincerely thanking all the team members for their great contribution, dedication, and hard work since the beginning of our event preparation and up until now. More importantly, we are thankful to the Steering Committee, who have advised us with their board experience and expertise.

Thank you to the Government of Indonesia (BRIN and LLDIKTI III), the Corporate of Mitra Keluarga, health related industries, and partners (Universiti Sultan Zainal Abidin, Malaysia; Taipei Medical University, Taiwan; Chulalongkorn University, Thailand; Groningen University, the Netherlands; La Trobe University, Australia; Universitas Indonesia; Universitas Muhammadiyah Semarang; Health Students Association or ISMAKES, West Java Province, Indonesia; Professionals Organization: PPNI, PATELKI, PERSAGI, IAI, PERGIZI PANGAN Indonesia; and Media Partner: GIZI GAMA) for supporting our event. Thank you for all participants, and hope you enjoy the conference.

Thank You.
Wassalammu'ailaikum, wr.wb. Salam Sejahtera, Om Swastyastu, Namo Buddhaya, Salam Kebajikan.

Chairperson

Guntari Prasetya, S.Gz., M.Sc

Sekolah Tinggi Ilmu Kesehatan
MITRA KELUARGA

Kampus Merdeka
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THE 3rd INTERNATIONAL ALLIED HEALTH STUDENTS CONFERENCE (IAHSC) 2023

"Innovation and Future Prospect in Health for Well-being"

Increasing the participation of the health students, academe, and professionals in scientific development based on health innovation

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Important Dates:

- Early Bird Registration : July 3-31, 2023
- Abstract submission : July 3 - August 28, 2023
- Abstract Acceptance Notification : September 1, 2023
- Oral Video Submission : September 1-10, 2023
- Full Paper Proceeding/Manuscript Journal Article Submission : September 1-October 1, 2023

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Wednesday, 13th September (Day 1) 2023
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Day 2 : 08.00 am - 16.30 pm

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ISSN 3031-0911

Nursing

Analysis Of The Application Of Blowing Balloon Therapy To Improve Breath Patterns In Children With Pneumonia At Hospital X Bekasi
Susi Hartati*, Dewati Wahyu Indah Sari 1-5

Application of Postural Drainage and Chest Physiotherapy To Increase Airborne Clearance in Children With Bronchopeneumonia
Susi Hartati*, Nidia Ainun Rindiani 6-11

The Effectiveness of Education With Video Media on The Knowledge and Attitudes of Family Planning Dropout Acceptors in The Work Area of The Central Kaliabang Health Center, Bekasi City
Parikesit Nuril Kartika*, Edita Astuti Panjaitan, **Lina Herida Pinem**……...12-16

The Relationship Between Utilization of the Maternal Child Handbook (MCH) with The Health Status of Pregnant Women at the Pengasinan Health Center in Bekasi City 2023
Fardilla Rochman Utami *, Edita Astuti Panjaitan, **Lina Herida Pinem**.....17-21

Correlation Of Pregnancy Risk Awareness With The Constancy Antenatal Care Expectant Two And Three Trisemester In Public Health Center Jatimulya
Amalia Rizky Atikah, Lina Herida Pinem, Edita Astuti Panjaitan……22-26

Pharmacy

Prescribing patterns of anti-hypertensive medications at PHC clinic Pedurungan, Semarang
Winda Dwi Astuti , Paulina Maya Octasari * 27-35

Determination of hydroquinone levels in whitening serum circulating at market place x using the uv-vis Spectrophotometric method
Desalfa Pafitriano* , Melania Perwitasari, Intan Kurnia Putri,Maya Uzia Beandrade. 35-42

Acute Toxicity Test of Probiotic Juice Red Guava, Lemon, Melon, and Apple With Bacteria Starter Lactobacillus casei on Female *Balb/C* Mice
Desi Retnosari* , Reza Anindita, Melania Perwitasari, Nofria Rizkia Amalia Harahap 43-46

Phytochemical screening of *Kaempferia galanga* l. Rhizome in Banguntapan, Bantul, Yogyakarta
Muhammad Fadhiel Ihsan*, Reza Anindita, Intan Kurnia Putri, Noor Andryan Ilsan 47-50

ISSN 3031-0911

Synergism test on date seeds (*Phoenix dactylifera* L.) And peanut shells (*Arachis hypogea* l.) Extract on antioxidant activity
Galuh Anjani Garnisaputri , Intan Kurnia Putri*, Melania Perwitasari , Dede Dwi Nathalia ...51-59

Effect of stevia sugar on innovation of guava, apple, lemon, and melon juice probiotic drink using *Lactobacillus casei* bacteria on amount Lactic acid bacteria
Mety Nur Ivani*, Reza Anindita, Maulin Inggriani, Maya Uzia Beandrade..... 60-63

Formulation test of facial serum mangosteen rind extract (*Garcinia Mangostana*) and niacinamide
Mia Marlina, Maya Uzia Beandrade*, Dede Dwi Nathalia , Reza Anindita..... 64-68

Acute toxicity test of probiotic red guava, apple, lemon, melon juice with bacteria starter *Lactobacillus acidophilus* on male Balb/c. Mice
Reza Anindita* , Nurin Natasha, Nofria Rizkia Amalia Harahap , and Ariska Deffy Anggarany 69-72

Synergism test on dates (*Phoenix dactylifera* l.) Fruit extract and soybean (*Glycine max* (l.) Merr.)) Nuts extract on antioxidant activity
Sinta Nuriyah Awaliah , Intan Kurnia Putri* , Melania Perwitasari, Maya Uzia Beandrade ... 73-80

Determination of Hydroquinone Content in Whitening Toner Sold in Online Market Place X by Reverse Phase High Performance Liquid Chromatography (HPLC)
Stevani Hana Marumata*, Melania Perwitasari, Intan Kurnia Putri, Maya Uzia Beandrade .. 81-87

Formulation and evaluation test of lip balm from ethanol extract of fruit (*Salacca zalacca* (Gaertener) Voss.)
Syarifah Purbaningrum*, Maya Uzia Beandrade, Melania Perwitasari, and Reza Anindita 88-93

Physical Evaluation Test Of Serum Facial Ethanol Extracts Of Purple Passion Fruit Peel (*Passiflora edulis* Sims)
Unggul Fernando Sinambela*, Maya Uzia Beandrade2, Reza Anindita, Intan Kurnia Putri.... 94-98

ISSN 3031-0911

Nutrition

Relationship of Body Mass Index-For-Age to Body Fat Percentage Using Relative Fat Mass in Teenagers in Surakarta
Nais Volum Firdaus*, Tonang Dwi Ardyanto , Isna Qadrijati 99-103

Quantitative Test Of Quercetin Content Of Rome Beauty Apple Flour Extract Using The High-Performance Liquid Chromatography Method
Dwi Wulandari Aaas Hamka,Dhani Redhono Harioputro,Ratih Puspita Febrinasari 104-111

The Effect of Body Image and Pocket Money on Food Choice Attitudes in Obseous Adolescents In Surakarta
Afifah Istiqomah*, Eti Poncorini Pamungkasari , Sri Mulyani 112-117

The Effect of Nutrition Education Through Flashcard Media on Knowledge of Iron Sources Food Consumption in Adolescent Women at SMP N 33 Bekasi City
Miswah Alfaresti Sutrisna*, Arindah Nur Sartika..... 118-122

Literature Review: Effectiveness of Online Nutrition Education on Mother Nutrition Knowledge of Stunting Children
Alfi Rahmatillah Nur Fithriah* , Sumardyono, Anik Lestari 123-128

The relationship between intake of iron, vitamin D and nutritional status on the incidence of anemia in pregnant women at Kebak Kramat 1 community health center, Karanganyar
Baarizah Febriana Badri * , Dono Indarto, Tonang Dwi Ardyanto 129-136

Analysis of the effectiveness of herbal drink consumption on emesis gravidarum in pregnant women in the senapelan, Pekanbaru city
Desy Winda*, Endah Purwani Sari, Fatma Richa Rahmana, Mona Dewi Utari 137-140

Assessment of the needs and role of nutritionists in schools in indonesia? How important? : A literature review
Imtiyaz Irbatunnisa* , Delita Septia Rosdiana, Anisa Nur Wulan, Nanda Fitria Salsabila, Tsaltsa Najwa Fitria 141-146

ISSN 3031-0911

The Effect of Adding Mocaf Flour *(Modified cassava flour)* and Kepok Banana Peel *(Musa paradisiaca forma typica)* on Vla Pie as Alternative Snack for Hypertension Patients
Laurentine Stefanny Arfenda*, Tri Marta Fadhilah 147-151

Anti-Proliferative Activity of Fragrant Rice MRQ 74 (Mas Wangi) Water Extract Against Colon Cancer Cell Lines (Ht-29)
Nur Anis Aliah binti Azhar, Norhayati Abd Hadi, Mohd Adzim Khalili Rohin, Norhaslinda Ridzwan Ruzaina Ishak, Atif Amin Baig 152-161

The Effect of Online Games Tumpeng Gizi Seimbang to Balanced Nutrition Knowledge, Fat Intake, and Carbohydrate Intake in Overweight Adolescents in High School Surakarta
Nur Sitra Buamona* , Sapja Anantanyu, Suminah 162-168

Syzygium Polyanthum as a Potential Hemoglobin Booster for Iron Deficiency Anemia Due to its High Vitamin C Content
Rhisma Mutiara Devi,* Adi Magna Patriadi Nuhriawangsa, Setyo Sri Rahardjo 169-172

The relationship between intake of iron, vitamin D, and nutritional status on the incidence of anemia in pregnant women at the Kebak Kramat 1 community health center, Karanganyar
Baarizah Febriana Badri * , Dono Indarto , Tonang Dwi Ardyanto 173-180

The Relation Of Nutritional Status And Fat Percentage Towards Blood Pressure Women Aged 45-65 Years Old In Posbindu, Klaten, Central Java, Indonesia
Sophia Denta Prapdika Sari*, Ida Nurwati, Ratih Puspita Febrinasari 181-186

The Effect Of Ethanol Ants Nest Extract (Myrmecodia pendans) On Fasting Blood Sugar Levels In Mice Obesity With Type 2 Diabetes
Rizky Alfiqi Nuriza* , Setyo Sri Rahardjo , Lilik Wijayanti..... 187-192

Preliminary Test of Edamame Jelly Anti Hypertension: The Analysis Of Acceptability and Nutritional Quality of Edamame Jelly
Yualeny Valensia*, Budiyanti Wiboworini, Nur Hafidha Hikmayani 193-198

Relationship Between Dietary Diversity And Body Weight Monitoring With The Incidence of Overnutrition in Stikes Mitra Keluarga Students
Vini Andriani Taher*and Guntari Prasetya 199-208

Relationship Between Knowledge and Physical Activity with Nutritional Status Among High School Teachers in Surakarta
Laras Eka Nur Hasanah*, Yulia Sari , Ratih Puspita Febrinasari…….209-215

ISSN 3031-0911

Medical Laboratory Technology

Identification of *Vibrio* sp. in Green Clam (*Perna viridis*)
at West Bekasi Traditional Market
Andien Endina, Nadia Laura Agustin Simanjuntak, Maulin Inggraini,* Noor Andryan Ilsan 216-219

Examination of Nail Fungus in Garbage Transporters using
Saboraud Dextrose Agar Culture Media
Gefira Syahrotul Aini, Maulin Inggraini* , Noor Andryan Ilsan 220-225

Potential of Mangosteen Peel Extract (*Garcinia mangostana*) as
Alternative Dye to Eosine in Papanicolaou Buccal Smear Method
Vanesa Mariana , Ria Amelia* , Siti Nurfajriah..... 226-231

Correlation Between Neutrophil Lymfocyte Ratio (Nlr) And Platelets Values in
Dengue Hemorrhagic Fever Patients at Private Hospital Jatiasih Bekasi
Period 2019-2021
Yuniar Rohma Maulida, Ria Amelia*, Elfira Mayasari 232-236

Analysis Of The Application Of Blowing Balloon Therapy To Improve Breath Patterns In Children With Pneumonia At Hospital X Bekasi

Susi Hartati [1*], Dewati Wahyu Indah Sari [2]

[1,2,3,4] Department of Nursing, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga Bekasi, Indonesia
*Correspondence author : susi@stikesmitrakeluarga.ac.id

Abstract

Pneumonia is usually marked by coughing and runny nose to shortness of breath which is a well-known disease in the community and easily attacks children, this disease mainly attacks the children's lung organs. One of the main nursing problems that arise due to pneumonia is an ineffective breathing pattern, where ventilation is not optimal. As a nurse, to provide non-pharmacological therapy in the form of innovative balloon-blowing therapy to improve oxygenation status in pediatric patients, especially those with breathing pattern problems. This innovation was carried out using the case study method for 3 patients. Balloon blowing therapy which is carried out by inhaling air from the nose and expelling it through the inflated mouth then the air coming out of the mouth is put into the balloon until the balloon is inflated, with the inclusion criteria applied to children aged 3-12 years, experiencing signs of respiratory distress, children with a diagnosis medical pneumonia and bronchopneumonia and cooperative. This balloon-blowing therapy was evaluated by looking at the respiratory frequency for 3 days with an observation sheet and an assessment of the clinical respiration score (CRS). The results for 3 days in the three patients showed a decrease in respiratory frequency with an average of before the action of 24 x/minute and after the procedure 21 x/minute, and the evaluation of the three patients included mild respiratory disorders. Based on evaluation results for 3 days with three patients, it was concluded that this balloon-blowing therapy improved the breathing pattern of pediatric patients by decreasing the respiratory rate to become slow and regular.

Keywords: *pneumonia, blowing balloon, breathing patterns, respiration frequency, clinical respiration score (CRS)*

Introduction

Pneumonia attacks children very easily, this disease is mainly attacked by children's respiratory tract, especially the lungs (UNICEF, 2019). Children with weak immune systems will easily experience respiratory tract infections which are characterized by fever, coughs, colds, rapid breathing and shortness of breath (Ministry of Health, 2019). The following manifestations are signs of pneumonia, which is caused by the bacteria *Streptococcus pneumoniae* (WHO, 2021). Pneumonia attacks the respiratory tract caused by the presence of Pneumococcus, Staphylococcus, Streptococcus bacteria and viruses that enter the child's body **(Johns Hopkins Medicine, 2022).**

In 2019, pneumonia contributed to the death rate in the world with an incidence reaching 740,180 **(WHO, 2021).** Pneumonia is estimated at around 19,000 in 2018 contributing to the mortality rate. Worldwide, it shows that 71 children in Indonesia are infected with pneumonia every hour **(UNICEF, 2019).** According to health profile data in 2019, around ± 400,000 cases of pneumonia occurred in Indonesia **(Ministry of Health, 2019).** The findings of pneumonia cases in 2021 in Bekasi City reached 1,544 cases **(Dinkes, 2021).** Data on the incidence of children aged 3-12 years at Hospital X Bekasi with pneumonia and bronchopneumonia from January to April 2023 were 440 cases.

The process of pneumonia starts from inflammation which results in an increase in sputum production until signs of symptoms appear and problems arise and one of these problems is ineffective breathing patterns, so pneumonia can be treated with immunization and pharmacological treatment, in the form of antibiotics, oxygen therapy and therapy. mucolytic inhalation to dilute sputum **(Suryono, 2020; UNICEF, 2020).**

The non-pharmacological management that can reduce one of them is nursing intervention that can be given to those experiencing this problem based on research on the application of playing balloon blowing (balloon therapy) which can improve oxygenation status in children with respiratory problems and can be used as an approach to traumatic care for children due to the effects of hospitalization, according to research by **Smita *et al.* (2018),** giving balloon therapy to 60 children for 10 minutes every day in 1 session, evaluation of respiratory frequency and oxygen saturation showed significant differences between before and after with p value < 0.0001 (**Smita *et al*., 2018).**

The impact of pneumonia that is not treated properly can result in infections that spread to all organs in the child's body, lung abscesses and pleural effusions or accumulation of fluid in the lining of the lungs **(Suryono, 2020).** This is an undesirable thing if pneumonia is not resolved and can even contribute to the mortality rate. in children in the world (Rich & Melgar, 2021).

At the East Bekasi Private Hospital Based on the reasons for writing above, the author draws the conclusion that he is interested in providing nursing care to children related to pneumonia accompanied by the application of additional implementation in the form of balloon blowing therapy.

Methods

The method used was a case study with 3 child respondents, the respondents were given a balloon blowing therapy intervention whose mechanism was like deep breathing exercises for adults but modified for children with balloon blowing games. The action is carried out by inhaling air from the nose and exhaling it through the protruding mouth, then the air that comes out of the mouth is put into a balloon until the balloon inflates, the air that enters the balloon expands the balloon to the end of the balloon **(Lee *et al*., 2019).**

The procedure is carried out for 3 days, in one day one treatment is given with one inflatable balloon, it can be done in the morning, afternoon or evening, the balloon is inflated until air is filled to the tip of the balloon (inflates) to a size of ±10cm, one session is carried out for 10 minutes interspersed breathing, if the child's response begins to become tired, the action is allowed to stop for 1 minute and then resume **(Harsismanto *et al.*, 2021).** The action begins 1 hour after the child eats and takes medicine (Nithya, 2019). The aim of this therapy is to improve oxygenation status by achieving an optimal ventilation process, reducing respiratory performance, breathing becomes slow and deep so that oxygen transport is better **(Junaidin, 2021).** This innovation is given to children with pneumonia to overcome the oxygenation problem of the patient's breathing pattern, the aim of which is to improve inspiration and expiration so that ventilation is adequate. Inclusion criteria for this case study include children aged 3-12 years, children experiencing signs of respiratory problems, children with medical diagnoses of pneumonia and bronchopneumonia, cooperative children and parents willing to be respondents. Meanwhile, the exclusion criteria used were children who had allergies to latex, children with mental retardation, congenital mouth and nose disorders, problems with the heart, kidneys and chest trauma, receiving other complementary therapy and children with post-oral surgery.

The results evaluation instrument looks at respiratory frequency and uses a clinical respiratory score (CRS) checklist sheet to see the results of pediatric patients' scores in the categories of mild (score <3), moderate (score 4-7) or severe (score 8-12) respiratory disorders. In the CRS instrument, respiratory rate, auscultation results, use of accessory muscles for breathing, consciousness, oxygen saturation and skin color are assessed by assessing a score of 0, 1 or 2 in the classification of each patient's condition. This sheet is used to assess the success of providing nursing care for three days to patients based on changes in the classification of respiratory disorders.

A process evaluation instrument to see improvements in the patient's breathing pattern, seen through the respiratory rate (RR) in the categories of normal, tachypnea and bradypnea which are grouped based on the age of children 3-6 years and children 6-12 years **(Hafen & Sharma, 2022; Reenee, 2022).** In the normal breathing category for children 3-6 years old it is 22-34 x/minute while in children 6-12 years it is 18-30 x/minute, the respiratory frequency in the tachypnea category in children 3-6 years old is >34 x/minute while in children 6-12 years >30 x/minute and respiratory frequency in the bradypnea category in children 3-6 years <22 x/minute while in children 6-12 years <18 x/minute.

Results and Discussions

The results of applying blowing balloon therapy to the 3 patients for 10-15 minutes over a period of 3 days were assessed in the morning, afternoon or evening, looking at the results of evaluating the respiratory frequency process and evaluating the results of assessing respiratory disorders. In patient An. A, aged 9 years 9 months and female, obtained the following results:

Table 1. Evaluation of Process and Results in An. A

Date	Process Evaluation (RR)		Evaluation of Results (Respiratory Disorders)	
	Before	After	Before	After
23/05/2023	RR: 23 x/minute (normal)	RR: 23 x/ minute (normal)	Mild respiratory distress (score 1)	Mild respiratory distress (score 1)
24/05/2023	RR: 22 x/ minute (normal)	RR: 22 x/ minute (normal)	Mild respiratory distress (score 1)	Mild respiratory distress (score 1)
25/05/2023	RR: 22 x/ minute (normal)	RR: 21 x/ minute (normal)	Mild respiratory distress (score 1)	Mild respiratory distress (score 1)

In the first case managed by An. A on the first day, May 23 2023, before being given the action and after being given the action, evaluate the child's RR process in the normal category and evaluate the results before the action is taken for the child in the category of mild respiratory disorders by showing a score of 1 in the RR category aged > 5 years. On the first day there was an increase in SaO2 from previously 96% to 97%, crackles were still scattered on auscultation, chest and nostril retraction were absent, the child's consciousness was normal and the skin color was normal.

On the second day, May 24 2023, before the action was given and after the child was given an evaluation of the RR process in the normal category as well as an evaluation of the results before the action was taken the child was in the mild respiratory disorder category by showing a score of 1 in the RR category aged > 5 years. On the second day there was an increase in the previous SaO2 from 95% to 95%, crackles were still scattered on auscultation, chest and nostril retraction were absent, the child's consciousness was normal and the skin color was normal. On the second day there were no significant changes.

On the third day, May 25 2023, before the action was given and after the action was given, evaluate the child's RR process in the normal category and evaluate the results before the action was taken for the child in the mild respiratory disorder category by

showing a score of 1 in the RR category aged > 5 years. However, on the third day there was a decrease in RR before 22 x/minute while after 21 x/minute and an increase in SaO_2 before 94% after 96%, crackles were still scattered on auscultation, chest and nostril retraction were absent, the child's consciousness was normal and the skin color was normal. Conclusions obtained from giving balloons for 3 days to An. A, there was a decrease in RR from 23 x/minute to 21 x/minute and the category for 3 days was mild respiratory problems. In patient An. AL, 4 years old, female, obtained the following results:

Table 2. Evaluation of Process and Results in An. AL

Date	Process Evaluation (RR)		Evaluation of Results (Respiratory Disorders)	
	Before	After	Before	After
27/05/2023	RR: 25 x /minute (normal)	RR: 25 x/ minute (normal)	Mild respiratory distress (score 0)	Mild respiratory distress (score 0)
28/05/2023	RR: 23 x/ minute (normal)	RR: 22 x/ minute (normal)	Mild respiratory distress (score 0)	Mild respiratory distress (score 0)
29/05/2023	RR: 22 x/ minute (normal)	RR: 21 x/ minute (normal)	Mild respiratory distress (score 0)	Mild respiratory distress (score 0)

In the case of the first management of An. AL on May 27 2023, the first day before the action was given and after the action was given, the child's RR process evaluation was in the normal category and the evaluation of the results before the child's action was carried out in the mild respiratory disorder category showed a score of 0. On the first day there was an increase in SaO2 before 96% to 99% , on auscultation the rhonchi are still scattered, there is no retraction of the chest and nostrils, the child's consciousness is normal and the skin color is normal.

On the second day, May 28 2023, before the action was given and after the action was given, the child's RR process evaluation was in the normal category and the evaluation of the results before the child's action was carried out in the mild respiratory disorder category showed a score of 0. However, there was a decrease in the RR from 23 x/minute to 22 x / minute, and SaO_2 increased from 98% to 99%, crackles were still scattered on auscultation, chest and nostril retraction were absent, the child's consciousness was normal and the skin color was normal.

On the third day, May 29 2023, before being given action, the child's RR process evaluation was in the normal category after being given action into the bradypnea category. Meanwhile, evaluation of the results before and after the child's treatment in the category of mild respiratory problems showed a score of 0. There was a decrease in RR from 22 x/minute to 21 x/minute, and SaO_2 increased from 97% to 99%, crackles were still scattered on auscultation, chest and nostril retractions are absent, the child's consciousness is normal and the skin color is normal. Conclusions obtained from giving balloons for 3 days to An. AL, decreased RR from 25 x/minute to 21 x/minute and category for 3 days of mild respiratory disorders. In patient An. H, aged 5 years 8 months and male, obtained the following results:

Table 3. Evaluation of Process and Results in An. H

Date	Process Evaluation (RR)		Evaluation of Results (Respiratory Disorders)	
	Before	After	Before	After
30/05/2023	RR: 24 x /minute (normal)	RR: 23 x/ minute (normal)	Mild respiratory distress (score 1)	Mild respiratory distress (score 1)
31/05/2023	RR: 24 x/ minute (normal)	RR: 22 x/ minute (normal)	Mild respiratory distress (score 1)	Mild respiratory distress (score 1)
01/06/2023	RR: 21 x/ minute (normal)	RR: 20 x/ minute (normal)	Mild respiratory distress (score 1)	Mild respiratory distress (score 1)

In the case of the first management of An. H on May 30 2023, the first day before the action was given and after the action was given, evaluate the child's RR process in the normal category and evaluate the results before the child was taken in the mild respiratory disorder category by showing a score of 1. On the first day there was a decrease in the RR of 24 x/minute to 23 x/minute and SaO_2 increases before 96% to 99%, crackles are still scattered on auscultation, chest and nostril retraction are absent, the child's consciousness is normal and the skin color is normal.

On the second day, May 31 2023, before the action was given and after the action was given, the child's RR process evaluation was in the normal category and the evaluation of the results before the child's action was carried out in the mild respiratory disorder category showed a score of 1. However, there was a decrease in the RR from 24 x/minute to 22 x/minute, the SaO_2 increased from 96% to 99%, crackles were still scattered on auscultation, chest and nostril retraction were absent, the child's consciousness was normal and the skin color was normal.

On the third day, June 1 2023, before the action was given and after the action was given, evaluate the child's RR process in the bradypnea category and evaluate the results before the child was given action in the mild respiratory disorder category by showing a score of 1. There was a decrease in the RR from 21 x/minute to 20 x/ minutes, SaO2 increased from 97% to 99%, crackles were still scattered on auscultation, chest and nostril retraction were absent, the child's consciousness was normal and the skin color was normal. Conclusions obtained from giving balloons for 3 days to An. H, decrease in RR from 24 x/minute to 20 x/minute and category for 3 days of mild respiratory disorders.

The results of the case study on the three managed patients who were observed and assessed for 3 days with nursing problems with ineffective breathing patterns carried out the application of innovative independent nursing actions using the balloon blowing technique. It was found that the patient's breathing pattern could be improved to become more regular by taking deep breaths and visible exhalations. from a decrease in respiratory frequency. And the responses from the three patients were very happy and enthusiastic when playing with balloons, always looking for time to play with balloons again, overcoming atraumatic care in pediatric patients.

According to WHO, the incidence of pneumonia can cause mortality in children. In 2019, 740,180 children aged < 5 years died due to pneumonia (WHO, 2014, 2022). Children aged 2-5 years are most commonly infected with the respiratory virus streptococcus pneumoniae, especially children aged 5-13 years are most often infected with mycoplasma pneumoniae (**Ebeledike & Ahmad, 2023).** The estimated number of pneumonia cases each year reaches 120 million **(WHO, 2022).** According to **WHO (2022),** pneumonia occurs in pre-school children because their immune system is not yet mature (WHO, 2020), while according to **Ningrum (2019)** in school-aged children, pneumonia can occur because there are several other factors besides immune system and recurrent pneumonia, it can also be caused by environmental factors and house conditions, according to research on ventilation area, wall type, humidity temperature and residential density **(Ningrum, 2019).**

The priority problem found in the three patients was ineffective breathing patterns caused by airway obstruction. Ineffective breathing patterns are ineffective ventilation exchange between oxygen and carbon dioxide **(Mashudi, 2021; PPNI, 2016).** The nursing problem of ineffective breathing patterns in cases of patients with pneumonia is a problem characterized by, this nursing problem is a priority because it has problems with the vital system, namely oxygenation, which has a very important role in regulating ventilation, the release of carbon dioxide and the entry of oxygen into the body, oxygen It is very necessary for metabolism, if oxygen is not met within 4 seconds then the tissue and cells in the brain can be damaged **(Mashudi, 2021).**

There was an increase in oxygen saturation in the case study results, this is in line with research by **Nugroho *et al.* (2018)** the balloon blowing activity carried out on 22 children aged 3-5 years had a significant effect on increasing oxygen saturation with a p value of 0.000 and according to Nugroho, balloon blowing play can be used to treat children with an atraumatic care approach, which is in line with the study In the third case of giving balloon blowing therapy, the patient looked enthusiastic and happy and always looked for the nurse who gave the balloon **(Nugroho *et al.*, 2018).**

Based on the results of the case study, in line with research by **Kharvi *et al.* (2021)** this balloon therapy affects oxygen saturation and respiratory frequency, because by inhaling and then exhaling you can clear the airways, expand the lung parenchyma, increase respiratory muscles, increase expiratory volume because the exhaled air lengthens, when the volume of the balloon is completely filled with when air is exhaled, the respiratory muscles also increase (diaphragm), so that as the ventilation cycle lengthens the contraction of the intercostal muscles increases.

There was a decrease in respiratory frequency in the results of the case study in line with the research of **Raja & Arunachalam (2022).** There was a significant difference in the change in respiratory frequency in 20 children aged 3-6 years with balloon therapy with a significant p value < 0.05 seen from the respiratory frequency before and after providing therapy **(Raja & Arunachalam, 2022).**

The results of the case study are in line with research by **Yunica (2021),** balloon blowing therapy is similar to breathing relaxation, which can increase the muscles in the intercostals and increase the elevation of the diaphragm and ribs. By blowing balloons, the lungs absorb oxygen and release more carbon dioxide from the lungs, becoming a training effort. breathing for the lungs by taking in and releasing air **(Dwi *et al.*, 2020; Yunica, 2021).**

Based on previous research, **Tunik (2017)** is in line with the results of the case study, because during this balloon blowing exercise it will release carbon dioxide trapped in the lungs during exhalation and enter oxygen into the blood during the inhalation phase, this balloon exercise also has an effect on oxygen saturation because it can increase ventilation. alveoli, maintain good gas exchange by removing air trapped in the alveoli so that oxygen saturation can increase **(Dwi *et al.*, 2020; Oktaviani *et al.*, 2021; Tunik & Khoiriyati, 2017).**

According to the author's assumption, this balloon blowing therapy affects the child's oxygenation status, especially on the oxygenation pattern because of the ventilation process where the inspiratory and expiratory phases are optimal, the release of CO_2 that is retained when inflating the balloon, the expiratory phase is prolonged so that CO_2 expenditure is optimal and during the inspiration phase, O_2 is absorbed. becomes better, intercostal muscle contraction increases causing ventilation in the alveoli to increase, breathing performance decreases so that the breathing pattern becomes regular.

Conclusions

For 3 days, the three patients showed a decrease in respiratory frequency with an average RR before the procedure of 24 x/minute and after the procedure of 21 x/minute, and the evaluation of the three patients included mild respiratory disorders. The results for 3 days saw an increase in oxygen saturation with an average of 96% for the three patients before the procedure and 98% after the procedure. The application of blowing balloon therapy is effective in improving breathing patterns, there are no obstacles to the implementation and this therapy can be continued in the pediatric inpatient room at the X Private Hospital, East Bekasi.

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Application of Postural Drainage and Chest Physiotherapy To Increase Airborne Clearance in Children With Bronchopeneumonia

Susi Hartati [1*], Nidia Ainun Rindiani[2]

[1,2]Department of Nursing, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga Bekasi, Indonesia
*Correspondence author : susi@stikesmitrakeluarga.ac.id

Abstract

There is still a high incidence of pneumonia in toodlres. Pneumonia is an infectious disease of microorganisms that affects one or both lungs, pneumonia that inflames the alveoli (tiny air sacs) inside the lungs is a type of bronchopneumonia. This disease causes symptoms of productive cough and shortness of breath with ineffective airway clearance nursing problems. This study aims to analyze the application of postural drainage and chest physiotherapy to improve airway clearance in toddlers with bronchopneumonia. This research uses a descriptive case study with three subjects according to the inclusion criteria is toddlers aged 1-5 years, have medical diagnosis of bronchopneumonia, with problem ineffective airway clearance nursing, and The family signed informed consent to do the therapy. The researcher also determined the exclusion criteria such as patients who suffered chest drain, haemodynamic instability, bone fragility or rib fractures, and any other contraindication to chest physical therapy. Three case studies were carried out in X private hospital in East Bekasi. The inverstions with postural drainage is a technique laying to certain positions to drain secretions from your airways using gravity, the position is adjusted based on the location of the rhonchi sound. Also with chest physiotherapy is a nursing action include percussion (clapping) and vibration. Postural drainage and chest physiotherapy interventions were carried out twice a day in the morning and evening for 3 days. The results are two cases out of three, there was a decrease in the respiratory distress score and there were no additional breath sounds and they were able to cough effectively. In conclusion, Postural drainage and chest physiotherapy are effective in improving airway clearance in toddlers with bronchopneumonia. For this reason, nurses are expected to be able to provide postural drainage therapy and chest physiotherapy to increase airway clearance.

Keywords: *Postural drainage, chest physiotherapy, Airway clearance, Toddlers with bronchopneumonia*

Introduction

In Indonesia, pneumonia is the leading cause of death for toddlers with a percentage of 5.05% (Kemenkes RI, 2021). Pneumonia case finding among toddlres in Indonesia reached 50-70% from 2015 to 2019, which is a significant increase compared to the pneumonia coverage among toddlers from 2010 to 2014, which was 20-30%. In 2020, pneumonia coverage among toddlers in Indonesia decreased to 34.8%, due to the Covid 19 stigma, which resulted in a 30% decrease in visits to health centers for children coughing or having difficulty breathing. In Indonesia, the province of West Java has the 10th highest number of pneumonia cases in toddlers e with a coverage of 31.2%, namely 4,898,913 incidence cases, 68 deaths and 987,630 nonpneumonia cough cases in 2020 (Kemenkes RI, 2021). In Bekasi city, there were 1,544 pneumonia cases among toddlers (4.6%) and 55 children suffered from severe pneumonia (Kemenkes, 2020).

Pneumonia is caused by viral, bacterial and pathogenic infections that cause peripheral alveolar inflammation and consolidation due to the filling of the alveoli cavity by exudates (Chroneos, 2017). Pneumonia is classified based on the location of the infection, namely lobar pneumonia where infection occurs in one or both lung lobes, lobular pneumonia or bronchopneumonia, which often occurs at the end of the bronchioles, with spots due to mucopurulent exudate blockage, and intertitial pneumonia or bronchiolitis where the inflammatory process occurs in the alveolar wall and peri and interlobular tissues (Wahyuni *et al.*, 2023). Pneumonia in children results in an increase in excessive mucus production in the lungs due to the inflammatory process. Airway clearance is a condition in which the respiratory tract is free from the accumulation of secretions (Hanafi & Arniyanti, 2020). Children do not have the ability to cough perfectly so that airway clearance is not perfect. Complications that occur if the ineffectiveness of airway clearance is not treated will result in hypoxia (Sukma, 2020).

Pharmacological therapy of pneumonia in children is antibiotic therapy, antipyretic analgesics, bronchodilators and mucolytics (Meriyani *et al*., 2016). In addition, there are non-pharmacological therapies in the form of passive postural drainage and active chest physiotherapy to improve children's airway clearance. (Kusuma et al., 2022). According to researches Nurhayati *et al.,* (2022) that the application of chest physiotherapy is effective in improving

airway clearance in children. In the study Shutes *et al.* (2019) explained that physiotherapy as an effective measure to overcome airway clearance problems, therefore researchers evaluated airway clearance in children using scoring *Airway Clearance and Expansion Index* (ACE-I). Researcher Nayani *et al.*(2018) also make evaluations using scoring *The Clinical Respiratory Score* (CRS) to measure respiratory distress by categorizing mild, moderate and severe respiratory distress. In addition, international studies have also shown that chest physiotherapy is effective as a non-pharmacological therapy in children with respiratory distress (Chaves *et al*., 2019).

Based on a preliminary study, the incidence of toddlres with pneumonia at Mitra Keluarga Hospital in East Bekasi from January to April 2023 was 182 children. Meanwhile, 126 toddlers with bronchopneumonia were recorded. In the study, postural drainage and chest physiotherapy were effective in reducing additional ronchi sounds, breathing frequency and nasal lobe breathing (Andersson-Marforio *et al*., 2020). The application of postural drainage and chest physiotherapy in the room has not been a routine action by nurses because chest physiotherapy is an action in the physiotherapist room. However, based on the Indonesian Nursing Intervention Standards, physiotherapy becomes a nursing therapeutic intervention. Therefore, as a nurse who plays a role as a care giver, this nursing intervention can be done to overcome the problem of ineffective airway clearance in toddlers with pneumonia.

Methods

The analysis method used is the case study method. The data obtained from the nursing care approach, consisting of assessment, determining diagnoses based on the Indonesian Nursing Diagnosis Standards (SDKI), nursing interventions, implementation of postural drainage and chest physiotherapy and nursing evaluation. Nursing care was carried out on 3 toddlers with inclusion criteria, namely the medical diagnosis of bronchopneumonia, the nursing problem of ineffective airway clearance, children aged 1-5 years and the family agreed. The exclusion criteria were refusing to be treated and there were contraindications to chest physiotherapy such as heart failure, asthmatic status, shock and massive bleeding, severe lung infections, rib fractures, postoperative wounds and lung tumors. Postural drainage given position with sitting head held by pillow with 30 degrees and chest physiotherapy given with clapping and percution. Implementation carried out is postural drainage and chest physiotherapy ffective to be given 2x/day for 15 minutes at 1.5 - 2 hours before or after meals in three days. The evaluation used uses the Airway Clearance and Expansion Index/ACE-I evaluation instrument, ACE-I scoring indicators consist of sound breath score 0 normal or vesikuler, score 1 ronchi, score 2 ronchi with effective cough and score 3 ronchi with ineffective cough (Shutes *et al*., 2019) also the respiratory distress category The Clinical Respiratory Score/CRS, the CRS assessment indicators consist of Respiratory rate score 0 is less then 30×/minute, score 1 is 30-40×/minute, score 2 is over 40×/minute, indicators ausculatation score 0 is good air movement expiratory scattred ronchi, socre 1 is depressed air movement, ronchi rales/crackles, score 2 is severe ronchi, indicators use of accessory muscles score 0 is no use retractions, score 1 is moderate intercostal retractions, score 2 is severe intercostal retractions, indicators of mental status score 0 is normal, score 1 is irritable, agigated, retless, score 2 lethargic, indicators of spO2 score 0 is more than 95%, score 1 is 90-95%, score 2 is less than 90 %, the last is indicators of color score 0 is normal, score 1 is pale to normal, score 2 is cyanotic. The CRS was analysed as a continuous variable less than 3 is mild respiratory distress, 4-7 is moderate respiratory distress and 8-12 is severe respiratory distress (Nayani et al., 2018) evaluated before and after the action is taken. Case studies were conducted from May 23, 2023 - June 03, 2023.The results of nursing care that has been carried out are then analyzed qualitatively by comparing theory and previous research.

Results and Discussions

Based on age and gender, the case studies conducted in this research are shown in the table below:

Table 1. Respondent Characteristics

No.	Initials	Gender	Age
1	An.S	Male	3 years old
2	An.A	Male	3 years old
3	An.N	Female	5 years old

The number of respondents in this case study were 3 children with a medical diagnosis of bronchopneumonia and a nursing problem of airway clearance, namely An. S, male, 3 years old, An. A, male gender, age, 3 years and An. N,

female, 5 years old. After postural drainage and chest physiotherapy with a frequency of twice a day for 3 days, an evaluation was carried out before and after the action using an assessment score. Clinical Respiratory Score / CRS (Shutes *et al*., 2019) and using Airway Clearance and Expansion Index / ACE-I (Nayani *et al.*, 2018) the results are as follows:

Table 2. Evaluation of Respiratory Distress Level and Breathing Sounds on Day 1

No.	Initials	Before Interventions	After Interventions
1	An.S	Score CRS: Mild Distress Respiratory Score ACE-I 3: Ronchi with ineffective cough	Score CRS: Mild Distress Respiratory Score ACE-I 3: Ronchi with ineffective cough
2	An.A	Score CRS: Mild Distress Respiratory Score ACE-I 3: Ronchi with ineffective cough	Score CRS: Mild Distress Respiratory Score ACE-I 3: Ronchi with ineffective cough
3	An.N	Score CRS: Mild Distress Respiratory Score ACE-I 3: Ronchi with ineffective cough	Score CRS: Mild Distress Respiratory Score ACE-I 3: Ronchi with ineffective cough

Based on the table above in An.S before the postural drainage and chest physiotherapy actions the results of the assessment of RR 21 ×/minute, oxygen saturation 98% with a CRS score of mild respiratory distress and an ACE-I Ronchi score with an ineffective cough. Then after performing postural drainage and chest physiotherapy, the results of the assessment of RR 21 ×/minute, oxygen saturation 100% with a CRS score of mild respiratory distress and an ACE-I Ronchi score with an ineffective cough. In An.A before the action of postural drainage and chest physiotherapy, the results of the assessment of RR 24 ×/min, oxygen saturation 98% with a CRS score of mild respiratory distress and an ACE-I Ronchi score with an ineffective cough. After the action of postural drainage and chest physiotherapy, the results of the assessment of RR 24 ×/minute, oxygen saturation 100% with a CRS score of mild respiratory distress and an ACE-I Ronchi score with an ineffective cough. Also in an.N before the postural drainage and chest physiotherapy measures were carried out the results of the assessment of RR 25 ×/min, oxygen saturation 98% with a CRS score of mild respiratory distress and an ACE-I Ronchi score with an ineffective cough. Then after performing postural drainage and chest physiotherapy the results of the assessment of RR 25 ×/minute, 100% oxygen saturation with a CRS score of mild respiratory distress and an ACE-I Ronchi score with an ineffective cough.

Table 3. Evaluation of Respiratory Distress Level and Breathing Sounds Day 2

No.	Initials	Before Interventions	After Interventions
1	An.S	Score CRS: Mild Distress Respiratory Score ACE-I 3: Ronchi with ineffective cough	Score CRS: Mild Distress Respiratory Score ACE-I 3: Ronchi with ineffective cough
2	An.A	Score CRS: Mild Distress Respiratory Score ACE-I 3: Ronchi with ineffective cough	Score CRS: Mild Distress Respiratory Score ACE-I 2: Ronchi with effective cough
3	An.N	Score CRS: Mild Distress Respiratory Score ACE-I 3: Ronchi with ineffective cough	Score CRS: Mild Distress Respiratory Score ACE-I 2: Ronchi with effective cough

Based on the table above in An.S before the postural drainage and chest physiotherapy actions the results of the assessment of RR 25 ×/minute, oxygen saturation 98% with a CRS score of mild respiratory distress and an ACE-I Ronchi score with an ineffective cough. Then after performing postural drainage and chest physiotherapy, the results of the assessment of RR 21 ×/minute, oxygen saturation 100% with a CRS score of mild respiratory distress and an ACE-I Ronchi score with an ineffective cough. In An.A before the action of postural drainage and chest physiotherapy, the results of the assessment of RR 24 ×/min, oxygen saturation 98% with a CRS score of mild respiratory distress and an ACE-I Ronchi score with an ineffective cough. After performing postural drainage and chest physiotherapy, the results of the assessment of RR 23 ×/min, oxygen saturation 100% with a CRS score of mild respiratory distress and an ACE-I Ronchi score with an effective cough. Also in an.N before the postural drainage and chest physiotherapy measures were carried out the results of the assessment of RR 25 ×/min, oxygen saturation 98% with a CRS score of Mild Disturbance of Breathing and an ACE-I Ronchi score with an ineffective cough. Then after performing postural drainage and chest physiotherapy the results of the assessment of RR 23 ×/min, 100% oxygen saturation with a CRS score of mild respiratory distress and an ACE-I Ronchi score with an effective cough.

Table 3. Evaluation of Respiratory Distress Level and Breathing Sounds on Day 3

No.	Initials	Before Interventions	After Interventions
1	An.S	Score CRS: Mild Distress Respiratory Score ACE-I 3: Ronchi with ineffective cough	Score CRS: Mild Distress Respiratory Score ACE-I 3: Ronchi with ineffective cough
2	An.A	Score CRS: Mild Distress Respiratory Score ACE-I 2: Ronchi with effective cough	Score CRS: Normal Respiratory Score ACE-I 0: Normal/Vesikuler
3	An.N	Score CRS: Mild Distress Respiratory Score ACE-I 2: Ronchi with effective cough	Score CRS: Normal Respiratory Score ACE-I 0: Normal/Vesikuler

Based on the table above in An.S before the postural drainage and chest physiotherapy actions the results of the assessment of RR 25 ×/minute, oxygen saturation 98% with a CRS score of mild respiratory distress and an ACE-I Ronchi score with an ineffective cough. Then after performing postural drainage and chest physiotherapy the results of the assessment of RR 20 ×/minute, 100% oxygen saturation with a CRS score of mild respiratory distress and an ACE-I Ronchi score with an ineffective cough. In An.A before the action of postural drainage and chest physiotherapy the results of the assessment of RR 22 ×/minute, oxygen saturation 98% with a CRS score of mild respiratory distress and an ACE-I Ronchi score with an ineffective cough. After the action of postural drainage and chest physiotherapy, the results of the assessment of RR 20 ×/minute, oxygen saturation 100% with CRS score No Breathing Disorders and ACE- I Normal / Vesicular score. Also in an.N before the postural drainage and chest physiotherapy measures were carried out the results of the assessment of RR 23 ×/min, oxygen saturation 98% with a CRS score of Mild Disturbance of Breathing and an ACE-I Ronchi score with an effective cough. Then after the action of postural drainage and chest physiotherapy the results of the assessment of RR 20 ×/minute, 100% oxygen saturation with CRS score No Breathing Disorders and ACE- I Normal / Vesicular score.

In two of the three cases, the assessment results before postural drainage and chest physiotherapy showed an increase in airway clearance. After the action of postural drainage and chest physiotherapy, two of the three children experienced an increase in airway clearance. The application of position, percussion and vibration in children can mobilize secret in the airway so as to increase airway patency. Increased airway patency where RR is normal, regular breathing rhythm, no ronchi and able to remove sputum are indicators of increased airway clearance (Febriyani *et al.*, 2021). In addition, physiologically, percussion techniques in chest physiotherapy cause waves in the chest wall, namely amplitude and frequency so that the consistency and location of the secret changes (Tehupeiory & Sitorus, 2022). Percussion technique is a manual technique in the form of a pat on the chest / back under the patient's arm with the aim of loosening thick and sticky mucus from the side of the lungs. While the vibration technique is a vibration technique to push the secret out. Then in the application of postural drainage where the position is adjusted to the secret retained in the child, the position setting is opposite to the location of the segment where the secret accumulation occurs (Hanafi & Arniyanti, 2020). Research Wardiyah *et al*. (2022) showed that the application of chest physiotherapy was effective in clearing airway secretions. In the study Pangesti & Setyaningrum, (2020) showed similarly that non-pharmacological techniques of chest physiotherapy effectively address airway clearance in children with airway disease. In the study Ken *et al.*(2022) showed that chest physiotherapy therapy was effective in overcoming airway clearance problems in patients with pneumonia. In the study Syafiati & Nurhayati (2021) also showed that chest physiotherapy is effective for children aged 3-6 years with ineffective airway clearance problems. Other studies have shown that postural drainage and chest physiotherapy have an effect on improving airway clearance with differences in sputum production and ronchi sounds that become vesicular in children with bronchopneumonia (Sarina & Widiastuti, 2023).

In the first case, An.S, the evaluation of the child still did not experience an increase in airway clearance, this was due to the intensity of providing chest physiotherapy which was not continuous due to the child who was not cooperative during the implementation due to the impact of hospitalization experienced by the child (Tehupeiory & Sitorus, 2022). In addition, the child's inability to cough effectively is to inhale through the nose and exhale through the mouth to form the letter (Hanafi & Arniyanti, 2020). The ability to cough effectively is also influenced by age, where age is a factor in whether the patient can be taught to cough effectively or not. In addition, there is the factor of long treatment or entering the last month of treatment (Widiastuti & Siagian, 2019). Similar to the case of An.S due to the age of 3 years who have not been able to follow the direction of effective coughing and a long history of lung infection. Therefore, the application of postural drainage and physiotherapy in this case should be longer because the longer the intervention is carried out, the more changes in airway clearance will be shown (Tehupeiory & Sitorus, 2022). The application of postural drainage and chest physiotherapy is a recommendation for independent nursing actions in the room to improve airway clearance effectively.

Conclusions

Based on the results of the case study analysis of the application of postural drainage and chest physiotherapy in three bronchopneumonia children with ineffective airway clearance problems, it was found that two children out of three children who performed postural drainage and chest physiotherapy 2 times a day for 3 days had an impact on the score of respiratory distress to decrease and additional breath sounds were absent and could cough effectively so that the goal of improved airway clearance was achieved. Whereas one child did not experience significant improvement which was influenced by factors of the child's condition, history of recurrent lung infections and the length of chest physiotherapy itself It can be concluded that the application of postural drainage and chest physiotherapy is effective in improving airway clearance in children with bronchopneumonia.

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The Effectiveness of Education With Video Media on The Knowledge and Attitudes of Family Planning Dropout Acceptors in The Work Area of The Central Kaliabang Health Center, Bekasi City

Parikesit Nuril Kartika[1]*, Edita Astuti Panjaitan[2], Lina Herida Pinem[3]

[1,2,3] Department of Nursing, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Bekasi, Indonesia
*Correspondence: parikesitnuril@gmail.com

Abstract

Population growth in Indonesia has increased, and the Indonesian Government has established a helpful program to emphasize the population, namely the Family Planning program. It is known that many family planning acceptors have dropped out. Family planning dropout is the event of stopping the use of previously used contraceptives. One factor that influences dropout is the need for more knowledge. This research aimed to determine the effectiveness of education using video media on the knowledge and attitudes of dropout family planning acceptors in the work area of the Central Kaliabang Community Health Center, Bekasi City. The method used in this research is a pre-post test. The results of this research are that there is educational effectiveness at the level of knowledge before and after education. In contrast, there is no educational effectiveness in attitudes before and after education. This research concludes that there is no effectiveness of education using video media on the knowledge of family planning acceptors who have dropped out, and there is no education using video media on the attitudes of family planning acceptors who have dropped out.

Keywords: *attitudes, dropout, education, family planning, knowledge, videos.*

Introduction

The Indonesian Government has established a helpful program to increase population size, namely the Family Planning. It is known that many family planning acceptors experience discontinuation or drop out. The large number of dropout incidents will result in an increase in the population, which will have an impact on community welfare. *Education* is an activity used to motivate increasing knowledge, changing attitudes, and changing behavior/actions correctly. Information will be provided during education on how couples choose which contraceptives to use according to their needs, assisting couples in using appropriate contraceptives, and explaining the advantages and disadvantages of the chosen contraceptives. This research aims to determine the effectiveness of education using video media on the knowledge and attitudes of family planning acceptors who have dropped out.

Methods

This research design was used to test the influence of health education on the knowledge and attitudes of dropout family planning acceptors in the work area of the Central Kaliabang Community Health Center, North Bekasi City. This research uses a quantitative method with an intervention study approach with pre-experimental and planning methods, namely, one group pre-test and one group post-test, by providing measurements before the intervention is carried out and comparing the measurements after the intervention. The incident group's knowledge level will be measured using a questionnaire before the intervention is carried out. The aim of the intervention is that it can influence or change the dropout event variable in the treatment group. After receiving treatment, the dropout event group will be measured again with a questionnaire.

Results and Discussions

Respondent Characteristics

The number of respondents in this study was 39 people. The following is the distribution of respondents based on the respondent's age, occupation, education, parity status/number of children, history of family planning use, type of family planning used previously, and partner support, which can be seen in **Table 1.**

Table 1. Characteristics of Research Respondents (N=39)

Variable	N	%
Age		
Late Teenagers (17-25)	3	7.7
Early Adulthood (26-35)	15	38.5
Late Adulthood (36-45)	16	41.0
Early Seniors (46-55)	5	12.8
Total	39	100.0
Work		
Doesn't work	32	82.1
Work	7	17.9
Total	39	100.0
Education		
Low (Not elementary school)	2	5.1
Intermediate (Junior High School)	33	84.6
Higher (Diploma-Bachelor)	4	10.3
Total	39	100.0
Parity Status		
Primipara (1)	10	25.6
Multiparous (2 or more)	29	74.4
Total	39	100.0
History of birth control use		
Yes	39	100
No	0	0
Total	39	100.0
Types of Contraception		
Family planning pills	8	20.5
KB injection	20	51.3
Implants/Implants	1	2.6
IUD/IUD	10	25.6
Total	39	100.0
Reasons for Dropping Out		
Weight Changes	8	20.5
Health problems	9	23.1
Menstrual Cycle Problems	22	56.4
Total	39	100.0
Spousal Support		
No	4	10.3
Yes	35	89.7
Total	39	100.0

Source: Primary Data, 2023

Table 1 shows that the highest percentage of respondents are late adults aged 36-45 years, namely 41.0%, and the lowest age group is the late teens aged 17-25 years, which amounted to 7.7%. Thirty-two respondents (82.1%) worked, and 7 (17.9%) did not. The educational level of the respondents varied. As many as 33 respondents (84.6%) had secondary education, and only 2 respondents (5.1%) had a low level of education. The parity status of respondents was dominated by multipara (74.4%). All respondents in this study had previously used contraception. Injection contraception is the highest type of contraception used, namely 51.3%, and only 1 respondent (2.5%) uses an implant. The most significant reason respondents experienced family planning dropout was menstrual cycle problems, with 22 respondents (56.4%) and 8 respondents experiencing weight changes (20.5%). The majority of respondents received support from their partners when using contraception, namely 89.7%, and only 10.3% of respondents did not receive support from their partners.

Respondent's Knowledge Level

The respondents' knowledge in this study was measured before and after video education. Data regarding the level of knowledge before and after education can be seen in table 2

Table 1. Level of Knowledge Before and After Education

Variable	Before		After		p value
	n	%	n	%	
Knowledge level					0,000*
Low (<60)	8	20.5%	0	0	
Medium (60-79)	17	43.6%	0	0	
High (80-100)	14	35.9%	39	100.0	
Total	39	100.0	39	100.0	

*Wilcoxon test

Based on the table above, the level of knowledge of respondents shows that there are differences before and after the intervention. Before being given health education, it was found that 17 respondents had moderate knowledge, 14 respondents had high knowledge, and 8 respondents had low knowledge. After being given education, none of the respondents experienced a decrease in their level of knowledge. A total of 35 respondents experienced increased knowledge, and 4 respondents had a constant level of knowledge. So, based on the results of statistical testing using the Wilcoxon test, a significant result of p=0.000 or a smaller value of α=0.005 was obtained, so H0 was rejected, and H1 was accepted. This shows that there is effectiveness in education using video media on the knowledge of dropout family planning acceptors in the work area of the Central Kaliabang Community Health Center, Bekasi City.

Attitude

The respondents' attitudes in this study were measured before and after the video education. Data regarding attitudes before and after education can be seen in **table 3.**

Table 3. Respondents' Attitudes Before and After Education

Variable	Before		After		p value
	n	%	n	%	
Attitude					0.058*
Low (<60)	1	2.6%	0	0	
Medium (60-79)	32	82.1%	28	71.8%	
High (80-100)	6	15.4%	11	28.2%	
Total	39	100.0	39	100.0	

***Wilcoxon test

Based on the table above, it was found that there were differences in respondents' attitudes before and after the intervention. Before being given health education, 32 respondents had a medium attitude, 6 had a high attitude, and 1 had a low attitude. After health education, 29 respondents' attitudes remained unchanged, 8 respondents' attitudes improved after being given education, and 2 respondents experienced a decrease in attitudes. In statistical testing using the Wilcoxon test, significant results were obtained at p=0.058 or greater than the value α=0.005, so H0 was accepted, and H1 was rejected. This shows that there is no effectiveness of education using video media on the attitude of family planning acceptors who drop out in the work area of the Central Kaliabang Community Health Center, Bekasi City.

Selected respondents are respondents who meet the inclusion and exclusion criteria. This study found that the highest percentage of respondents were late adults aged 36-45 years, namely 41.0%, and the lowest age group was the late teens aged 17-25 years, namely 7.7%. This is by the fertile age group, namely, 15-49 years old (Harahap, 2022). Results from the research Nurjannah & Susanti (2018) there is no relationship with the incidence of dropout of contraceptive users. Even though respondents were divided into working and non-working categories, this did not influence respondents in choosing not to use contraception or dropping out. In this modern era, a person can obtain

information through any means, including social media or the internet, which can be accessed via cell phone. Homemakers usually get information through experience and limited information compared to working mothers. Working mothers have a more comprehensive range of information so that they can exchange information or experiences with their co-workers. In the digital era, the internet allows anyone to access all information, including homemakers. Respondents who are not working also find it easy to obtain information through counseling delivered by the local health center (Lasmini *et al*., 2021).

Parity is the number of births experienced by a mother, including miscarriages or births of live and stillborn babies. Parity can also be interpreted as the status of a mother, which is related to the number of children born (Nurjannah & Susanti, 2018). Partner support is a form of emotional, informational, instrumental assistance and appreciation from partners for using contraception. Husband's support is provided with emotional support from the husband's participation in discussions related to family planning, informational support provided in the form of advice and opinions given by the husband to his partner can determine the use of the type of contraception, appreciation support given in the form of accompanying the wife to carry out routine control or reuse contraceptives, instrumental support provided in the form of materials or fees provided to obtain contraceptive services (Amraeni, 2022).

Success in implementing the Family Planning program depends on community participation. It is hoped that a high level of community participation in the Family Planning program will be achieved by its objectives. Community participation in family planning programs must be a driving force to increase women's empowerment to make decisions for themselves and their families (Amraeni, 2022). The media used to increase knowledge will be more effective if you use video rather than other educational media because video is a media that is attractive and does not make you bored; it contains audiovisuals, as well as pictures and direct explanations by educators so that education is accessible for the audience to understand in increasing knowledge (Nurrita, 2018).

Audiovisual media is a medium for conveying educational material through sight and hearing. Audiovisual media lets someone hear, see, observe something, and visualize what is on the audience's mind. Video media can be provided effectively because the material's content is accompanied by a method for installing family planning and an explanation of family planning with animation and sound. Video media has the advantage of attracting the audience's attention; the images displayed can provide messages indirectly but are accessible for the audience to understand. Conveying messages using images helps the audience concentrate more on delivering the material and develop imagination and motivation (Lasmini *et al*., 2021).

In the research carried out by Kartikawati *et al.* (2021), The attitude of respondents who were given health education using video media increased by an average of 22.31, and the interest of respondents after being given the education was that several respondents intended to use the IUD. Video media is considered more effective in improving respondents' attitudes. The research carried out by Rokayah and Kurniawati (2015) showed that most respondents had a negative attitude, 59.2%, and a positive attitude, 40.8%. This negative attitude causes acceptors to drop out because they do not understand the information related to family planning program education. Attitude plays a vital role in everyday life; if a person's attitude has been formed, it must be accompanied by changes in behavior towards something. In determining the response of dropout acceptors, the more positive the attitude, the easier it will be to understand, accept, and return to using contraception. However, on the other hand, if the negative response is lower, it will be challenging to encourage acceptors to return to using contraception. (Ernita Amru, 2017).

In line with theory (2017), Health education is an effort to change a person's knowledge, attitudes, and habits to achieve health goals. Health education is provided to individuals, families, and communities to help them achieve healthy living behavior. With health education, individuals, families, and communities can change how they think and behave and change men's behavior to resolve health problems. Health education raises public awareness of lifestyle and habitual mistakes to influence people's desire to change to a healthy life. Health education plays a role in helping patients monitor their health condition by influencing and strengthening decisions about actions that suit them. The research by Martiana *et al.* (2022) shows that increasing knowledge does not always align with changing attitudes. This research showed an increase in attitudes after being given education compared to before. Positive attitudes have increased significantly, while negative attitudes have decreased, although not significantly. Factors that influence attitude change are only sometimes in line with increased knowledge. In this study, it is possible to change attitudes due to changes in knowledge. However, it cannot be ensured that attitude changes occur due to increased knowledge in the intervention group.

Conclusions

There is the effectiveness of education using video media on the knowledge of drop out family planning acceptors in the work area of the Central Kaliabang Community Health Center, Bekasi City. There is no effectiveness of education using video media on the attitude of family planning acceptors who drop out in the work area of the Central Kaliabang Community Health Center, Bekasi City.

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The Relationship Between Utilization of the Maternal Child Handbook (MCH) with The Health Status of Pregnant Women at the Pengasinan Health Center in Bekasi City 2023

Fardilla Rochman Utami[1]*, Edita Astuti Panjaitan[2], Lina Herida Pinem[3]

[1,2,3] Department of Nursing, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Bekasi, Indonesia

Abstract

Introduction: Mother Mortality Rate (MMR) and Infant Mortality Rate (IMR), which continue to increase, make the government take various ways to reduce these rates. Utilization of the MCH handbook is one of the government's efforts to suppress MMR and IMR. From the results of the preliminary study, it is known that many mothers still rarely read their MCH books, and some mothers have risks in their pregnancies. Objective: To identify the relationship between using MCH handbooks and maternal health status at Pengasinan Bekasi Community Health Center 2023. **The method** of this research uses quantitative research methods. This research design uses a cross-sectional design to determine the correlation between the use of MCH handbooks and the mother's health status. The population was 95 respondents. The sample in this research was taken using a purposive sampling technique. After calculating using Issac Michael's formula, the sample size was 76 respondents. The data normality test was carried out, and the p-value of the Kolmogorov test was $0.000 \leq \alpha$ (0.05). It was stated that the data distribution was not expected, so the bivariate test used was the Spearman correlation test. T**he result** of this research is a relationship between the variable utilization of MCH handbooks and the health status of mothers at the Pengasinan Community Health Center with p value $0.028 \leq \alpha$ (0.05). The direction of the relationship obtained from the value of r is 0.253, which is positive, meaning that the higher the utilization of the respondent's MCH handbook, the higher the health status and vice versa.

Keywords: *Utilization of MCH handbook, health status of pregnant women, pregnancy*

Introduction

Mortality in mothers and babies in Indonesia has increased from year to year. The Maternal Mortality Rate (MMR) is the ratio of maternal deaths in every 100,000 live births, and the Infant Mortality Rate (IMR) is the ratio of infant deaths per 1,000 live births. The decline in MMR and IMR cases indicates the success of implementing the Sustainable Development Goals (SDG) program.

According to the Ministry of Health of the Republic of Indonesia, in 2018 the number of MMR was 4,226 people and decreased in 2019 to 4,221 people. Then, in 2020, the number of MMR increased by 4,627 people; in 2021, the number of MMRs increased sharply to 7,389 people (Ministry of Health of the Republic of Indonesia, 2021). Data in Bekasi City in 2018 showed that the number of reported MMR was 18 people out of 100,000 birthing or pregnant women. In 2019, the reported MMR was 16 out of 100,000 giving birth to pregnant women, and in 2020, 15 out of 100,000 birthing or pregnant women (Bekasi Health Office, 2021).

There have been many methods and efforts by the government to reduce the MMR. The use of Maternal and Child Health books is one of several programs run by the government to reduce MMR cases. The policy on the use of the MCH handbook is regulated in the Decree of the Minister of Health Number 284/MENKES/SK/III/2004, which states that the MCH handbook can be used for the information for mothers, health workers, families and the community in knowing health status, health documentation, early detection related risks, conducting counselling and monitoring toddler growth and development. The benefits of using the correct MCH handbook can make mothers and their families understand more about the health of mothers and children, increase community movement and empowerment to live healthier lives, increase public awareness of quality services, increase health surveillance and monitoring and increase health information (Hanum & Safitri, 2018). The MCH Handbook was used for the first time in Indonesia in 1994, with the first trial being conducted in Salatiga, Central Java. At that time, Indonesia and an organization in Japan, namely the Japan International Cooperation Agency (JICA), developed the Pink Book or MCH Handbook. In 1997, almost every district/city in Central Java had put into practice the use of the MCH handbook. In 2001, almost all districts or cities in Indonesia used the MCH handbook (Suparmi *et al*., 2018).

Based on the results of health research in 2013, 19.2% of pregnant women did not have an MCH Handbook, as many as 39.9% of pregnant women could not show the MCH Handbook, and as many as 40.9% of pregnant women have and can show the MCH Handbook (Ministry of Health RI, 2014). Meanwhile, in 2018, 30% of pregnant women did not have an MCH Handbook, as many as 10% of pregnant women could not show an MCH Handbook, and as many as 60% of pregnant women had and could show an MCH Handbook (Ministry of Health of the Republic of Indonesia, 2019).

The results of a preliminary study at the Pengasinan Bekasi Health Center of 13 pregnant women, 12 of them said they had MCH Handbooks but rarely read MCH Handbooks. 13 of the pregnant women interviewed had a history of pregnancy complications, namely 1 person with anaemia and 2 more people with an upper arm circumference of more than 23.5 cm. Pregnant women rarely read MCH Handbooks because they are busy taking care of the house, children and husband, so they rarely have free time to read MCH books. Health workers at the Puskesmas have socialized the use of the MCH handbook. Every pregnancy check-up, immunization, posyandu, and counselling activity for mothers, constant reminders to complete and read the MCH handbook. However, in practice, pregnant women have not used the MCH Handbook.

Methods

This research uses quantitative methods. This research uses a cross-sectional design to determine the correlation between using MCH handbooks and the mother's health status. This research was conducted at the Pengasinan Community Health Center, Bekasi. The population was 95 respondents. The sample in this research was taken using a purposive sampling technique. After calculating using Issac Michael's formula, the sample size was 76 respondents. The inclusion criteria of pregnant women, pregnant women who own MCH Handbooks, pregnant women in the Pengasinan Health Center working area, pregnant women who are willing to be respondents and the exclusion criteria in this study are mother pregnant women who do not have an MCH handbook and pregnant women who are not willing to be respondents. The research was conducted from April – July 2023.

The independent variable in this study is the use of MCH Handbooks, which is assessed using the MCH Handbook utilization questionnaire, and the dependent variable in this study is health status, which is assessed with the Poedji Rochjati Score Card (KSPR) to determine the risk to pregnant women. Interpretation of the KIA book utilization questionnaire, MCH handbook utilization is negative if the score value is ≤ 50 and positive MCH handbook utilization if the score value is > 50 (Munna et al., 2020). To interpret the KSPR score, it is Very Highrisk Pregnancy (KRST): Score ≥ 12 (Red), High-Risk Pregnancy (KRT): Score 6-10 (Yellow), Low-Risk Pregnancy (KRR): Score 2 (Green) (Hastuti *et al.*,2018). The analysis carried out in this research was univariate (frequency distribution) and bivariate (Spearman correlation test).

Results and Discussions

Result of this study consist of univariat analysis and bivariat analysis

The characteristics of the respondents in this study include; age, occupation and education

Table 1. Frequency distribution of age, occupation, education

Variabel	Frequency	Percentage
Usia:		
No Risk	57	75 %
Risk	19	25%
Total	76	100%
Occupation :		
No Job	68	89,5%
Have a Job	8	10,5%
Total:	76	100%
Educational Level:		
Elementary School	9	11,8%
Junior High School	12	15,8%
Senior High School	45	59,2%
Collage	10	13,2%
Total	**76**	**100%**

Based on the data above, it can be seen that as many as 57 respondents (75%) are in an age that is not at risk, and as many as 19 respondents (25%) are in an age that is at risk for pregnancy. The majority of the respondents who work are homemakers who live at home, so they are categorized as not working as many as 68 respondents (89.5%) and for respondents who work as many as 8 respondents (10.5%). The last education that most respondents took was high school, with a total of 45 respondents (59.2%), followed by junior high school with 12 respondents (15.8%), then tertiary institution with 10 respondents (13.2%), and most The least is elementary school as many as 9 respondents (11.8%).

In the research conducted, the respondents were 76 pregnant women. The characteristics of the respondents collected include age, occupation, education, utilization of the MCH handbook, and health status. This research found that most respondents were of a non-risk age, did not work or were homemakers, their last education was high school, their use of MCH Handbooks was good, and their health status was at risk. In research conducted Farida (2016), 63 respondents (58.3%) used MCH handbooks, and those who did not use MCH handbooks were 45 respondents (41.7%). Age 20-35 is still in the productive age, so many respondents who are at that age still use the MCH handbook. This is also in line with research conducted by Napitupulu *et al*. (2018), as many as 95 respondents (63%) of pregnant women aged 20 -35 used the MCH Handbook, and 56 respondents (43%) did not. At the age of 20, respondents tend to use the MCH Handbook more because they feel that pregnancy checks are essential, unlike respondents over 30, who tend to use the MCH Handbook less. They feel they have enough experience in pregnancy because, on average, they already have children. This is different from research conducted Herfanda & Subiyatun (2021), which states that working mothers make more use of the MCH handbook, with 35 respondents (58.4%) working. Her work makes working mothers more aware of the importance of information related to pregnancy, which is also in the MCH handbook.

The last education that most respondents took was high school, with a total of 45 respondents (59.2%), followed by junior high school with 12 respondents (15.8%), then tertiary institution with 10 respondents (13.2%), and most The least is elementary school as many as 9 respondents (11.8%). The higher the education you have, the better your knowledge will be. It is hoped that better information transformation will be achieved. However, this comes back to each individual. This is in line with research conducted by Munna *et al*. (2020), which stated that as many as 30 respondents (66.7%) of respondents with a senior high school education used the MCH handbook. A mother's education influences the breadth of insight she has. Knowledge or information obtained from formal education is expected to facilitate the transformation of the knowledge gained. This research shows that good use of MCH Handbooks occurs because the educational background of the majority of respondents is good. So that there is awareness of the importance of information related to pregnancy. With good information about pregnancy, it is hoped that it will impact their health status.

Utilization of MCH Books

Table 2 Frequency distribution of KIA book use

Utilization Of MCH Books	Frequency	Percentage
Negative	13	17,1%
Positive	63	82,9%
Total	76	100%

Based on the table above, it is known that 63 respondents (82.9%) used MCH Handbooks positively, and as many as 13 respondents (16.9%) used them negatively. This is in line with research conducted by Jannah (2015), which was conducted on 180 respondents. It was found that 134 respondents (74.4%) used the MCH handbook, and 46 respondents (25.6%) did not use the MCH handbook. A good use of the MCH handbook comes from self-awareness to learn about pregnancy. The respondent's last education influences pregnant women's use of the MCH handbook. So, mothers with higher education levels use the MCH handbook more. Insufficient use of MCH books can be caused by a lack of awareness and sensitivity of pregnant women towards their pregnancy (Rahmi *et al*., 2018).

From this study, it is known that the higher the last education attained by the respondents, the better the utilization of the MCH handbook. The majority of respondents are in the productive age (20-30 years), making the respondents make good use of the MCH handbook. In addition, most respondents are housewives who only stay at home every day and take care of household affairs, children, and husbands, giving respondents little free time to read the information in the MCH handbook. Even though the MCH book is not the primary source of information used by pregnant women, the MCH book has a good influence on its users.

Health Status

Table 3 Frequency distribution of maternal health status

Health Status of Pregnant Woman	Frequency	Percentage
Very High Risk Pregnancy	21	27,6%
High Risk Pregnancy	23	30,3%
Low Risk Pregnancy	32	42,1%
Total	**76**	**100%**

Based on the table above results, it is known that KSPR assesses the health status of pregnant women to detect risks to the respondent's pregnancy. A total of 21 respondents (27.3%) were pregnant women at very high risk, 23 respondents (30.3%) were pregnant women at high risk and 32 respondents (42.1%) were at low risk. Maternal health status is essential to know in pregnant women. With early detection using the easy-to-use Poedji Rochjati Score Card (KSPR), it is hoped that pregnant women will be able to know the risk status of their pregnancy. The risks in pregnancy are dynamic according to the mother's condition. Usually, acceptable mothers in the first trimester of pregnancy can change their health status as their gestational age increases (Putri & Purnomo, 2017). If the mother is in poor health, in which case her pregnancy is at risk, the fetus may also experience complications in its development. Good health status in pregnant women can be realized if the mother regularly undergoes pregnancy checks according to government recommendations at least 6 times during pregnancy so that if there are complications in her pregnancy, they can be detected and treated early, in addition to a healthy diet, adequate rest and management. Good stress will make the mother and fetus healthier. In the research conducted, it was found that the majority of respondents were in a health status that was at high risk of pregnancy. The behavioural factor is the patient's belief that she is sure of the condition of her pregnancy because most patients have been pregnant before.

Bivariate analysis was used to determine whether there was a relationship between the use of MCH Handbooks and the health status of pregnant women. The test used in this bivariate analysis is the Spearman correlation test. Because the results of the data normality test obtained the Kolmogorov test results p – value 0.000 <α (0.05), it can be concluded that the data on utilization of the MCH handbook and health status were not normally distributed. Therefore, the test used is the Spearman correlation test.

Table 4. Correlation between Utilization of the MCH Handbook and Mother's Health Status

Variable	R value (Coefficient of Correlation	P Value
Utilization of KIA books with Health Status of Pregnant Women	0,253	0,028

From the table above, it is known that there is a relationship between the variable utilization of the MCH handbook and the health status of pregnant women with a p-value of 0.028 ≤ α (0.05). The direction of the relationship obtained from the r value is 0.253, which is positive because the higher the utilization of the respondent's MCH handbook, the higher the health status and vice versa. In this research, it was discovered that the majority of the use of MCH books was good, but the health status of the majority of pregnant women was still at high risk. According to the researcher's assumption, this occurs because most respondents' parity status is multigravida, which initiates respondents to make more use of the MCH book from this experience in their current pregnancy. Some respondents' adherence to the MCH handbook was sourced from the information or knowledge they received from various media. Pregnancy records in the MCH handbook are also instrumental in liaison between health workers. According to the researchers' assumption, this happened because most of the respondents were pregnant with their second or third child, so in previous pregnancies, they already had an MCH Handbook. This makes respondents understand more about the contents of the MCH Handbook.

Utilization of the MCH handbook is one of the priority programs promoted by the Indonesian government. Effective use of MCH books by health workers and pregnant women can prevent risks to the mother's pregnancy (Mayang *et al*., 2019). Several things, such as knowledge, time availability, family support, attitudes etc influence utilization of the MCH handbookes. In line with research conducted by Napitupulu *et al.* (2018), of 183 respondents, 96 respondents (52.5%) used the MCH Handbook well, and 87 respondents (47.5%) did not use the MCH Handbook well, the average of respondents in the study are at a good level of knowledge and utilize the MCH handbook. The majority of pregnant women in this study were of productive age and still had a high level of awareness of information about their pregnancy.

The health status of pregnant women in this study experienced high-risk pregnancies. *Maternal health status* is a dynamic condition that can change during pregnancy. Pregnant women with pregnancies at risk will affect the condition of the fetus. So that the mother and fetus are healthy during pregnancy, several immunizations are carried out according to the gestational age. From the records in the MCH handbook, the average respondent has carried out immunization according to the age of his pregnancy. However, many respondents did not know the blood supplement tablet (TTD) control box in the MCH Handbook because the side effects of TTD, which caused nausea and constipation, made respondents rarely consume it, so many respondents experienced anaemia during their pregnancy.

According to the assumptions of the researchers, the use of the MCH handbook, which was already suitable for the respondents, did not rule out the possibility of them experiencing a risky pregnancy. Because even though the respondents made good use of the information in the MCH Handbook, many of their pregnancies were unplanned. Many of the respondents did not use family planning, causing many of them to experience unplanned pregnancies at an age when they were at risk of becoming pregnant. Based on the assessment using KSPR, it is known that several factors make the majority of respondents have risky pregnancies. The mother's age at pregnancy is too old, a history of miscarriage and fetal death in the womb, a history of anaemia during pregnancy experienced by the mother, a history of cesarean section in a previous pregnancy, a pregnancy gap that is too long (>10 years), and having more than 4 children.

Limitations in this study include : data collection in this study was carried out door to door, not collecting respondents in one place. So it requires good time management because researchers are also still following learning on campus. The obstetric and genetic history experienced by pregnant women greatly influences the KSPR assessment. This makes the risk points higher for pregnant women. So that the majority in this study, the health status of pregnant women is at risk. To reduce points or result scores in the KSPR assessment, the inclusion criteria must be added to the gravida status

Conclusions

The results of statistical tests using the sperm correlation test showed that the p-value = 0.028, and when compared with $\alpha = 0.05$, the p-value is $0.028 < \alpha = 0.05$. So, it can be concluded that there is a relationship between the utilization of the MCH handbook and health status.

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Correlation Of Pregnancy Risk Awareness With The Constancy Antenatal Care Expectant Two And Three Trisemester In Public Health Center Jatimulya

Amalia Rizky Atikah[1], Lina Herida Pinem[2]*, Edita Astuti Panjaitan[3].

[1,2,3] Department of Nursing, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Bekasi, Indonesia
*Correspondence author : lina@stikesmitrakeluarga.ac.id

Abstract

Antenatal care is regular care for pregnant women to monitor the emergence of diseases or adverse conditions that will occur during pregnancy until delivery. Antenatal care is also a The role of nurses is to provide education to pregnant women, husbands, families, and relatives about pregnancy, childbirth, and child care. Pregnant women's compliance with antenatal care will help reduce maternal and infant mortality. Adherence to Antenatal Care can be demonstrated through the frequency of maternal visits to health services. Factors that can influence the frequency of maternal visits are knowledge about danger signs of pregnancy, age, pregnancy parity, history of illness, employment, and distance traveled to health facilities. Nationally, the indicator for coverage of antenatal visits has reached the target. There is an increase in coverage of antenatal visits in 2021 compared to 2020, namely from 79.36% with a target of 80% to 88.13% with a target of 85%. The research was conducted to determine whether there was a relationship between the level of knowledge about the danger signs of pregnancy and compliance with antenatal care for pregnant women in the second and third trimesters at the Jatimulya Community Health Center. This research uses quantitative methods. The research design used by researchers is a cross-sectional design where the results of the research design are measured and observed simultaneously. The instrument used by researchers is a questionnaire in the form of questions on paper. The variable used for the questionnaire is the level of knowledge about signs. danger of pregnancy and compliance with antenatal care. When conducting research analysis using the SPSS 25.0 chi-square test. The results of the research show that knowledge about the danger signs of pregnancy influences compliance with antenatal care in pregnant women in the second and third trimesters, which is indicated by a p-value of 0.000 (<0.05), so there is a relationship between the level of knowledge about the danger signs of pregnancy and compliance with antenatal care. in second and third-trimester pregnant women.

Keywords: *Antenatal Care; Danger Signs of Pregnancy; Level of Knowledge ; Pregnancy; Trimester*

Introduction

Antenatal care carried out before giving birth is the key to keeping the mother and baby healthy and making the pregnancy enjoyable. Antenatal Care is a preventive effort by an obstetric health service program to optimize the reduction of maternal and infant mortality through several routine examination activities during pregnancy to prevent pregnancy complications. Some women have health problems that arise during pregnancy and there are also women who had health problems before pregnancy that could end in complications. Pregnancy complications are health problems that occur during pregnancy and can have a significant impact on the mother and baby. The level of education is one of the factors that can influence the provision of antenatal care because if pregnant women's knowledge is poor it will result in non-compliance with carrying out pregnancy checks so that complications in pregnancy are not detected early. This will result in slow treatment of complications which can result in the death of the mother or baby. This study aims to determine whether there is a relationship between the level of knowledge about the danger signs of pregnancy and the presence of Antenatal Care for pregnant women in the 2nd and 3rd trimester at the Jatimulya Community Health Center.

Methods

This research uses quantitative methods, quantitative research that applies quantification to each variable. The research design uses a cross-sectional design which carries out measurements and observations simultaneously. The variables in this study consist of 2 variables, namely the level of knowledge about the danger signs of pregnancy as the independent variable and compliance with antenatal care as the dependent variable. This research uses univariate and bivariate analysis. Univariate analysis was carried out to describe the characteristics of respondents, namely education level, age, pregnancy parity, history of illness, occupation, and distance traveled to health service facilities. Bivariate analysis was carried out to determine the relationship between the two variables using the chi-square test. This study had a sample of 85 respondents using a purposive sampling technique. The population in this study was 340 second and third-trimester pregnant women. The sample characteristics are pregnant women in the second and third trimesters, pregnant women who live in Jatimulya, Bekasi subdistrict, can read and write and are willing to be respondents. The location of Jatimulya Health Center is Jl. Jatimulya Raya No. 500, Jatimulya, South Tambun District, Bekasi Regency, West Java, 17510. Data collection was carried out using a questionnaire to determine the characteristics of respondents and answers to the research questionnaire. The researcher asked permission from the head of the Jatimulya Community Health Center.

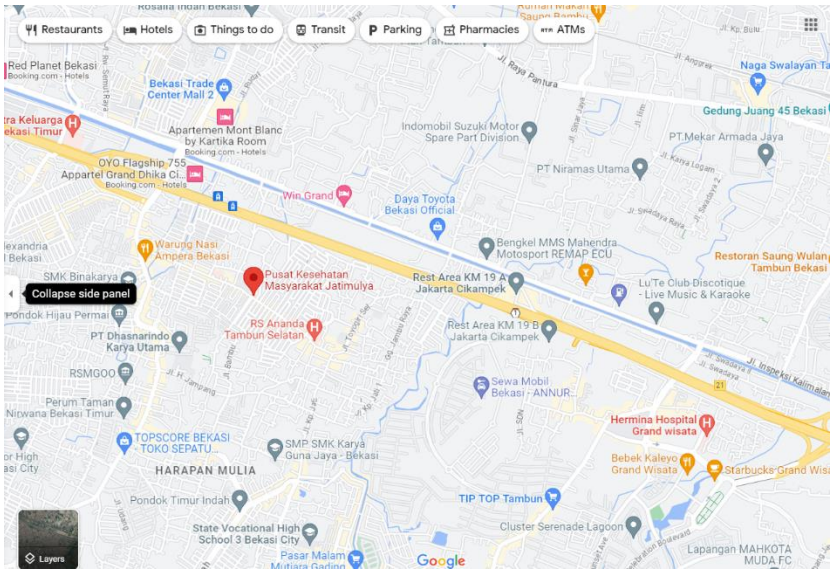

Figure 1. Location of Community Health Center showing sampling location: 6°16'10"S 107°01'26"E

Results and Discussions

In this study, univariate data consisted of age, education level, occupation, pregnancy parity, history of illness, employment, and distance traveled to health facilities which are depicted in the table below:

Table 1. Frequency Distribution of Respondent Characteristics At UPTD Jatimulya Community Health Center

Carakteristics	Frequency	Percentage (%)
Level of education		
Elementary school	2	2,4
Junior high school	17	20,0
Senior High School	50	58,8
College	16	18,8
Total	**85**	**100**
Paritas		
Primipara	26	30,6
Multipara	52	61,2
Grandemultipara	7	8,2
Total	**85**	**100**
Disease History		
There isn't any	83	97,6
Minor Illness	1	1,2
Serious Illness	1	1,2
Total	**85**	**100**
Job-status		
Housewife	69	81,2
Private sector employee	11	12,9
Businessman	3	3,5
Teacher	2	2,4
Total	**85**	**100**
Age		
No risk	80	94,1
Risky	5	59.9
Total	**85**	**100**
Distance		
Distance to health service facilities		
Far >3km	2	2,4
Near <1km-3km	83	97,6
Total	**85**	**100**
Knowledge level		
Not enough	28	32,9
Good	57	67,1
Total	**85**	**100**
Compliance Level		
Not obey	31	36,5
Obedient	54	63,5
Total	**85**	**100**

Based on the data presented in Table 1, it appears that among the 85 participants, the majority of pregnant women had completed high school, with 50 of them (58.8%) achieving this level of education. Of the respondents, 52 (61.2%) had already given birth to at least one child.

Interestingly, 97.6% of the participants had no prior health conditions. The majority of the pregnant women were housewives, comprising 69 of the respondents (81.2%). Most of the participants who were not at risk of pregnancy, around 80 of them (94.1%), were between 20 and 35 years old. Furthermore, 97.6% of the respondents lived within a distance of 1-3 kilometers from their homes. It was promising to discover that 57 of the pregnant women respondents (67.1%) had a good understanding of the danger signs of pregnancy. Additionally, 54 pregnant women (63.5%) were found to adhere to antenatal care.

Table 2. Relationship Between Level Of Knowledge About Danger Signs Of Pregnancy With Pregnant Women's Antenatal Care Compliance Second And Third Trimester At UPTD Jatimulya Health Center

Knowledge Level	Disobedient		Obedient		Percentage		CI	p-value
	n	%	n	%	n	%		
Not Enough	19	67.9	9	32,1	28	100	**7,917 (2,863-21,891)**	**0,000**
Good	12	21,1	45	78,9	57	100		
Total	**31**	**89**	**54**	**11,1**	**85**	**100**		

Based on Table 2, it can be seen that the majority of pregnant women who do not comply with antenatal care checks with a poor level of knowledge are 19 respondents (67.9%), and pregnant women who do not comply with a good level of compliance are 12 respondents (21.1%). Meanwhile, there were 9 respondents (32.1%) who complied with antenatal care examinations with poor knowledge, and 45 respondents (78.5%) who complied with antenatal care examinations with good knowledge.

Knowledge is an indicator of someone taking action. When someone has good knowledge of health, that person will understand the importance of maintaining health and motivate themselves to apply it in their life. Knowledge is needed as support in growing self-confidence as well as attitudes and behavior every day, knowledge is the main factor that supports a person's actions **(Citrawati & Laksmi, 2021)**. In this study, the majority of pregnant women who did not comply with antenatal care checks with a poor level of knowledge were 19 respondents (67.9%), and pregnant women who did not comply with a good level of compliance were 12 respondents (21.1%). Meanwhile, there were 9 respondents (32.1%) who complied with antenatal care examinations with poor knowledge, and 45 respondents (78.5%) who complied with antenatal care examinations with good knowledge. The results of the chi-square test obtained a p-value of 0.000, which means that there is a relationship between the level of knowledge about the danger signs of pregnancy and pregnancy checks for pregnant women in the second and third trimesters.

Citrawati's (2021) shows that data analysis between the level of knowledge and antenatal care visits shows that the p-value is 0.00, which means that the p-value < α 0.01, so statistically there is a significant relationship between the level of knowledge of 59 pregnant women about antenatal care. of pregnancy, check-up visits at the community health center appeared to be frequent II. From the research results, it was found that out of 30 respondents, 25 (83.3%) respondents had good knowledge of making regular antenatal care visits, while 5 (16.7%) respondents had poor knowledge of making irregular antenatal care visits. **Lilis Nugrawati's research (2023)** showed that the knowledge of pregnant women about the danger signs of pregnancy in the year obtained knowledge in the good category was 42 respondents (63.6%) and with sufficient knowledge was 24 respondents (36.4%). it can be concluded that the majority of pregnant women in Malaysia received the highest number of ANC registrations, namely 42 respondents who complied with the law (63.6%) and 24 respondents who did not (36.4%). As a result, it can be concluded that the majority of pregnant women in Gorontalo Regency participate in antenatal care (ANC) by established rules and standards. Based on analysis using the chi-square statistical test with a p-value of 0.000 (or 0.05), it can be concluded that there is knowledge of pregnant women about the number of people who have registered for antenatal care (ANC) at the Limboto Community Health Center, Gorontalo. The results of **Hariani & Syafriani's (2021)** research are not from previous research. The results of the research showed that 16 (72.7%) of the 22 respondents with a poor level of knowledge and non-compliant ANC visits were 16

(72.7%) compared to 10 respondents with a good level of knowledge and 2 (20%) non-compliant ANC visits. Mothers whose level of knowledge is not good are more likely to not make ANC visits. The Chi-Square test results obtained p value = 0.008 < 0.05, so it can be concluded that this is caused by the mother's very low curiosity. According to the researchers' analysis, the level of knowledge is very influential in providing routine antenatal care, because the better a person's knowledge, the more motivated they are to know their condition so that they can minimize undesirable things, If pregnant women have low knowledge, they will not have curiosity about the condition of their pregnancy. Good knowledge is essential for maintaining good health habits. Research shows that pregnant women with poor knowledge about antenatal care are less likely to comply with check-ups. However, those with good knowledge are more likely to adhere to medical check-ups. Having good knowledge motivates individuals to prioritize their health, leading to better outcomes.

Conclusions

The research results show that there is a relationship between the level of knowledge about dangerous signs of pregnancy and compliance with antenatal care in pregnant women in the second and third trimesters, a p-value of 0.000 (<0.05) was obtained. So, it is hoped that future researchers will be able to share more about things that can influence knowledge about the dangerous signs of pregnancy which might influence compliance in providing antenatal care. In summary, antenatal care plays a crucial role in monitoring the health of pregnant women and identifying potential risks that may arise during pregnancy and delivery. The study found that the majority of participants had a higher level of education and belonged to the upper middle class. Additionally, most respondents were multiparous and had no history of disease. The study also revealed that 81.2% of the respondents were housewives, aged between 20 and 35 years old, and lived within a kilometer of the hospital. Furthermore, 67.1% of them had good knowledge of antenatal care, and 63.5% were compliant with it. The findings suggest that there is a significant correlation between the level of knowledge about dangerous pregnancy signs and compliance with antenatal care during the second and third trimesters, with a p-value of 0.000 (<0.05). Further research should identify factors that influence a pregnant woman's knowledge of dangerous pregnancy signs and their compliance with antenatal care.

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Prescribing patterns of anti-hypertensive medications at PHC clinic Pedurungan, Semarang

Winda Dwi Astuti[1], Paulina Maya Octasari[2]*
[1,2] Department of Pharmacy, Politeknik Katolik Mangunwijaya, Indonesia
*Correspondence author : pm.octasari@gmail.com

Abstract

Hypertension is one of the leading causes of premature death worldwide and can cause complications such as stroke, coronary heart disease and kidney failure. The number of hypertension sufferers in 2018 was higher than in 2013 at 8.31%. Control of hypertension includes non-pharmacological therapy and pharmacological therapy. Pharmacological treatment must be supported by adequate drug availability, so knowing the drug prescribing pattern in a health facility is necessary. This study aims to determine the design of antihypertensive prescribing at the Pedurungan PHC Clinic, Semarang. This research is a type of descriptive observational study using retrospective data in the form of prescription sheets containing antihypertensives from October to December 2022. The data taken in this study were age, gender, antihypertensive name, and number of antihypertensive items. Data were analyzed by classification according to gender, age, antihypertensive name, antihypertensive combination, and antihypertensive group, and quantitatively by calculating the percentage. The results showed 137 prescriptions with 187 antihypertensive agents within the period. Hypertension in women was higher than in men (52.83%). The highest prevalence of hypertension was 56 – 65 years old. The pattern of prescribing resulted in the monotherapy being higher than combination therapy. Amlodipine 10 mg (28.26%) was the most widely used in a single treatment. The antihypertensive groups showed that calcium channel blockers had the highest presentation (49.63%) while angiotensin-converting enzyme inhibitors were used the least, namely 2.19%. The most widely used antihypertensive combination therapy is a combination of two types of antihypertensive groups, namely calcium channel blockers and angiotensin receptor blockers, with a percentage of 15.33%. This research concluded that monotherapy was more used than combination therapy, and amlodipine 10 mg was the highest presentation in hypertension patients at PHC Clinic, Pedurungan, Semarang.

Keywords: *prescribing patterns; antihypertensive ; agents ; PHC Clinic*

Introduction

Hypertension is a non-communicable disease that is one of the leading causes of premature death worldwide. Data from the World Health Organization in 2018 shows that the global prevalence of hypertension is 22% of the world population. Southeast Asia is in the third-highest position, with a prevalence of 25% of the total population (Ministry of Health, Republic of Indonesia, 2019). The survey shows that in Indonesia, the prevalence of hypertension starting from the age of 18 was 34.11% in 2018. This prevalence was higher than in the survey in 2013, namely 25.8%, so the total increase in hypertension prevalence was 8.31% (Ministry of Health Republic of Indonesia, 2018). Age ≥ 35, the risk of developing hypertension increases with age. Systolic pressure increases by an average of 20 mmHg and continues to increase after the age of 70 years because, with increasing age, the body's abilities and mechanisms decrease slowly, one of which is an increase in peripheral vascular resistance (obstruction of blood flow in peripheral blood vessels) in arteries (Calvin, 2018).

Hypertension can be divided into two types: hypertension of unknown cause (primary hypertension) and hypertension of known cause (secondary hypertension). The symptoms of hypertension are almost like other diseases, such as dizziness, headaches, and blurred vision, so most hypertension sufferers are unaware of their disease. Risk factors for hypertension that cannot be changed include heredity (genetics), age and gender. In contrast, risk factors that can be changed include lifestyle-related factors such as smoking, alcohol consumption, lack of physical activity, consumption of foods high in sodium, low consumption of fruit and vegetables, stress and obesity (Ministry of Health Republic of Indonesia, 2018).

Hypertension threatens public health because it can potentially cause complications such as stroke, coronary heart disease and kidney failure (Dipiro *et al*., 2015). Hypertension must be controlled to prevent and reduce morbidity, complications, and death. These steps can be grouped into pharmacological and non-pharmacological approaches. Non-pharmacological approaches to lower blood pressure are losing weight, reducing stress, not smoking, avoiding alcohol, and doing sports activities, which can also reduce blood pressure (Fauziah, 2021), and limiting salt intake to less than 5 grams per day (WHO, 2012).

Pharmacological approaches are carried out with antihypertensive medication therapy. Antihypertensives are divided into several groups, namely diuretics, beta blockers, alpha II adrenergic agonists, calcium antagonists (Calcium channel blockers/CCB), Angiotensin-converting enzyme inhibitors (ACE-Inhibitors), Angiotensin receptor blockers (ARB) (BPOM RI, 2014). Initial treatment for hypertensive patients can begin by controlling blood pressure regularly

and periodically checking their health at the health center, clinic or hospital at least once a month (Ministry of Health Republic of Indonesia, 2019).

PHC Clinic, Pedurungan, Semarang, is a clinic that provides health services supported by diagnostic medical procedures and drug services. Every month, an average of 662 prescriptions are received at the pharmacy, and 117 (18%) prescriptions contain antihypertensives. Hypertension treatment is long-term treatment, even for life. Patients are required to take medication regularly even if there are no symptoms. The availability of antihypertensives in pharmacies or clinics must be guaranteed. Based on this background, research must be conducted on antihypertensive prescribing patterns at the PHC Clinic, Pedurungan.

Methods

This type of research is descriptive observational research using retrospective data. The data used in the research were obtained from patient prescription sheets containing antihypertensives in October – December 2022 at the PHC Clinic, Pedurungan, Semarang. The data taken in this study were the date and number of prescriptions, patient characteristics (age and gender), type of antihypertensive and number of antihypertensive items. The data obtained were analyzed qualitatively to be classified according to gender, age, antihypertensive, combination of antihypertensives and antihypertensive group. Data was also processed quantitatively by calculating the percentage.

Results and Discussions

The Characteristics of the Patient

Based on data obtained from antihypertensive prescriptions at the PHC Clinic in October – December 2022, there were 137 prescriptions. The data is then processed quantitatively and qualitatively. The collected data was analyzed qualitatively to be classified according to gender, age, type of antihypertensive, combination of antihypertensives and antihypertensive group. The distribution of patients based on gender and age can be seen in **Table 1**.

Table 1. The characteristics of hypertension patients in October – December 2023 at PHC Clinic.

No.	Range of Age (years)	The number of patients		Percentage (%)		Total (%)
		Men	Women	Men	Women	
1.	35 - 45	14	4	10,22	2,92	18 (13,14)
2.	46 - 55	12	8	8,76	5,84	20 (14,60)
3.	56 - 65	9	28	6,57	20,44	37 (27,01)
4.	> 65	32	30	23,36	21,89	62 (45,25)
Total		**67**	**70**	**48,17**	**51,83**	**137 (100)**

In Table 1, it is found that the distribution of hypertensive patients at the PHC Pedurungan Semarang Clinic shows a relatively significant increase in the number of female patients in the range 56 – 65 years old, namely 20.44%, wherein the 35 – 45 year age range there are only 2.92% of patients. This result was influenced by menopause, which generally occurs at 45-55 years (Purnama, 2017). Levels of the hormone estrogen influence the incidence of hypertension one estrogen. In women, the hormone estrogen plays a role in increasing High-Density Lipoprotein (HDL) levels. HDL cholesterol is a protective factor in preventing the atherosclerosis process. The protective effect of estrogen in women prevents the risk of hypertension. The estrogen hormone levels will decrease when women enter menopause, so women become more susceptible to hypertension (Kusuma *et al*., 2016). The number of male patients begins to increase in the age range of 35-45 years, in line with research by Aristotle (2018) in Palembang, which shows that gender influences the incidence of hypertension. Hypertension in men can be triggered by unhealthy lifestyle behaviors such as smoking and psychological factors that cause stress.

The total number of hypertensive patients aged > 65 years has increased. This shows that hypertension is a degenerative disease that tends to get worse over time due to decreased function of the body's organs. According to the data in the research above, the older the number of hypertensive patients increases. This shows that the risk of hypertension increases because as you get older, your blood vessels experience changes in structure, becoming stiffer, which tends to cause higher pressure. After all, blood is forced through the vessel's narrow blood (Adam, 2019).

The Prescribing Patterns of Antihypertensive

Data obtained from 137 prescriptions contained 184 antihypertensive items. The percentage of antihypertensives based on drug type at the PHC Pedurungan Semarang Clinic for October - December 2022 can be seen in **Table 2.**

Table 2. The Prescribing Patterns based on the antihypertensive drugs.

No.	The name of drugs	Drugs potency (mg)	The number	Percentage (%)

1.	Amlodipine	10	52	28,26
2.	Amlodipine	5	43	23,37
3.	Candesartan	16	15	8,15
4.	Candesartan	8	11	5,98
5.	Valsartan	80	11	5,98
6.	Spironolactone	25	9	4,89
7.	Bisoprolol	2,5	8	4,35
8.	Bisoprolol	5	8	4,35
9.	Furosemide	40	8	4,35
10.	Irbesartan	300	5	2,72
11.	Nifedipine	30	5	2,72
12.	Losartan	50	2	1,09
13.	Captopril	25	1	0,54
14.	Carvedilol	6,25	1	0,54
15.	Clonidine	0,15	1	0,54
16.	Imidaprilat	5	1	0,54
17.	Olmesartan	40	1	0,54
18.	Ramipril	10	1	0,54
19.	Telmisartan	80	1	0,54
Total			184	100

Table 2 shows that amlodipine 10 mg and amlodipine 5 mg have a usage percentage of more than 20% of the total antihypertensive items. Amlodipine 10 mg is the most widely used because blood pressure targets are not achieved in the first treatment, so that the initial drug dose can be increased. Amlodipine is the most frequently prescribed CCB group because it is relatively affordable. It also has the advantage of its pharmacodynamic and pharmacokinetic properties.

Amlodipine has a long half-life with a duration of lowering blood pressure for 24 hours, high bioavailability and a long duration of drug action, allowing for once-a-day dosing (Chan *et al.*, 2016). Administration of amlodipine is recommended at night because it can reduce the risk of death, the risk of type 2 diabetes and the routine of waking up due to the habit of most people who tend to take medication before bed. This can benefit patient compliance in taking medication (Admaja, 2020). Amlodipine has a working mechanism of relaxing blood vessel arterioles. Amlodipine is vascular-selective, preventing blood pressure from dropping suddenly (Tandililing *et al.*, 2014). Based on the data collected, the percentage of antihypertensives based on the group at the PHC Pedurungan Semarang Clinic for October – December 2022 can be seen in **Table 3.**

Table 3. The number of antihypertensive drugs in October – December 2022 at PHC Clinic.

No.	The antihypertensive groups	The number	Percentage (%)
1	Calcium chanel blocker (CCB)	100	54,35
2	Angiotensin receptor blocker (ARB)	46	25,00
3	Beta Blocker	17	9,24
4	Diuretic	17	9,24
5	ACE Inhibitor	3	1,63
6	Agonis α II adrenergic	1	0,54
Total		184	100

The percentage of the antihypertensive class that is most widely used is the CCB group at 54.35%. This is in line with research by Andriyana (2018), which states that the antihypertensive often prescribed for elderly patients is Amlodipine, which is included in the CCB group as much as 32.78%. The CCB group is often used as therapy for elderly patients because it is a class of antihypertensives that has good clinical management of hypertension either alone or in combination and has been proven to be safe and effective in lowering blood pressure with good tolerance (Ardhany *et al*., 2018). This is also in line with research by Alaydrus and Tobing (2019), which explains that as many as 60% of the antihypertensive therapy chosen is Amlodipine, which is included in the CCB group for elderly patients because the use of CCB class antihypertensives has a working mechanism by relaxing the heart muscle and smooth muscle by inhibiting calcium activity or inhibiting the flow of calcium into the heart muscle and arterial blood vessel

walls (Dipiro *et al*, 2015). The CCB group provides more vasodilation benefits in arterial blood vessels, so it is effective for use in elderly patients because, in the elderly, there is stiffness of the blood vessels (Mason, 2012).

The use of α II adrenergic agonist antihypertensives is the lowest in percentage because the mechanism of action of this group is directly on the central nervous system, inhibits the action of hormones, can reduce sympathetic nerve activity, affects smooth muscle function, which can cause side effects, unusual fatigue or depression, sexual dysfunction, and sleep disorders, including nightmares (Ministry of Health Republic of Indonesia, 2019). The collected data was grouped, and the percentage of antihypertensive types obtained based on single and combination administration can be seen in Table 4. Table 4 shows more antihypertensive administration when a single therapy is administered (71,53%). Pharmacological therapy for hypertension begins with a single drug because monotherapy can reduce systolic blood pressure by around 7-13 mmHg and diastolic blood pressure by around 4-8 mmHg. After receiving therapy within one month, if the blood pressure target is not achieved, the initial drug dose can increase. Adding other drug classes from first and second-line therapy can minimize the side effects of drug interactions (Putri *et al*., 2019). Monotherapy administration is given to patients whose blood pressure tends to be better controlled. Patients with high levels of compliance with antihypertensive use show better blood pressure control than those with low compliance. Giving antihypertensives in a single form is generally because the patient is still in the early stages of treatment for stage 1 hypertension. This is in line with research presented by Sinata and Rahmadani (2021) regarding the description of the use of antihypertensives at the Sidomulyo Outpatient Health Center Pekanbaru in 2020.

Table 4. The Patterns of Antihypertensive Prescription in October – December 2022 at PHC Clinic, Pedurungan, Semarang.

Type of therapy	Type of groups	The number	Percentage (%)	Total (%)
Monotherapy	CCB	68	49,63	71,53
	ARB	13	9,49	
	Diuretic	7	5,11	
	Beta Blocker	7	5,11	
	ACE Inhibitor	3	2,19	
Combination				
2	CCB + ARB	21	15,33	24,09
	CCB + Betablocker	3	2,19	
	CCB + Diuretic	3	2,19	
	ARB + Betablocker	3	2,19	
	ARB + Diuretic	3	2,19	
3	CCB + ARB + Betablocker	2	1,46	3,65
	CCB + ARB + Diuretic	2	1,46	
	ARB + Betablocker + Diuretic	1	0,73	
5	CCB + ARB + Betablocker + Agonis α II + Diuretic	1	0,73	0,73
Total		**137**	**100**	**100**

The use of single antihypertensives at the PHC Pedurungan Semarang Clinic is mainly in the CCB group (49.63%). In line with this research, hypertension is mainly experienced by elderly patients because CCB has a working mechanism that provides more vasodilation benefits in arterial blood vessels, so it is effectively used in elderly patients because, in the elderly, there is stiffness of the blood vessels (Mason, 2012). CCB can also inhibit voltage-sensitive calcium channels, thereby reducing the entry of extracellular calcium into heart cells and blood vessel walls (Dipiro *et al*., 2015). The ACE Inhibitor group is the least prescribed, namely (2.19%), because the side effects of these drugs can cause dry cough, weakness, dizziness or headaches, and increased blood potassium levels, so ACE Inhibitors are rarely chosen as therapy, especially in elderly patients (Ministry of Health Republic of Indonesia, 2019).

Table 4 also shows that the two-combination therapy is the most widely used in the patient. It is ARB and CCB. The Joint National Committee (JNC) VIII suggests that selecting the type of drug combination needs to consider the patient's clinical condition. A combination of two antihypertensives is given to stage 2 or stage 1 hypertension patients who do not reach the blood pressure target with a single administration of antihypertensives. It aims to lower blood pressure and help reduce the side effects of drug use when compared to using high-dose single antihypertensive therapy. Combination therapy is an option for patients who find it challenging to achieve blood pressure targets or for patients with many indications who require several antihypertensives (Ansa *et al.*, 2010). Combination therapy can use two or more different classes of antihypertensives depending on the patient's clinical condition. The combination of CCB and ARB (15,33%) is more often given to hypertensive patients because it is well tolerated at the beginning of treatment and is superior in inhibiting renin-angiotensin aldosterone stimulation (RAAS), as well as reducing oxidative stress and arterial rigidity, reducing the risk of stroke progression and vascular dementia (Putri *et al.*, 2019). The combination of CCB and ARB has the advantage of lowering blood pressure and preventing high-risk cardiovascular events in

hypertensive patients (JNC-8, 2013). The same thing was also stated that CCB does not increase the incidence of mortality in CKD patients, so the combination of ARB and CCB can be a therapeutic option to be considered to obtain a better antihypertensive effect and cause lower metabolic events (Kinanti *et al.*, 2022).

CCB has a working mechanism that inhibits the movement of calcium from blood vessels to the heart muscle and smooth muscle and can reduce peripheral resistance so that blood pressure can decrease. In contrast, ARB has a working mechanism by directly inhibiting angiotensin II type I receptors to reduce blood pressure. These ARBs have milder side effects, such as ACE inhibitors, than other groups. The combination of CCB + ARB is effective because the incidence of peripheral edema due to CCB use can be overcome by ARB with joint vasodilation of veins and arteries so that blood pressure can be reduced and side effects can be suppressed (Wulandari *et al.*, 2021).

Based on a combination of three types of antihypertensives at the Pedurungan PHC Clinic, Semarang, for October – December 2022, which can also be seen in Table 4. The distribution of types of antihypertensives based on the combination of three drug items shows that data on most combinations use the CCB, ARB, Betablocker and CCB, ARB, Diuretic groups (1,46%). The combination of three types of drugs can indicate that the patient is suffering from stage 2 hypertension and does not reach the blood pressure target with the combination of two antihypertensives, especially in patients with compelling indications that aim to avoid further organ damage (Pikir *et al.*, 2015). Choosing the type of combination must also pay attention to the patient's comorbidities so that the right group can be selected, such as an effective ARB for hypertensive patients with kidney disorders, an effective ACE inhibitor for hypertensive patients with accompanying diabetes mellitus, an effective Betablocker for hypertensive patients with impaired heart function (Ansa *et al.*, 2010). The background patients with a combination of three antihypertensives have comorbidities with impaired heart function, so it is necessary to add beta-blocker and diuretic antihypertensives. The purpose of adding Betablocker to the combination of CCB and ARB aims to slow the heart rate and reduce the pressure on the heart muscle when it beats while having the effect of widening the blood vessels, resulting in a decrease in blood pressure and a reduced burden on the heart in pumping blood throughout the body (Sari *et al.*, 2020).

The addition of diuretics to the combination of CCB and ARB is to control blood pressure by inhibiting the absorption of sodium, chloride and potassium salts and releasing more salt and water from the blood vessels into the urine. Blood pressure can be reduced by reducing the amount of fluid in the blood vessels (Fa'idah, 2016). Diuretics can also inhibit vasoconstriction (narrowing) of blood vessels so that blood vessels become loose and can reduce blood pressure (Ministry of Health, Republic of Indonesia, 2019).

Table 4 also shows that only one patient took five antihypertensive drugs (0,73%). This could indicate the presence of persistent hypertension. Persistent hypertension is blood pressure that does not reach the target BP <140 mmHg and BP <90 mmHg, even though you have received three or more types of antihypertensives of different classes at the maximum dose, and the patient has carried out lifestyle modification recommendations (Ministry of Health Republic of Indonesia, 2019). The background patients who received a combination of five antihypertensives in this study had other comorbidities, such as hypercholesterolemia and diabetes mellitus. Cases of diabetes mellitus with uncontrolled blood sugar can cause damage to small and large blood vessels, which triggers plaque formation in the blood vessels or atherosclerosis. This triggers blood to thicken, making the heart work harder and increasing blood pressure so that up to five antihypertensives are needed in combination (Mandaya, 2021).

The addition of α II agonists to the combination therapy of CCB, ARB, Betablocker and Diuretic is an antihypertensive that stimulates alpha-2 adrenoceptor receptors in the central nervous system (brain and spinal cord). Alpha-2 receptors are found on cells in the sympathetic nervous system. When alpha-2 receptors are stimulated, there is an obstacle to releasing norepinephrine, which reduces sympathetic activity. This plays a role in widening blood vessels to reduce blood pressure (Ministry of Health, Republic of Indonesia, 2019).

For hypertensive patients with comorbid diabetes mellitus, adding Betablocker is not recommended because it can have a camouflage effect on diabetes mellitus levels or mask the side effects of hypoglycemia from antidiabetic drugs but can be given as a last resort for patients who also experience impaired heart function or heart failure, in patients Betablocker heart can maintain the stability of the heart rhythm and muscle (Dungan *et al.*, 2019).

Conclusions

The pattern of antihypertensive prescribing at the PHC Pedurungan Semarang Clinic for the period October – December 2022 shows that most hypertension therapy uses a single therapy (71.53%), the most widely used group is the CCB group (49,63%), the most widely used antihypertensive was amlodipine 10 mg (28.26%). The suggestions given by this research can still be developed further by looking at the effectiveness of therapy (the success of therapy after one month or more of antihypertensive use).

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Determination of hydroquinone levels in whitening serum circulating at market place x using the uv-vis Spectrophotometric method

Desalfa Pafitriano[1*], Melania Perwitasari[2], Intan Kurnia Putri[3] ,Maya Uzia Beandrade[4]
[1,2,3,4] Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga Bekasi, Indonesia
*Correspondence author : desalfapaf17@gmail.com

Abstract

Hydroquinone is a dangerous substance in cosmetic products that has been banned for use on the face and skin, even though hydroquinone is relatively high in society. This study aimed to determine the hydroquinone content in whitening serum circulating in the marketplace using the UV-Vis spectrophotometry method. This type of research is descriptive and non-experimental. Samples of 12 whitening serums circulating in marketplace X were selected by purposive sampling. Qualitative analysis of organoleptic tests and color tests using FeCl3. Quantitative analysis of the determination of hydroquinone levels in whitening serum by UV-Vis spectrophotometry. The wavelength detection of 295 nm resulted from a linear regression formula of Y = 0.0331x + 0.0697 with a correlation coefficient (r) = 0.9997. Qualitative analysis of the organoleptic tests showed that 4 of the 12 samples were positively hydroquinone in samples S1, S2, S11 and S12 with a sticky to very sticky texture and a pungent odor. The results of the color test showed that 2 of the 12 samples suspected of being positive for hydroquinone in samples S3 and S11 showed a dark brown color. Method validation in the specificity test has a maximum wavelength of 294 nm at 4, 10, and 14 ppm concentrations. Precision test yields % RSD 0.10%. The accuracy test produces a %recovery value of 77.98%. The quantitative analysis test results for determining hydroquinone levels in whitening serum circulating in marketplace X using the UV-Vis spectrophotometry method showed that all 12 serum samples contained hydroquinone with levels ranging from 2.20 to 3.77%. From this study, it can be concluded that all bleach serum samples circulating in marketplace X contained hydroquinone levels that did not meet the Food and Drug Supervisory Agency requirements.

Keywords: prescribing patterns; antihypertensive ; agents ; PHC Clinic

Introduction

With the development of science and technology, people's living needs are also increasing. This need is not only for clothing, food and education; the need for appearance has become a daily need, especially for women. Some women want clean, smooth, firm and beautiful skin (Adriani & Safira, 2019). One of the many forms of cosmetic preparations that have been developed recently is serum. Because serum has a high concentration of active ingredients, the effect is more easily absorbed, spreads quickly on the surface of the skin and can brighten facial skin (Carolin, 2020).

From June 2020 to September 2021, Badan Pengawas Obat dan Makanan (Food and Drug Administration of Indonesia) found 72 dangerous products circulating on the market, 18 of which were cosmetic products containing dangerous ingredients, namely hydroquinone (BPOM, 2021). Based on this statement, it has been proven that the use of hydroquinone is still high on the face and skin in the community and does not meet the requirements of the Food and Drug Supervisory Agency. In this case, it is vital to carry out this research to ensure that cosmetic products without a BPOM distribution permit registration number containing the dangerous substance hydroquinone are not suitable for use on the skin, especially whitening serums circulating in marketplaces in Bekasi Regency.

According to Putri and Wahyudiani (2021), hydroquinone has unwanted effects when used for a long time, including irritative dermatitis, contact dermatitis, and ochronosis. Hydroquinone can treat skin problems caused by melanin accumulation hyperpigmentation, such as the appearance of uneven black spots on the skin. Research conducted by Arwibiyantari (2018) analyzed hydroquinone in whitening serum using UV-Vis spectrophotometry in 2 samples with results of 0.146% and 0.138%. In this study, the UV-Vis spectrophotometric method was used to determine serum levels of bleach circulating on the market because it has the advantage of being used for the analysis of various organic and inorganic substances, being selective, having high accuracy with a relative error of 1 - 3%, being able to determine the quantity of the substance. Which is small, and the results are accurate because the numbers listed are read directly, recorded automatically and printed in digital numbers (Rohmah *et al.*, 2021). Therefore, research regarding determining hydroquinone levels in whitening serum circulating in the marketplace using the UV–vis spectrophotometric method is fundamental.

Methods

The design of this research is non-experimental descriptive. This research was conducted at the Mitra Keluarga STIKes Pharmaceutical Chemistry Laboratory, Bekasi, West Java Province, from January to April 2023. The sample population in this study were all cosmetic shops in the Bekasi Regency area, which were in marketplace X. The tools

used include analytical scales, steam cups, measuring cups, beaker glasses, funnels, 50 mL measuring flasks, 10 mL measuring flasks, 100 mL measuring flasks, watch glasses, stir sticks, micropipettes, tips, cuvettes, glass, filter paper. , test tube, test tube rack, drip plate, spatula, dropper pipette, Erlenmeyer 50 mL, and UV–vis spectrophotometry.

The first way of working is organoleptic qualitative analysis; samples were subjected to organoleptic testing using the body's senses. The parts of the body that have a role are the eyes, ears, sense of taste, sense of smell, and sense of touch (Simaremare, 2019), and the second way of working in qualitative analysis is the color test on hydroquinone using FeCl3 by pipetting 5 mL of serum sample and dissolving 5 mL of methanol p.a until dissolved, adding 1% FeCl3 reagent. Five drops. The sample can be concluded to be positive for hydroquinone if the color changes from green to black (Rahmadari *et al.*, 2021). Next, the first quantitative analysis is carried out: making a standard hydroquinone solution by weighing 50 mg of hydroquinone, putting it into a 50 mL measuring flask, and dissolving it with methanol p.a. until the limit mark. The solution was shaken until homogeneous, and a concentration of 1000 ppm was obtained. The second quantitative analysis, the preparation of a 20 ppm hydroquinone standard solution, is carried out with a concentration of 1000 ppm that has been made. A 50 mL volumetric flask is prepared, then 2 mL of input is taken, which is taken using a pipette. Add methanol p.a to the mark and shake until dissolved. Then, 20 ppm of standard hydroquinone solution is obtained (Sahumena, 2019).

Determination of the maximum wavelength was carried out by inserting a standard solution of 20 ppm into a cuvette using a blank solution, namely methanol p.a. absorbance was measured with a wavelength of 200-400 nm. The maximum wavelength is determined by looking at the highest absorbance value. A 20 ppm standard curve was made by pipetting 2, 3, 4, 5, 6 and 7 mL of 20 ppm standard solution, each put into a 10 mL volumetric flask, adding methanol p.a. to the limit mark. The concentrations obtained were 4, 6, 8, 10, 12, and 14 ppm. Each of these concentrations was measured at the previously obtained wavelength using a blank methanol solution. The standard standard curve is obtained by plotting the concentration with absorbance (Sahumena, 2019).

In this research, a method validation test was carried out. The specificity test was carried out with hydroquinone concentrations of 4.10 and 14 ppm inserted into the cuvette to see the absorption spectrum formed at the maximum wavelength obtained (Guy, 2006). Linearity test was carried out with concentrations of 4, 6, 8, 10, 12 and 14 ppm measured maximum wavelength. Linear regression optimization made a relationship curve between the x-axis hydroquinone levels, namely absorbance. The results obtained were selected with good correlation coefficient relations and carried out three replications. The accuracy test was carried out using the addition method. A sample of 1 mL of whitening serum is taken, put into a 100 mL volumetric flask and added methanol p.a until the mark is marked and shaken until dissolved. Then take as much as 10 mL using a measuring pipette, put it into a 50 mL volumetric flask and add 100 ppm standard hydroquinone, pipettes 2, 4, and 6 mL to produce concentrations of 4, 8, and 12 ppm. In a 50 mL volumetric flask containing each - methanol p.a was added to each concentration along with the sample to the limit mark, the absorbance value obtained by UV-Vis spectrophotometry was measured and carried out three times. The absorbance value obtained was plotted with the linear regression equation and can produce hydroquinone concentrations, which will be determined as % recovery. The precision test was carried out with a hydroquinone solution with a concentration of 4 ppm, and then the absorbance measurement was carried out at the maximum wavelength with six replications (Sahumena, 2019).

Then, the final test of quantitative analysis, namely the determination of hydroquinone levels, was carried out by taking 1 mL of bleaching serum sample, adding methanol p.a to 100 mL in a measuring cup, then filtering the solution and taking 10 mL and adding methanol p.a in a 50 mL volumetric flask to the mark limit. Then, fill it into a cuvette and measure it using UV-Vis spectrophotometry at a wavelength of 295 nm. Each sample was carried out three times.

Results and Discussions

This study obtained samples of whitening serum products from shops in Bekasi Regency at marketplace X at a price of Rp. 3,000 – Rp. 30,000, which had sold ≥100 pcs and did not have a BPOM permit number, then 12 samples were analyzed based on the inclusion criteria. The organoleptic test is a test using the body's senses, which consist of the sense of sight, the sense of taste, the sense of smell and the sense of touch (Simaremare, 2019). In **Table 1**, the organoleptic test results can be seen.

Table 1. Organoleptic test results for whitening serum

Sample Code	Color	Scent	Texture
S1	Pink	Fragrant and pungent	Thick and sticky
S2	Bright Pink	Fragrant and pungent	Very thick and sticky
S3	Bright Yellow	Fragrant and pungent	Thick, slightly runny and not overpowering
S4	Clear White	Fragrant and not	Thick and a little sticky

		stinging	
S5	Bright Pink	Fragrant and pungent	Runny and a little sticky
S6	Yellow	Fragrant and pungent	Thick, slightly runny and not sticky
S7	Deep Pink	Fragrant and pungent	Thick and not sticky
S8	Pink	Fragrant and pungent	Thick, a little runny and sticky
S9	Yellow	Fragrant and pungent	Thick and not sticky
S10	Pink	Fragrant and pungent	Watery and not sticky
S11	Cloudy Pink	Fragrant and pungent	Thick and sticky
S12	Clear White	Fragrant and pungent	Thick and sticky

In **Table 1.** The organoleptic test of samples S1, S11, and S12 smells good, does not sting and has a sticky, thick texture. Sample S2 smells good and stings. The texture is thick and sticky. Research by Yusuf *et al*. (2017) showed that serum preparations that did not contain hydroquinone showed clear organoleptic test results, had no odor and had a thick texture. The results of a study by Simaremare (2019) show that hydroquinone samples have a sticky texture and a pungent odor. This color test aims to determine the presence of phenol groups in hydroquinone, which reacts with FeCl3. The color test in this study can be seen **Figure 1** ; **Table 2.**

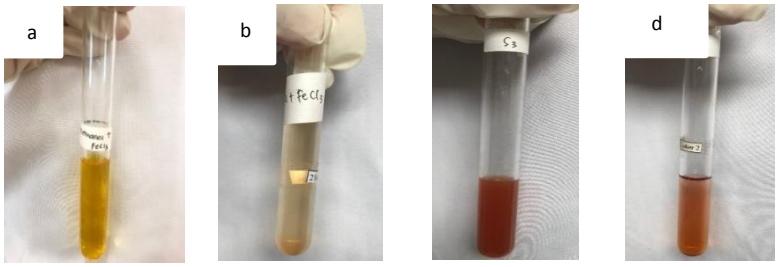

Figure 1. Results of the bleach serum color test with 1% FeCl3. a) Negative control, b) Positive control, c) sample S3, d) sample S11

Table 2. Color Test Results with 1% FeCl3

Sample code	**Color before addition of $FeCl_3$**	**Color after addition of $FeCl_3$**	**Results**
Control +	Clear	Brown	+
Control -	Clear	Dark Yellow	-
S1	Pink	Orange	-
S2	Bright Pink	Yellow	-
S3	Bright Yellow	Deep Brown	+
S4	Clear White	Yellow	-
S5	Bright Pink	Dark Pink	-
S6	Yellow	Yellowish Brown	-
S7	Deep Pink	Orange	-
S8	Pink	Dark Pink	-
S9	Yellow	Yellow	-
S10	Pink	Orange	-
S11	Cloudy Pink	Dark Brown	+
S12	Clear White	Yellow	-

Using a cuvette that already contains a blank solution, namely methanol pro analysis, the absorbance is set to 0 (zero) absorbance. The blank solution is a different solution to the sample, but is made in the same way for the purpose of comparison. The blank solution is entered into UV-Vis spectrophotometry for each absorbance measurement, with the aim being that only the absorbance or uptake of the desired substance is measured (Mustika, 2018). The maximum wavelength obtained is 295.0 nm. This is included in the theoretical requirements, according to USP (2020)

hydroquinone has a theoretical wavelength of 293 nm ± 2 nm. The maximum wavelength chosen is 295 nm, because methanol has a UV cut off of 215 nm, if the maximum wavelength chosen is close to the solvent's UV cut off, it will interfere with the wavelength reading of the analyte.

Determination of Hydroquinone Standard Curve

In the test, a standard curve for hydroquinone is then created by measuring the absorbance of the standard hydroquinone solution in maximum wavelength that has been obtained. Absorbance (y) obtained is then plotted against the concentration of the standard solution (x). Determining sample levels using the linear regression method is a parametric method with the independent variable (sample concentration) and the dependent variable (sample absorbance) using the curve regression line equationstandard solution. The sample concentration can then be calculated based on the standard curve equation obtained (Arifiyana *et al.*,2019)

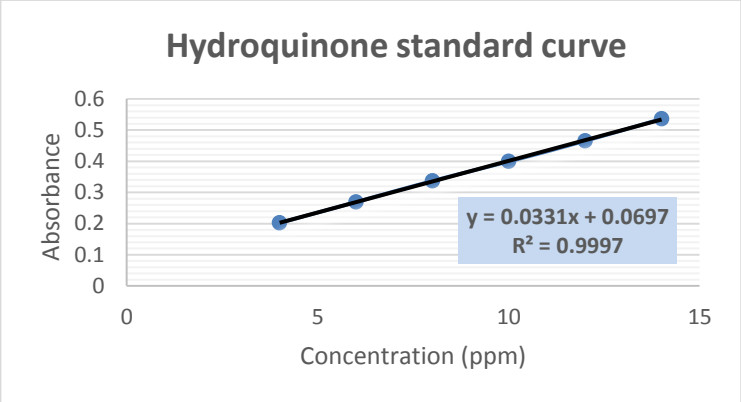

Figure 3. Hydroquinone Standard Standard Curve

Hydroquinone standard curves were prepared with concentrations of 4, 6, 8, 10, 12 and 14 ppm. Based on standard curve measurements, it was found that the equation results from the three replications had a correlation that met the requirements. The results of the linear regression equation Y = 0.0331x + 0.0697 were found.

Linearity Test

Linearity is a measure of how well the standard curve relates response (y) to concentration (x). Linearity is calculated statistically through a correlation coefficient (r) (Rahmadari *et al.*, 2021). This study produces a correlation coefficient of r = 0.9997. The value of r ≥ 0.99 indicates that there is a linear relationship between the concentration of the analyte and the measured absorbance based on Lambert Beer's law (Miller, 2014). The increasing levels of hydroquinone in solution, therefore the absorption also increases, because of the correlation relationship what happens is linear then the Lambert-Beer Law is in place materialized (Saraswati, 2022). This proves that the curve Hydroquinone calibration provides good linearity and a reliable method used appropriately to determine hydroquinone levels in whitening serum.

Specificity Test

Based on Table 3, it can be seen that with an increase in concentration there is also an increase in the absorbance value with a fixed maximum wavelength value in the specificity test.

Table 3. Specificity Test Results

Hydroquinone concentration (ppm)	Maximum absorbance value	Average± SD	Maximum wavelength
	0,155	0,159 ± 0,0050	294 nm
4	0,156		294 nm
	0,166		294 nm
	0,511		294 nm
10	0,524	0,521 ± 0,0069	294 nm
	0,527		294 nm
	0,735		294 nm
14	0,786	0,772 ± 0,0261	294 nm
	0,794		294 nm

The specificity test uses concentrations of 4, 10 and 14 ppm, because they represent the lowest, medium to highest concentrations and are carried out three times to get accurate results. From the research that has been carried out, it can be concluded that the higher the concentration, the greater the absorbance and the higher the concentration, the maximum wavelength will not shift towards bathochromic or hypsochromic. According to USP (2020) the maximum wavelength of hydroquinone is 293 nm ± 2 nm.

Accuracy Test

Based on Table 4. The results of the accuracy test determination showed that the % recovery value was 73.64 – 83.80% with an average value of 77.98%.

Table 4. Accuracy Test Results

Concentration (ppm)	Absorbance (sample)	addition	Absorbance (sample rate)	addition	Measurable standard	% *recovery*	Average % *recovery* ± SD
4	0,210	0,320	4,24	7,56	3,32	83,08	82,07 ± 0,942
	0,216	0,325	4,42	7,71	3,29	82,33	
	0,221	0,328	4,57	7,80	3,23	80,82	
8	0,210	0,405	4,24	10,13	5,89	73,64	74,90 ± 1,083
	0,216	0,414	4,42	10,40	5,98	74,77	
	0,221	0,423	4,57	10,67	6,10	76,28	
12	0,210	0,516	4,24	13,48	9,24	77,04	76,96 ± 0,314
	0,216	0,520	4,42	13,60	9,18	76,54	
	0,221	0,528	4,57	13,85	9,27	77,29	
			Average (% *Recovery*)				**77,98**

Accuracy is the thoroughness of the analysis method used or closeness measurement results with the values accepted by Fahira *et al.*, (2021). Accuracy itself is a statement as a percentage of acquisition return (% recovery) of the added analyte. In this research using the standard addition method (standard addition), addition method a known standard solution is added to the sample which are reviewed to reduce errors caused by various matris (Sahumena, 2019). Based on research conducted at concentrations of 4, 8 and 12 ppm, an average % recovery value was found to be 77.98%. These results can be said to be in accordance with the requirements for good accuracy, namely 60 – 115%. This proves that the method used has accurate measurements by showing the degree of equality of the average compared to the actual value and that the method has good accuracy.

Precision Test

Based on Table 5. The results of the precision test showed that % RSD was 0.10%. According to Syahriana *et al.* (2019) a method is declared precise if the percent RSD value is ≤2%.

Table 5. Precision Test Results

No	Concentration (ppm)	Absorbance at λ 295 nm	Measurable levels (ppm)
1	4	0,226	4,72
2	4	0,245	5,30
3	4	0,254	5,57
4	4	0,265	5,90
5	4	0,273	6,14
6	4	0,283	6,44
		Average	**5,68**
		SD	**0,57**
		RSD (%)	**0,10**

In this study, precision was determined by measuring the absorbance of one concentration of a 4 ppm hydroquinone standard solution for 6 replications, because to determine whether the repeatability value was good or not. Based on the research that has been carried out, the results of the precision test show that the %RSD value is 0.10%.

Determination of Hydroquinone Levels

The results of determining hydroquinone levels in whitening serum circulating in market places in Bekasi Regency ranged from 2.20 – 3.77%. Of the 12 samples, they did not meet the requirements set by the Food and Drug Supervisory Agency. In **Table 6.** it can be seen as follows.

Table 6. Results of Determination of Hydroquinone Content

Sample	Replication	Absorbance	X (ppm)	Analyte mass (ug)	% rate (b/v)	Average (%) ± SD
S1	1	0,210	4,24	21,19	2,12	2,20 ± 0,068
	2	0,216	4,42	22,10	2,21	
	3	0,221	4,57	22,85	2,29	
S2	1	0,260	5,75	28,75	2,87	3,06 ± 0,137
	2	0,274	6,17	30,86	3,09	
	3	0,282	6,41	32,07	3,21	
S3	1	0,223	4,63	23,16	2,32	2,60 ± 0,218
	2	0,245	5,30	26,48	2,65	
	3	0,258	5,69	28,44	2,84	
S4	1	0,247	5,36	26,78	2,68	2,80 ± 0,105
	2	0,254	5,57	27,84	2,78	
	3	0,265	5,87	29,35	2,94	
S5	1	0,253	5,54	27,69	2,77	3,06 ± 0,218
	2	0,275	6,20	31,01	3,10	
	3	0,288	6,60	32,98	3,30	
S6	1	0,205	4,09	20,44	2,04	2,19 ± 0,131
	2	0,213	4,33	21,65	2,16	
	3	0,226	4,72	23,61	2,36	
S7	1	0,212	4,30	21,50	2,15	2,42 ± 0,222
	2	0,230	4,84	24,21	2,42	
	3	0,248	5,39	26,93	2,69	
S8	1	0,311	7,29	36, 45	3,65	3,77 ± 0,120
	2	0,317	7,47	37,36	3,74	
	3	0,330	7,86	39,32	3,93	
S9	1	0,218	4,48	22,40	2,24	2,69 ± 0,561
	2	0,225	4,69	23,46	2,35	
	3	0,300	6,69	34,79	3,48	
S10	1	0,255	5,60	27,99	2,80	2,96 ± 0,124
	2	0,266	5,93	29,65	2,97	
	3	0,275	6,20	31,01	3,10	
S11	1	0,229	4,81	24,06	2,41	2,51 ± 0,080
	2	0,236	5,02	25,12	2,51	
	3	0,242	5,21	26,03	2,60	
S12	1	0,273	6,14	30,71	3,07	3,21 ± 0,111
	2	0,283	6,44	32,22	3,22	
	3	0,291	6,69	33,43	3,34	

After carrying out method validation tests with the aim of prove that these parameters have met the requirements its use (Sahumena, 2019). The next testing stage is in progress The final step is determining hydroquinone levels. Hydroquinone levels were determined using long-wavelength UV-Vis spectrophotometry 295.0 nm where each sample was measured three time with the aim of showing more accurate results. According to the results of measurements and calculations that have been carried out, Hydroquinone levels were found in all twelve whitening serum samples obtained from the marketplace in Bekasi Regency with a range 2.20 – 3.77%. It can be shown that these 12 samples do not enter into the conditions set by BPOM. From 12 samples what has been analyzed are products that circulate freely in marketplace, this is conveyed by marking the product no there is a batch number, BPOM permit number and other markings only include the product name. Based on **Table 2.** The results of the color test using FeCl3 out of 12 samples only 2 samples showed a dark brown color so that the sample was suspected to be positive for hydroquinone. Based on research conducted by Simaremare (2019) the color test using FeCl3 6 from 8 samples was suspected to be positive for hydroquinone which produced a green to black color.

Conclusions

It can be concluded that the results of the qualitative organoleptic test of 4 of the 12 samples were suspected to be positive for hydroquinone and the color test for 2 of the 12 samples was suspected to be positive for hydroquinone with

a dark brown color change. Quantitative test using UV-Vis spectrophotometry for all 12 serum samples was positive for hydroquinone with levels ranging from 2,20 to 3,77%, meaning that the samples did not meet BPOM requirements number 17 of 2022 concerning Technical Requirements for Cosmetic Ingredients.

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Acute Toxicity Test of Probiotic Juice Red Guava, Lemon, Melon, and Apple With Bacteria Starter *Lactobacillus casei* on Female Balb/C Mice

Desi Retnosari, [1*], Reza Anindita[2], Melania Perwitasari[3], Nofria Rizkia Amalia Harahap[4]

[1,2,3,4] Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Bekasi, Indonesia

[*]Correspondence author: desiretnosari05@gmail.com

Abstract

Introduction: Probiotic drinks are drinks that have health effects and contain living microbes in them. These drinks are popular among the public, and have started to consume these drinks. Probiotic drinks contain probiotic agents made through fermentation, one of which is Lactobacillus casei. Before consumption, probiotic drinks will undergo preclinical testing, one of which is in vivo. This study aimed to determine if the purpose was to determine if there was an acute toxicity effect after being given a juice probiotic drink. **Methods:** This quantitative research type applied research applied research was a quantitative research type applying an experimental study design. The variables used are independent and dependent. This study used 15 female white mice (Balb/c) divided into five groups. The test material used was the Probiotic drink, administered orally at 5, 50,300, and 2000 mg/KgBW doses and distilled water as the control group. The test animals were observed individually for 14 days. The variables observed were clinical symptoms like seizures, saliva, tremors, limp, and walking using the stomach. **Results:** There are no toxic symptoms that occurred in mice Balb/C like seizures, saliva, tremors, limp, and walking using the stomach during 14 days of observation. **Conclusion:** There were no clinical symptoms that occurred in the toxicity test. This study concluded that probiotic drink juice with *L.casei* starter at doses of 5, 50, 300, and 2000 mg/KgBW did not cause a decrease in no toxic symptoms occurred in mice *Balb/*C.

Keywords: *Probiotic Drinks; Acute Toxicity; Fix Doses; Balb/C; Mice*

Introduction

Indonesian people already have new habits, such as consuming healthy foods that have more benefits for the body, which serves to help maintain a healthy body. Functional food is defined as food or drink that has undergone a modification and innovation process in the processing process (Rahmawati *et al*., 2021). According to the Food and Drug Supervisory Agency (2021), health supplements supplement nutritional needs, maintain health functions, and contain one or more ingredients in the form of vitamins and minerals combined with plants. Food or drink functions to be used as a supplement to replace synthetic drugs. It is necessary to carry out consumption safety tests, including selection stages, preclinical trials, standardization, and clinical trials. In efforts to carry out clinical trials, it is necessary to carry out preclinical trials first. According to Pradono *et al*. (2019), preclinical testing is a test used to collect information on toxicology and pharmacology to determine the efficacy and safety of a scientifically tested product through toxicity tests or activity tests. Drug toxicity tests are generally divided into 2: in vitro and in vivo. According to (BPOM 2022), a toxicity test is a test used to determine whether or not a substance has a toxic effect on biological systems and also to obtain data responses that are typical of the test preparation, where the data obtained will be used to provide information about the dangers of the prepared preparation and tested in case of exposure to humans to determine the user's dose for human safety. According to (BPOM 2020), toxicity tests on test animals are carried out as evidence of the safety of test preparation. The choice of the test is related to the purpose of a substance user and the possibility of a risk of exposure to humans. Research on animals can determine whether the material under study is safe or causes toxic effects on medication (Jawi, 2014).

According to Rahayu and Firman (2018), toxicity is a term used to compare a chemical substance with another. A substance will be said to be toxic (toxic) if the substance has the potential to harm the biological mechanisms of an organism. This toxic effect is determined based on the dose, the nature of the substance, and the form of the effect it causes. There are several acute toxicity tests, including subacute, acute, chronic and subchronic. *Subacute toxicity* is a test carried out to detect the presence or absence of toxic effects after administering the test preparation with repeated doses given orally to test animals for 28 days. This toxicity test aims to provide information on the toxic effects on the target organs it affects (Ritonga & Ayu, 2017). Acute toxicity is a test to detect toxic effects that appear within a short time after administering the test preparation by mouth (orally) in a single dose or repeated doses within 24 hours (Melisa *et al*., 2022). Chronic toxicity is a test to detect toxic effects that appear after administering the test preparation to test animals repeatedly for 9-12 months (BPOM, 2020). A subchronic toxicity test is a test of a compound that is given for 1-3 months with repeated doses to test animals (Wahyuni *et al*., 2017)

The probiotic drink to be developed in this study consists of several fruits, including red guava, lemon, melon, and apple, with a starter Lactobacillus casei using the fixed doses method. This research needs to carry out preclinical tests before being used by humans, one is an acute toxicity preclinical test using Balb/C strain female mice.

Methods

This research is quantitative experimental research. This research was conducted at the STIKes Mitra Keluarga, Pharmacology Laboratory, Bekasi, West Java, from January to March 2023. The samples in this research were guava fruit, apples, lemons, melons, and *Lactobacillus casei* bacterial cultures obtained from PT Agritama Sinergi Innovation and mice—balb/C females obtained from Karanganyar, Central Java.

This research began with selecting the sample used, namely the Jamale probiotic drink with doses of F1, F2, F3, and F4, with the dosage for each formula being as follows:

Fruits	Formula I (5 mg/KgBW)	Formula II (50 mg/KgBW)	Formula III (300 mg/KgBW)	Formula IV (2000 mg/KgBW)
Apple	6,5 mg	65 mg	390 mg	2700 mg
Red Guava	6,5 mg	65 mg	390 mg	2700 mg
Melon	6,5 mg	65 mg	390 mg	2700 mg
Lemon	6,5 mg	65 mg	390 mg	2700 mg
Sugar	1,3 mg	13 mg	78 mg	120 mg

The following method is the selection of test animals, where the test animals used are 15 healthy-bodied Balb/C female mice with a body weight of around 20-30 grams. Previously, mice would be acclimatized for 7 days, where this was intended, so that the animals could adapt to the atmosphere in the laboratory and the test animals could adapt to the new environment. This study used the Fixed Doses Method based on BPOM in 2022 using test animals of the same sex with doses in stages of 5, 50, 300 and 2000 mg/kgBB. Previously, the mice would be fasted for 3-4 hours, and drinking was allowed. Mice were divided into 5 treatment groups, where each group consisted of 3 female mice, which were determined randomly. Group 1 was the control group, and groups F1 5 mg, F2 50 mg, F3 300 mg, and F4 2000 mg were the treatment group.

Observations will be made after and before the dose is given. The behaviour of the mice was observed for 30 minutes after the dose was given, and observations of clinical symptoms were carried out every 4 hours once a day for 14 days. The observed toxic symptoms of mice were seizures, tremors, salivation, weakness, and walking using the stomach. For test animals that have been exposed to toxic effects after being given the appropriate dose of probiotic drink or found dead, the time of death of the mice must be recorded according to the date of death.

Results and Discussions

This research uses the Fixed Doses method, which uses test materials with a moderate degree of toxicity, and the dose used does not cause death, irritation, or corrosiveness (BPOM RI, 2022). The principle of the Fixed Dose method is to use dose levels of 5, 50, 300 and 2000 mg/kgBB. Before the primary test is carried out, a preliminary test is carried out to determine the dose that causes symptoms of toxicity (Lestari *et al*., 2022). Table 2 shows the results of this study, which showed toxic symptoms that occurred after administration of the probiotic drink.

Table 2. toxic symptoms that occurred after administration of the probiotic drink.

Doses	**Mice number**	**toxic symptoms**				
		Seizures	**Saliva**	**Tremors**	**Limp**	**Walking using stomatch**
Negatif control	25	-	-	-	-	-
	12	-	-	-	-	-
	1	-	-	-	-	-
5 mg/KgBW Doses	2	-	-	-	-	
	4	-	-	-	-	-
	5	-	-	-	-	-
50 mg/KgBW Doses	6	-	-	-	-	-
	9	-	-	-	-	-
	13	-	-	-	-	-
300 mg/KgBW Doses	14	-	-	-	-	-
	17	-	-	-	-	-

	18	-	-	+	+	-
2000 mg/KgBW Doses	19	-	-	-	-	-
	20	-	-	-	-	-
	22	-	-	+	+	-

Description: + (symptoms occur), - (no symptoms occur)

Table 2 shows that 2 mice showed toxic symptoms after being given juice at doses of 5 mg/KgBW, 50 mg/KgBW, 300 mg/KgBW, and 2000 Kg/BW, which had been observed for 14 days. It was found that mice number 18 at a dose of 5 mg/KgBW and 22 at a dose of 2000 mg/KgBB showed similar toxic symptoms in the form of a vibrating and passive stomach and hair loss in mice number 18 for 3 days. Clinical symptoms in mice number 18 and 22 were caused by stress because previously, the mice were stuck in the nets in the mouse cages, resulting in toxic symptoms that occurred during the observation. This differs from observations during acclimatization, where no mice had toxic symptoms.

The toxic symptoms or behaviour of mice were observed every day for 14 days. These toxic symptoms are observed through visualization or with the naked eye. To find out the symptoms that arise, they must be compared with the habits of the test animals during acclimatization and with the negative control group (Fithria *et al*., 2018). Factors that can make mice uncomfortable include air circulation, lighting and cages such as layout, materials and size (Mutiarahmi *et al*., 2021). Parameters observed for signs of toxicity include gait using the stomach, shaking, convulsions, salivation, and weakness. These toxic symptoms are observed visually, so they must be compared with the habits of the test animals during the acclimatization period and with the negative control group for each test group. The results of observations of the administration of Jamale probiotics are shown in Table 2, such as hair loss, body trembling, decreased appetite, and passivity on days 11 to 13, found in mouse number 18, body shaking, decreased appetite, and passivity. In mouse number 22 on day 2. It is suspected that the mice were pinched by the cage net, causing them to become stressed.

In this study, it was found that mice died at a dose of 2000 mg/KgBW in the Jamale probiotic drink at 08.00 after 5 days of observation. Death is the worst effect that can occur due to the toxic effects of an extract. Toxic doses can cause damage to the body's physiological functions, especially vital organs and body chemical functions, resulting in death. The results of this test are the effects that occur in mice when given juice using 4 doses with 1 control to compare the effects in the treatment group. In this study, 1 dead mouse was found, namely number 22. This is thought to be because the mouse was stressed after administering the drug. The mouse got caught in the lid of the mouse cage. It can be seen from the changes in the following days that toxic symptoms occur, such as body shaking (tremors), decreased appetite, hair loss, and inactivity. As a result, the mice died on the fifth day at 08.00 a.m. after being given juice. These characteristics are the same as mouse number 18, which was also caught on the 11th day (Asriningtyas and Daryanti, 2022). They observed that several factors, such as physical or psychological stimulation or both, caused the stressful conditions of mice. Physical stress is caused by exposure to a stressor that is harmful to the body, such as exposure to a cold environment, infection, and injury. It can be seen from the changes that occurred the following day, such as body shaking, decreased appetite, lack of activity, and hair loss for 3 days. However, mouse number 18 died on the 14th day of observation.

Conclusions

The probiotic juice drink given to female Balb/C mice at doses of 5 mg/KgBW, 50 mg/KgBW, 50 mg/KgBW, and 2000 mg/KgBW showed that there were no symptoms of toxicity which were observed during 14 days of observation. One mouse died at a dose of 2000 mg/KgBW, allegedly because it got caught in the cage when the mouse was actively moving, causing stress and death on the fifth day.

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Phytochemical screening of *kaempferia galanga* l. Rhizome in Banguntapan, Bantul, yogyakarta

Muhammad Fadhiel Ihsan[1*], Reza Anindita[2], Intan Kurnia Putri[3], Noor Andryan Ilsan[4]
[1,2,3] Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga Bekasi, Indonesia
[4]Department of Medical Laboratory Technology, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga Bekasi, Indonesia
*Correspondence author : muhammadfadhielihsan@gmail.com

Abstract

The rhizome of *Kaempferia galanga* L. is a medicinal plant with many benefits for human health. *Kaempferia galanga* L. rhizome extract has long been used in traditional medicine because it contains active compounds that have potential as medicinal ingredients. The place where *Kaempferia galanga* L. rhizomes are taken can affect the compounds contained therein. This research aimed to determine the secondary metabolites in *Kaempferia galanga* L. rhizomes harvested in Banguntapan, Bantul Regency, Yogyakarta. This type of research is qualitative and quantitative. The research design is experimental. The method used for extraction is maceration using 96% ethanol solvent. Phytochemical screening for alkaloids, phenols, flavonoids, essential oils, and tannins is carried out by adding color reagents and observing color changes in the ethanol extract of *Kaempferia galanga* L rhizomes. The data analysis used in this research is descriptive by interpreting the results of the tables presented in this research. The percentage yield from ethanol extraction of *Kaempferia galanga* L. rhizomes was 14.89%. The phytochemical screening test of the ethanol extract of *Kaempferia galanga* L. rhizomes showed that the test results with Mayer's reagent were positive for alkaloids (white precipitate), positive for FeCl3 for phenols and tannins (blackish green), concentrated HCl and Mg positive for flavonoids (orange), cheerful for anisaldehyde-sulphuric acid solution for essential oil (red). Screening results using Dragendorff and Wagner reagents produced negative alkaloids (no orange and brown precipitates). Based on the results of this research conclude that the ethanol extract of *Kaempferia galanga* L. from the Banguntapan area, Bantul Regency, Yogyakarta, positively had phytochemical contents such as alkaloids, phenols, flavonoids, essential oils, and tannins.

Keywords: banguntapan, ethanol, *Kaempferia galanga* L. rhizome, maceration, phytochemical screening.

Introduction

Indonesia is a country with a tropical climate that is rich in various types of plants, many of which are currently used as natural medicinal ingredients. One of the plants used as a natural medicine today is the rhizome of *Kaempferia galanga* L. The rhizome of *Kaempferia galanga* L. is an aromatic plant originating from India and cultivated, one of which is in Java (Subaryanti *et al*., 2020). Many benefits of the rhizome of *Kaempferia galanga* L. are often widely used by the public as herbal medicine and it is known that *Kaempferia galanga* L. rhizomes have analgesic, antibacterial, antidiarrheal, antioxidant, and anti-inflammatory effects (Cahyawati, 2020). The rhizome of *Kaempferia galanga* L. has 49 secondary metabolite components such as esters, terpenoids, steroids, tannins, flavonoids, polyphenols, sesquiterpenes, and monoterpenes which are mostly contained in its essential oil (Adianingsih *et al*., 2021).

The biochemical content of the rhizome of *Kaempferia galanga* L. depends on the cultivation method, the growing environment, and the variety so there may be differences in the biochemical content of *Kaempferia galanga* L. rhizomes from different regions (Utami *et al*., 2020). Central Java is the center of *Kaempferia galanga* L. rhizome cultivation, with production data obtained in 2018 by the Indonesian Central Bureau of Statistics of 8,759 tons per year (Adianingsih *et al*., 2021). The purpose of this study was to analyze the secondary metabolites contained in the ethanol extract of *Kaempferia galanga* L., which comes from the Banguntapan area, Bantul, Yogyakarta.

Methods

Sample preparation

This type of research is qualitative and quantitative, with the research design being experimental. The sample used was the rhizome of *Kaempferia galanga* L. 8-9 months old, harvested during the dry season in the Banguntapan area, Bantul Regency, Yogyakarta. The research was conducted at the STIKes Mitra Keluarga Phytochemical Laboratory.

Kaempferia galanga L. rhizome samples of as much as 5 kg of wet sorting were carried out by separating the rhizomes of *Kaempferia galanga* L. good with the rhizome of *Kaempferia galanga* L., which is rotten or damaged. Leaves, roots, and stems of *Kaempferia galanga* L. were cut with a knife and then washed (Hapsoh *et al*., 2010). The samples were chopped on *Kaempferia galanga* L. with a thickness of 3-4 mm. Then, the samples were dried in the hot sun for 4 days and covered with black cloth to avoid direct contact with sunlight. After that, drying is continued in the

oven for 2 days at 45˚C (Anggraini & Saputri, 2021). Dry sorting results were performed to select damaged or defective *Kaempferia galanga* L. rhizomes, such as charred, damaged rhizomes, or rhizomes that were not yet dry. The dry sorting results were pollinated using a blender, after which they were sifted using a mesh number 20 sieve (Azkiya *et al*., 2017).

Extraction

Simplisia rhizome of *Kaempferia galanga* L. as much as 200 grams dissolved in 1000 ml of 96% ethanol in a suitable airtight bottle, protected from sunlight. Maceration was carried out for 5 days and shaken every day, then continued with remaceration for 3 days. The results of maceration and remaceration are filtered using filter paper and stored in a brown bottle to avoid sunlight (Amelinda *et al*., 2018; Fajeriyati & Andika, 2017). The extraction results were then evaporated using a rotary evaporator to obtain a thick ethanol extract of *Kaempferia galanga* L. with a temperature setting of 48˚C, a pressure of 300 mbar, and a rotational speed of 90 rpm.

Phytochemical Screening

Phytochemical screening in this study was carried out by adding coloring reagents. In the phenolic compounds, the thick extract of *Kaempferia galanga* L. (0.5 gram) was added with FeCl3 (3-4 drops). A positive result for phenolic compounds will show a color change from bluish-black to dark-black. In the tannin compound, the extract (0.5 gram) is added to 10 mL of hot water and dripped with $FeCl_3$. A positive result will show a color change to black-green (Ningsih *et al*., 2020). Flavonoid compounds were screened by placing the extract (0.5 gram) in a test tube, adding 5 ml of ethanol, heating for 5 minutes, then adding 10 drops of concentrated HCl and 0.2 g of Magnesium powder. A positive result for flavonoids will show a color change to red-brown. In the alkaloid compound, the extract (0.5 gram) was put into a test tube along with 2 ml of chloroform and 10 ml of ammonia. The solution was then added 10 drops of $H2SO_4$ and shaken, then allowed to stand. The solution will be separated into 2 layers, then the $H2SO_4$ layer is transferred into 3 test tubes of 2.5 ml each and tested with Mayer, Dragendorff, and Wagner reagents. Positive results are indicated by a white precipitate in the Mayer reagent, a red-orange precipitate in the Dragendorff reagent, and a brown precipitate in the Wagner reagent (Rante *et al*., 2020). Screening of essential oil compounds was carried out by adding anisaldehyde-sulfuric acid to the extract (0.5 gram). Positive results of essential oils can be seen in visible light by producing blue, green, red, or brown colors, and some fluoresce in ultraviolet light with a wavelength of 366 nm (Hanani, 2016).

Data analysis

The data collected in this study is primary data from observations of the results of phytochemical screening tests. The data obtained is qualitative in the form of changes in sample color with positive or negative results. The resulting data from this study were processed and presented systematically in tables and figures and then described to determine the secondary metabolites in the ethanol extract of *Kaempferia galanga* L. rhizomes.

Results and Discussions

The extract yield calculation was carried out to determine the amount obtained by comparing the weight of the thick extract produced with the weight of the initial powder. The results of calculating the yield of *Kaempferia galanga* L. rhizome extract with a powder weight of 929 g and an extract weight of 138.32 g found that the extract yield percentage was 14.89%, which met the requirements set out in the Farmakope Herbal Indonesia, namely > 8.3 %. Phytochemical screening in this study was conducted to provide an overview of the secondary metabolite compounds contained in *Kaempferia galanga* L. rhizome extract. In this study, phytochemical screening was carried out qualitatively.

Table 1. Phytochemical Screening

No.	Compound	Reagent	Results	Information
1.	Alkaloids	Mayer	+	There is a white precipitate
		Dragendorf	-	The solution is not orange
		Wagner	-	The solution is not brown
2.	Phenol	$FeCl_3$	+	The solution is blackish-green
3.	Flavonoid	Concentrated HCl + Mg	+	Orange colored solution

	s			
4.	Essential oil	Anisaldehyde-sulfuric acid solution	+	Red in visible light
5.	Tannin	$FeCl_3$	+	The solution is blackish-green

Based on the results of the table above, the results of screening the ethanol extract of the rhizome of *Kaempferia galanga* L. with Mayer's reagent were positive for alkaloids (white precipitate), positive for $FeCl_3$ for phenol and tannin (blackish green), concentrated HCl and Mg positive for flavonoids (orange), anisaldehyde- positive sulfuric acid containing essential oil (red). Meanwhile, screening with Dragendorff and Wagner reagents produced negative alkaloids (no orange and brown precipitates).

Phytochemical screening is a qualitative preliminary test by observing the color reaction in *Kaempferia galanga* L. extract by adding specific reagents. The color change in the extract is caused by a chemical reaction between the compound detected and the reagent used (*Saputri*, 2021). Phytochemical screening in this study aims to identify or provide an overview of secondary metabolites in *Kaempferia galanga* L. rhizome extract with 96% ethanol solvent. The secondary metabolites identified include alkaloids, phenols, flavonoids, essential oils, and tannins.

Phytochemical screening in this study found that the ethanol extract of *Kaempferia galanga* L. rhizome positively contained alkaloids, phenols, flavonoids, essential oils, and tannins. Phytochemical screening of alkaloid compounds in this study used n-ammonia-chloroform to extract the sample which was then filtered and added H2SO4 acid to salt the alkaloids because alkaline alkaloids can dissolve in acidic solvents (Indarto, 2015; Puspa *et al*., 2017). After that, shake it so that two layers form. The top layer, the water phase, was tested with Dragendorff, Meyer, and Wagner reagents. These three tests showed positive results in the Meyer test with the formation of a white precipitate and negative results for the Dragendorff and Wagner tests. Based on Seniwaty *et al.* (2009) and Soniman *et al.* (2022), the reagent used is adjusted to the alkaloid's ability to combine with metals that have a high atomic weight, such as mercury, bismuth, tungsten, or iodine, in this case, mercury.

This phytochemical screening test for alkaloid compounds is based on the principle of a precipitation reaction that occurs by replacing a ligand. Alkaloids having a nitrogen atom with a lone pair of electrons can replace the iodo ion in the reagent. Dragendorff's reagent contains bismuth nitrate and potassium iodide, a solution of glacial acetic acid (potassium tetraiodobismutat (III)). Mayer's reagent contains potassium iodide and mercuric chloride (tetraiodomercurate (II)). Wagner's reagent contains potassium iodide (KI) and iodine (I_2), both of which react to produce brown I_3-. This precipitate is formed from a potassium-alkaloid complex, in which K+ ions form coordinate covalent bonds with nitrogen in the alkaloids (Julianto, 2016; Reiza *et al*., 2019).

Phytochemical screening for phenol and tannin compounds in this study was carried out using FeCl3 reagent, and positive results were found to be blackish green. FeCl3 reagent is widely used to identify polyphenolic compounds, including tannins. Therefore, other phenolic compounds in the extract can also give positive results. The color change is caused by FeC13 reacting with one of the hydroxyl groups in polyphenolic compounds (Sangi *et al*., 2008).

Phytochemical screening for flavonoid compounds in this study used the Wilstatter test method by adding magnesium powder and concentrated HCl to reduce the flavonoid compounds contained in the extract. The results of this test found a change in color to orange, which means it is positive for containing flavonoids. The cyanidin Wilstater test commonly detect compounds with an α-benzopyron nucleus (Julianto, 2016). The results of the phytochemical screening were also strengthened by research conducted by Hayati *et al*. (2015), which found that the phytochemical screening results were orange in color, and further identification using UV-Vis spectrophotometry suggested that the flavonoid compounds belonged to the flavonone or dihydro flavonol group.

Phytochemical screening of essential oil compounds in this study used anisaldehyde-sulfuric acid solution as a reagent and found positive results with a color change to red. *Sulfuric acid anisaldehyde* is a universal reagent used in identifying chemical compound classes of essential oil components. Sulfuric acid anisaldehyde reagent will change the color of the monoterpene alcohol and its ester compounds, cineol, citral, aldehyde, and citronellal. The color change to red is thought to be caused by the reaction of steroid/terpenoid compounds with anisaldehyde-sulfate reagent (Christiana & Soegianto, 2020; Syarifuddin & Sulistyani, 2019)

Conclusions

In summary, this study found that the presence of phytochemical contents such as alkaloids, phenols, flavonoids, essential oils, and tannins was detected in the ethanolic extract of *Kaempferia galanga* L by using the reagent test for each type of metabolites. According to the results, *Kaempferia galanga* L. has an antioxidant and antibacterial activity that helps to detect the bioactive principles and then develop products and medicines made from herbs, especially by using *Kaempferia galanga* L. Further studies should be conducted by doing extensive research and more detailed such as antibacterial and antioxidant tests using the ethanolic extract of *Kaempferia galanga* L.

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Synergism test on date seeds (*Phoenix dactylifera* l.) And peanut shells (*Arachis hypogea* l.) Extract on antioxidant activity

Galuh Anjani Garnisaputri[1], Intan Kurnia Putri[2]*, Melania Perwitasari[3], Dede Dwi Nathalia[4]

[1,2,3,4] Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Bekasi, Indonesia.
*Correspondence author : intan.kurnia.p@stikesmitrakeluarga.ac.id

Abstract

Free radicals can cause body tissue damage and cancer. Several compounds that can overcome tissue damage in the body are called antioxidant compounds. Antioxidant compounds can be found in various plants, such as dates and peanuts. The purpose of this study was to evaluate the antioxidant activity contained in date seeds and peanut skin as well as the synergistic effect of the combination of date seed extract and peanut shell extract. The samples used were ethanolic extract of date palm seeds and peanut shells by maceration extraction method. The Ferric Reducing Antioxidant Power (FRAP) method with UV-Vis spectrophotometry was applied to evaluate the antioxidant activity. The results of the phytochemical screening showed that the ethanol extract of date palm seeds and the ethanol extract of peanut shells contained flavonoid compounds characterized by a change in color to orange and phenolic compounds indicated by a change in color to black. The results of antioxidant activity using the FRAP method were 130.370% for date seed extract and 170.353% for peanut shell extract. Combining the ethanolic extract of date palm seeds and the ethanol extract of peanut shells obtained a percentage difference value of 78.575%. Peanut shell extract has higher antioxidant activity than date seed extract, and combining the two has a synergistic effect.

Keywords: Antioxidant activity; Cancer; Date seeds; FRAP; Peanut skin; Synergism

Introduction

Based on data from *the World Health Organization* (2022), there were a total of 396.914 cases of cancer in Indonesia and a total of 234.511 deaths. Deaths from cancer are expected to continue to increase to more than 13.1 million in 2030 (Ministry of Health, 2019). *Cancer* is a malignant tumor characterized by abnormal growth of body cells. The types of cancer most commonly found in patients in hospitals are breast cancer (28.7%) and cervical cancer (12.8%) (Ministry of Health of the Republic of Indonesia, 2018). One of the causes of cancer is free radicals, which attack cells in the human body and cause tissue damage. Compounds that are useful in helping overcome tissue damage caused by free radicals are antioxidants (Alghiffari, 2021).

Antioxidants are helpful in preventing cancer cells and work by capturing free radicals, so they can inhibit the proliferation of cancer cells and become anticancer agents. Antioxidant compounds can be obtained from synthetic compounds or natural compounds. Currently, people prefer to use traditional medicine because it is believed to have milder side effects than synthetic drugs. Indonesia has many types of plants that have potential as natural antioxidants, one of which is dates and peanuts (Santoso *et al.*, 2022).

Dates (*Phoenix dactylifera* L.) is a plant that can be used as a medicinal plants. Dates have been shown to have antioxidant, antimicrobial, and anti-inflammatory activity. The functional effects of dates are not only limited to the fruit but also to the seeds. In traditional medicine, date seeds treat various ailments, including cancer, digestive disorders, diarrhea, liver disorders, diabetes, and lung infections (Bouhlali *et al*., 2021). The antioxidant properties contained in date seeds can function to fight oxidative stress caused by free radicals. These date seeds are usually just waste and require further efforts to utilize them (Huang *et al*., 2020).

Peanuts (*Arachis hypogea* L.) have the same benefits as date seeds, namely that they can treat cancer and liver disorders (Fadly *et al.*, 2022). In addition, peanuts are widely known for their high content of phenolic compounds with antioxidant activity that is concentrated in the skin and has health effects on the human body (Lima & Alencar, 2022). So far, using peanuts is still limited to processing the seeds. In contrast, the use of peanut shells has not been maximized and is only considered waste, usually only for animal feed and fertilizer. It turns out that peanut skin contains a lot of flavonoids, and the most considerable flavonoid content is luteolin (Ichsani, 2021). Vrianty *et al*. (2019) stated that luteolin is an anti-inflammatory, antioxidant, and anticancer. Therefore, further efforts are needed to make peanut shells a helpful material.

Methods

In this study using the descriptive with design of research is pre-experimental. This research was carried out at the chemical laboratory of STIKes Mitra Keluarga in January – March 2023. The materials used were date palm seeds (phoenix dactylifera) type of Sukari, Peanut shells (Arachis hypogaea L.), 70% ethanol, 2,4,6-tripyridylstriazine (TPTZ) (Sigma), $FeCl_3$ (ferric chloride)(Brataco),and Vitamin C (L-Ascorbic Acid) (Sangon Biotech), Mg (Magnesium)

(Merck), HCl (hydrochloric acid), DMSO (Dymethyl sulfoxide), H_2SO_4 (Acetic acid), CH_3COONa (Sodium acetate), $FeSO_4$ (Brataco) and used several tools, namely test tubes, trays, test tube racks, plastic wrap, 60-80 mesh sieve, aluminum foil, knife, tissue, measuring cup,beaker glass,Spatula, Porcelain cup, Filter paper, Glass funnel, Stirring rod, Drop pipette, Vial bottle, Glass cuvette, Micropipette, Analytical balance, Microtip,Rotary evaporator(IKA), Oven, Vortex Mixer, UV-Vis Spectrophotometer (thermo).

Plant determination test

The dates seed and peanut shells used were subjected to a determination test at the National Research and Innovation Agency (BRIN), Bogor.

Preparation of Sample Extract

Date seeds are dried for 2 hours in an oven at 60°C. Furthermore, the date palm seeds are mashed using a blender until a fine powder is obtained. The fine powder of the date seeds was weighed as much as 250 grams (Siregar et al., 2018). Then maceration extraction was carried out soaked in 500 mL 70% ethanol. The yield was filtered using filter paper to separate the filtrate from the residue. The filtrate is concentrated usingrotary evaporatorsat 60°C at a speed of 90 rpm to obtain the ethanol extract of date palm seeds (Nazilah, 2019).

Peanuts were dried in an oven at 60°C for 3 days. After drying, the peanut shell simplicia is ground using a blender to make it into powder. Then the peanut shell powder was sieved using a 60 mesh sieve to obtain a fine peanut shell powder (Fadly *et al.*, 2022). Then maceration extraction was carried out by soaking peanut shell powder in 2 L of 70% ethanol and leaving it for 3 days. The juice is filtered and the residue is squeezed. The residue was re-macerated with the same solvent and left for 2 days. Then separate the filtrate and evaporate itrotary evaporatorsat a temperature of 60°C to obtain a thick ethanol extract of peanut shells (Kursia *et al.*, 2020).

Determination of Extract Water Content

The water content of the extract was tested using a moisturizer analysis tool by weighing 1 gram of extract of date palm seeds and peanut shells into a device that has been set at 105°C for 30 minutes (Susanto, 2021).

Phytochemical Screening

Phytochemical screening includes Flavonoid and phenol test. Flavonoid test on date seed extract and peanut shell extract was carried out by evaporating the extract until dry and then adding 2-3 drops of ethanol. After that, 0.2 gram of Mg powder and a few drops of 5M HCl were added. If a red, orange or purple color is formed, then there are flavonoid compounds (Ichsani, 2021). Phenol test on date seed extract and peanut shell extract was carried out by mixing the extract with 3-4 drops of 1% $FeCl_3$. The presence of phenol compounds can be seen from the formation of a blackish green color (Azizah & Widya Wati, 2018).

Preparation of Acetate Buffer

Acetate buffer with a pH of 3.6 was prepared from 0.155 grams of sodium acetate (CH_3COONa) added with 0.8 mL of concentrated acetic acid and dissolved with distilled water to exactly 50 mL in a measuring flask.

Preparation of 2,4,6-trippyridyl-striazine (TPTZ) Solution

TPTZ was weighed as 30 mg, dissolved in 40 mmol/L HCl to exactly 10 mL. A solution of 40 mmol/L HCl was prepared by dissolving 0.1656 mL of concentrated HCl in 50 mL of distilled water.

Preparation of FeCl3.6H2O Solution

FeCl3.6H2O was weighed as much as 0.27 grams, dissolved in distilled water in a measuring flask to exactly 50 mL.

Preparation of FRAP Reagent

FRAP reagent is prepared by mixing 25 mL of acetate buffer; 2.5 mL TPTZ solution; 2.5 mL FeCl3.6H2O solution. Then add distilled water to exactly 100 mL in the measuring flask.

Preparation and Testing of $FeSO_4.7H_2O$ Standard Solution

A total of 50 mg of $FeSO_4.7H_2O$ was dissolved in 50 mL of distilled water in a volumetric flask until a concentration of 1000 µg/mL was obtained. In a measuring flask 10 mL is made concentration of 40 – 140 µg/mL. From each concentration, 100 µL was taken and then put into a volumetric flask and 3 mL of FRAP reagent was added. After that, it was incubated for 10 minutes at 37°C. The absorbance of each concentration was read using a UV-Vis spectrophotometer with the maximum wavelength.

Maximum Wavelength Determination

The maximum wavelength was obtained by measuring the absorbance of the $FeSO_4.7H_2O$ standard with the highest concentration (140 µg/mL). 100 µL of the solution was taken, then 3 mL of FRAP reagent was added, then read at every wavelength of 580-610 nm.

Determination of Antioxidant Activity Values

A total of 10 mg of date seed samples was dissolved in distilled water to 10 mL (1000 µg/mL) in a volumetric flask. Then take a volume of 1 mL and then put it in a 10mL volumetric flask and dilute it with distilled water up to the boundary mark, so that a concentration of 100 µg/mL is obtained. A total of 20 mg of peanut shell sample was dissolved in distilled water to 10 mL (2000 µg/mL) in a volumetric flask. From these concentrations, 100 µL of each was taken plus 3 mL of FRAP reagent in a volumetric flask. After that, it was incubated at 37°C for 10 minutes. Then read the absorbance using a UV-Vis spectrophotometer with the maximum wavelength. Antioxidant activity was obtained from absorbance data against FeSO4.7H2O standard series and stated to be equivalent to ppm Fe2+. The percentage of FRAP activity is calculated according to the formula (Widowati et al., 2022):

$$\text{FRAP activity (\%)} = (A/B) \times 100 \qquad (1)$$

A : control absorbance, B : sample absorbance

Synergism Test

The synergism test was carried out by mixing date seed extract (100 µg/mL) and peanut shell extract (2000 µg/mL) in a ratio of 100: 100, then determining the synergistic extract by calculating using the following formula (Skroza et al., 2022).

$$\text{Difference (\%)} = \left[\frac{ab\ x\ 100}{(Individual\ a + individual\ b) \div n}\right] - 100 \qquad (2)$$

Differences: Effects resulting from a mixture of extracts a and b, Combination ab: Antioxidant activity (Fe^{2+} levels) in the mixture of extracts a and b, Individual a / b: Antioxidant activity (Fe^{2+} levels) in extracts a / b, n: The number of compounds in the mixture.

Results and Discussions

The test was carried out at the National Research and Innovation Agency (BRIN). The purpose of the determination test is to prevent sample errors and guarantee the correct identification of plants based on their taxonomy (Nurulita et al., 2019). The test results stated that the date seeds used in this study were date seeds with the Latin name Phoenix dactyliferaL., family Arecaceae. Meanwhile, the test results for peanut shells used in this research have Latin names Arachis hypogaeaL., Fabaceae family.

The results of thick date seeds extract are presented in Figure and the results of the characteristics of date seed extract are presented in Table 1.

Figure 1. Extract of Dates Seeds

Table 1. Results of the Characteristics of Date Seed Extract

Specification	Description
%yield	12.6%
Color	Brownish black
Smell	Typical of dates

The results of thick peanut shell extract are presented in Figure 2 and the results of the characteristics of peanut shell extract are presented in Table 2.

Figure 2. Peanut Shells Extract

Table 2. Results of Characteristics of Peanut Shells Extract

Specification	Description
%yield (Maceration 1)	1.25%
%yield (Maceration 2)	0.9%
Color	Chocolate
Smell	Typical of Peanut

The results obtained for the water content of date seed extract and peanut shell extract can be seen in table 3.

Table 3. Extract Water Content Test Results

Samples	Replication	Water Content (%)	Mean ± SD
Seed Dates Extract	1	3.68	3.74 ± 0.10
	2	3.68	
	3	3.86	
Peanut Shells Extract	1	6.73	6.70 ± 0.06
	2	6.73	
	3	6.63	

Phytochemical screening was carried out to qualitatively determine the content of active compounds in plants contained in date seed extract and peanut shell extract (Kusmiati et al., 2018). Phytochemical screening in this study was carried out on the flavonoid and phenol compounds. The flavonoid test was carried out by mixing the extract with 0.2 grams of Mg powder and several drops of 5M HCl. Samples containing flavonoid compounds obtained a color change to red, orange, or purple (Ichsani, 2021). Then the phenol test was carried out using FeCl reagent31%. The presence of phenolic compounds was indicated by the formation of a black color (Azizah *et al*, 2018). The phytochemical screening results obtained for date seed extract and peanut shell extract can be seen in table 4.

Table 4. Phytochemical Screening Results

	Flavonoid Test		Phenol Test	
Extract	**Before**	**After**	**Before**	**After**
Date Seeds	Yellow	(+) Orange	Yellow	(+) Black
Peanut Shells	Yellow	(+) Red	Yellow	(+) Black

Note : (+) = Compound detected, (-) = Compound not detected

Determine the maximum wavelength by reading the absorbance of FeSO standard standard$_4$.7H$_2$O with the highest concentration (140 µg/mL) in range 580 – 610 nm. The maximum wavelength obtained was 595 nm with an absorbance of 0.782. The maximum wavelength spectrum can be seen in Figure 3.

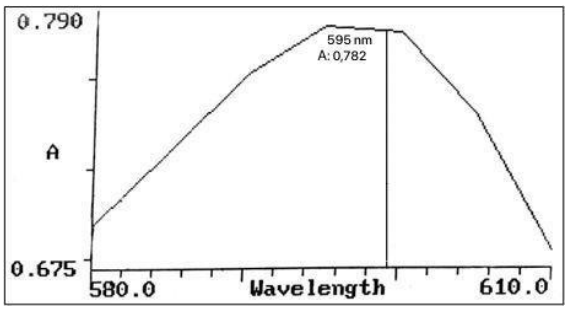

Figure 3. Maximum Wavelength Spectrum

The results of determining the standard curve absorbance were carried out in a series of solutions with a concentration of 50; 70 ; 100 ; 130 ; 140 µg/mL. The regression equation Y = 0.0032x + 0.1638 is obtained with a value of R^2 = 0.999, at a maximum wavelength of 595 nm, as shown in Figure 4. The standard curve concentration series absorbance table can be seen in table 5.

Table 5. Absorbance Data of FeSO$_4$.7H$_2$O Standard Curve

Concentration (µg/mL)	Absorbamce
50	0.328
70	0.384
100	0.482
130	0.585
140	0.611

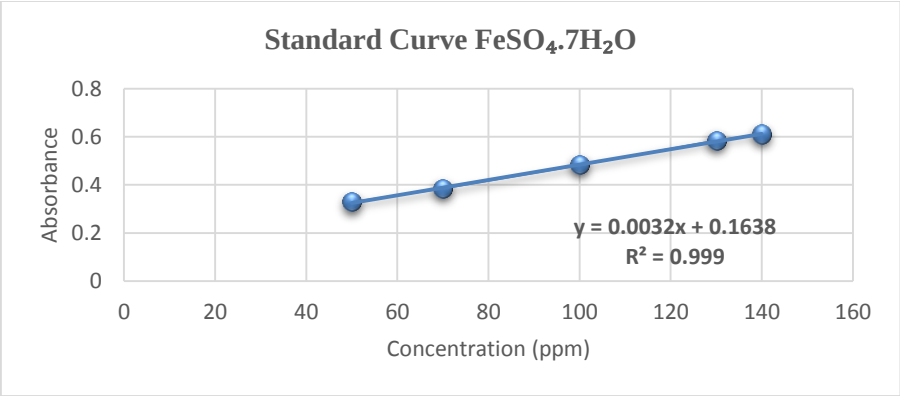

Figure 4. Standard Curve of FeSO$_4$.7H$_2$O

The control absorbance result was obtained at 0.611, while the sample absorbance was obtained by calculating the % FRAP activity to see how much antioxidant activity contained in date seed extract and peanut shell extract. The results of % FRAP Activity can be seen in table 6.

Table 6. FRAP Activity % Value

Formulas	Treatment		Fe^{2+} Rates (µg/mL)	Aktivity FRAP (%)	± SD
	Date Seeds Extract (%)	Peanut Shells Extract (%)			
F1	100	0	95,271	130,370	0,180
F2	60	40	100,062	126,240	0,313
F3	55	45	132,771	103,794	0,180

F4	50	50	107,771	120,118	0,180
F5	45	55	114,750	115,066	0,313
F6	40	60	80,375	145,131	0,313
F7	0	100	60,896	170,353	0,180

The synergism test was carried out in one step by mixing date seed extract and peanut shell extract in a ratio of 100:100. The synergism results of the two extracts can be seen in table 7.

Table 7. Synergism Test Values

Formulas	Treatment		Fe^{2+} Rates (µg/mL)	Difference (%)	± SD
	Date Seeds Extract (%)	Peanut Shells Extract (%)			
F1	100	0	95,271	-	0,180
F7	0	100	60,896	-	0,180
F8	100	100	139,438	78,575	0,313

The first stage in this research was the determination test for date palm and peanut plants which was carried out at the Bogorines BRIN Herbarium, Cibinong. This determination test aims to ensure the correct identity of the plants to be used in research, as well as to avoid errors in sampling for phytochemical analysis. The results of the determination test that has been carried out can be obtained with certainty that the date palm used in this study is the correct species Phoenix dactyliferaL. from the family Arecaceae. Then the peanut plants used in this research are the correct species Arachis hypogaea L. from the family Fabaceae.

Sample preparation of date seed extract and peanut shells was carried out by dried in an oven at 60°C for 2 hours for date seeds and 3 days for peanut shells. The drying process aims to reduce the water content in the date seeds and peanut shells which can inhibit the separation process and prevent the growth of bacteria and fungi. This drying process uses a temperature not exceeding 60°C because temperatures that are too high can damage the physiological properties of grains and thermolabile active compounds and can cause oil to be trapped inside the seed shell (Riyadi, 2018). Sample extraction was carried out using the maceration method. Maceration is the process of soaking samples with suitable solvents at room temperature so as to reduce the risk of damage to secondary metabolites (Ichsani, 2021). This method is safer because it can protect thermolabile active compounds such as flavonoids and phenols (Nazilah, 2019). The maceration process was carried out by soaking the samples in 70% ethanol solvent for 2x24 hours for date palm kernels and 3x24 hours for peanut shells and then re-macerated for 2x24 hours. The 70% ethanol solvent was chosen because it is polar, has a polarity index of 4.3 and has low toxicity.

Characteristic checks carried out on each extract include shape, color and odor as well as % yield. In the extract of date seeds, 30.4 grams were obtained from 250 grams of date seed powder, so the % yield obtained was 12.16%. Riyadi (2018) states that date seed extract has a yield value of 4.929%. Yield value calculated to determine the amount of extract obtained in each gram of date seed powder. Whereas in the first extract of peanut shell used for the qualitative test, 2.5 grams were obtained from 200 grams of peanut shell powder, so the % yield obtained was 1.25%. The condensed extract of the two peanut shells used for the antioxidant activity test was obtained as much as 1.8 grams from 200 grams of peanut shell powder so that the % yield obtained was 0.9%. Ichsani (2021) states that the % yield obtained from peanut shell extract is 28.553%.

The extract obtained is then tested for water content using a tool moisture analyzer which aims to provide a minimum limit for the amount of water content in the extract, the higher the water content, the easier it is for fungi and mold to grow so that it can reduce the biological activity of the extract during the storage period. The results of determining the water content of extracts of date palm seeds and peanut shells after 3x replication were obtained at 3.74 ± 0.10% and 6.70 ± 0.06%. This result meets the requirements set by BPOM, namely water content ≤ 10% to minimize mold growth so as to produce good storage and extract quality (Hanif *et al*., 2019).

Phytochemical screening was carried out to qualitatively determine the content of active compounds in plants (Nazilah, 2019). The results show of the phytochemical test that the ethanol extract of the two samples contained secondary metabolites, namely flavonoids and phenols. This is because the ethanol solvent extraction process can increase the permeability of the simplex cell wall to attract components of polar and semipolar compounds (Fikayuniar *et al*., 2022). Phytochemical screening of date seed extract obtained the same results as previous studies. Bouhlali *et al*

(2020) stated that the ethanol extract of date seeds contains flavonoid and phenol compounds. This can be influenced by environmental conditions where grown, harvest time, storage conditions, geographical origin, light, temperature, fertilizer and soil type and method of extraction. Then, phytochemical screening of peanut shell extract also obtained the same results as previous research. Franyoto *et al* (2019) stated that the ethanol extract of peanut shells contains flavonoids, tannins, saponins, alkaloids and terpenoids. Annadira (2021) stated that the ethanol extract of peanut shells also contains phenol compounds.

Testing the antioxidant activity of date seed extract and peanut shell extract using the FRAP method requires several stages, such as determining the maximum wavelength, determining the standard curve, and determining the antioxidant activity value of the sample. Determination of the maximum wavelength is carried out to determine the wavelength with the highest absorption. Determination of the maximum wavelength was carried out by measuring the absorbance of a standard solution of $FeSO_4.7H_2O$ with the highest concentration (140 µg/mL) in the range 580 – 610 nm. This is because $FeSO_4.7H_2O$ provides absorption in the wavelength range 580 – 610 nm. The wavelength measurement using a UV-Vis spectrophotometer gives the highest absorption at a wavelength of 595 nm with an absorbance of 0.782. The results obtained are the same as Nurhayati's *et a*l., (2022) obtained a maximum wavelength of 595 nm.

Making a standard curve with a standard solution of $FeSO_4.7H_2O$ was carried out with 5 series of concentrations. The choice of concentration is based on the Lambert-Beer law which states the absorption requirement is 0.2 – 0.8 to avoid photometric errors (Asmorowati & Lindawati, 2019). The standard curve absorbance measurement was carried out by repeating the test 3 times with the aim of obtaining more accurate data. The results of determining the $FeSO_4.7H_2O$ standard curve which can be seen in Figure 5.10 show that the concentration is directly proportional to the absorbance value. The greater the concentration of the standard standard solution $FeSO_4.7H_2O$, the higher the absorbance value produced (Rahayu *et al.*, 2021). When measuring the absorbance, the regression equation $FeSO_4.7H_2O$ was obtained, namely $y = 0.0032x + 0.1638$, where y is the absorbance and x is the concentration of antioxidant activity. The linearity value results are shown by the coefficient of determination (R^2) value of 0.999, where the condition for acceptance of a good coefficient of determination is ≥ 0.997. This shows that there is a linear relationship between the concentration of the $FeSO_4.7H_2O$ standard standard solution and the absorbance value. Linearity can be said to be good if it meets the acceptance requirements (Gayatri, 2021).

Determination of the value of antioxidant activity in this study using the FRAP method (Ferric Reducing Antiozidant Power). The principle of this method is the ability of the compound antioxidants in reducing the Fe^{3+}-TPTZ compound to Fe^{2+}-TPTZ (Wulandari, 2021). The more Fe^{3+} concentration reduced by sample becomes Fe^{2+} then the antioxidant activity of the sample also increases big (Pebriana *et al.*, 2019).

Determination of antioxidant activity in the form of absorbance. The absorbance was obtained when measuring the maximum wavelength of the $FeSO_4.7H_2O$ standard solution. The standard solution was reacted with FRAP reagent and then incubated at 37°C for 10 minutes. The aim of incubation is so that the antioxidant reaction process with FRAP reagent achieves a perfect reaction and is not influenced by stability factors. Antioxidant stability can be affected by several factors, namely light, oxygen, and pH. The value of the sample antioxidant activity is expressed in % FRAP activity with Fe^{2+} levels (µg/mL) using standard $FeSO_4.7H_2O$. The value of the antioxidant activity was determined from the two extract samples in 7 mixing stages (F1 – F7), where F1 only contained date seed extract and F7 only contained skin extract. peanuts, while F2 – F6 contains a mixture of extracts based on a predetermined formula. The results showed that F7 contained the highest FRAP activity value of 170.353%, which means that when the FRAP reagent was reacted with peanut shell extract it had a very strong antioxidant activity compared to date seed extract in F1 of 130.370%. Then when the extracts started to be mixed as in F2 – F6, the one with the highest FRAP activity value was F6 where date palm seeds were 40% and peanut shells were 60% which was 145.131%. This is directly proportional to the results of the activity of the peanut shell extract itself.

In this study, single extracts of date palm seeds and peanut shells had antioxidant activity. The ability as an antioxidant of these two plant extracts can still be improved, one way is by combining the extracts between the two. This is known as the synergistic effect. The synergism effect is the effect of two or more combinations of different types of antioxidants that have a greater effect than the effect of the individual antioxidants separately (Septiana *et al.*, 2020). Apart from producing a synergistic effect, the combination of extracts can also produce additive or antagonistic effects. In this study, the combination of date seed extract with peanut shell extract produced a synergistic effect with a % difference value of 78.575. The percentage value of this difference is used to determine antioxidant potential, namely having a synergistic effect if the value is positive (% difference > 0) or antagonistic if the value is negative (% difference <0).

Conclusions

Based on the results of quantitative tests using the FRAP method, samples of date seed extract have antioxidant activity with % FRAP activity of 130.370%. Then, based on the results of quantitative tests using the FRAP method, samples of peanut shell extract have antioxidant activity with % FRAP activity of 170.353%. The results obtained from the combination of date seed extract and peanut shell extract have a synergistic effect on increasing antioxidant activity with a % difference value of 78.575 because the % difference result is > 1.

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Synergism test on date seeds (*Phoenix dactylifera* l.) And peanut shells (*Arachis hypogea* l.) Extract on antioxidant activity

Galuh Anjani Garnisaputri[1], Intan Kurnia Putri[2]*, Melania Perwitasari[3], Dede Dwi Nathalia[4]

[1,2,3,4] Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Bekasi, Indonesia.
*Correspondence author : intan.kurnia.p@stikesmitrakeluarga.ac.id

Abstract

Free radicals can cause body tissue damage and cancer. Several compounds that can overcome tissue damage in the body are called antioxidant compounds. Antioxidant compounds can be found in various plants, such as dates and peanuts. The purpose of this study was to evaluate the antioxidant activity contained in date seeds and peanut skin as well as the synergistic effect of the combination of date seed extract and peanut shell extract. The samples used were ethanolic extract of date palm seeds and peanut shells by maceration extraction method. The Ferric Reducing Antioxidant Power (FRAP) method with UV-Vis spectrophotometry was applied to evaluate the antioxidant activity. The results of the phytochemical screening showed that the ethanol extract of date palm seeds and the ethanol extract of peanut shells contained flavonoid compounds characterized by a change in color to orange and phenolic compounds indicated by a change in color to black. The results of antioxidant activity using the FRAP method were 130.370% for date seed extract and 170.353% for peanut shell extract. Combining the ethanolic extract of date palm seeds and the ethanol extract of peanut shells obtained a percentage difference value of 78.575%. Peanut shell extract has higher antioxidant activity than date seed extract, and combining the two has a synergistic effect.

Keywords: Antioxidant activity; Cancer; Date seeds; FRAP; Peanut skin; Synergism

Introduction

Based on data from *the World Health Organization* (2022), there were a total of 396.914 cases of cancer in Indonesia and a total of 234.511 deaths. Deaths from cancer are expected to continue to increase to more than 13.1 million in 2030 (Ministry of Health, 2019). *Cancer* is a malignant tumor characterized by abnormal growth of body cells. The types of cancer most commonly found in patients in hospitals are breast cancer (28.7%) and cervical cancer (12.8%) (Ministry of Health of the Republic of Indonesia, 2018). One of the causes of cancer is free radicals, which attack cells in the human body and cause tissue damage. Compounds that are useful in helping overcome tissue damage caused by free radicals are antioxidants (Alghiffari, 2021).

Antioxidants are helpful in preventing cancer cells and work by capturing free radicals, so they can inhibit the proliferation of cancer cells and become anticancer agents. Antioxidant compounds can be obtained from synthetic compounds or natural compounds. Currently, people prefer to use traditional medicine because it is believed to have milder side effects than synthetic drugs. Indonesia has many types of plants that have potential as natural antioxidants, one of which is dates and peanuts (Santoso *et al.*, 2022).

Dates (*Phoenix dactylifera* L.) is a plant that can be used as a medicinal plants. Dates have been shown to have antioxidant, antimicrobial, and anti-inflammatory activity. The functional effects of dates are not only limited to the fruit but also to the seeds. In traditional medicine, date seeds treat various ailments, including cancer, digestive disorders, diarrhea, liver disorders, diabetes, and lung infections (Bouhlali *et al*., 2021). The antioxidant properties contained in date seeds can function to fight oxidative stress caused by free radicals. These date seeds are usually just waste and require further efforts to utilize them (Huang *et al*., 2020).

Peanuts (*Arachis hypogea* L.) have the same benefits as date seeds, namely that they can treat cancer and liver disorders (Fadly *et al.*, 2022). In addition, peanuts are widely known for their high content of phenolic compounds with antioxidant activity that is concentrated in the skin and has health effects on the human body (Lima & Alencar, 2022). So far, using peanuts is still limited to processing the seeds. In contrast, the use of peanut shells has not been maximized and is only considered waste, usually only for animal feed and fertilizer. It turns out that peanut skin contains a lot of flavonoids, and the most considerable flavonoid content is luteolin (Ichsani, 2021). Vrianty *et al*. (2019) stated that luteolin is an anti-inflammatory, antioxidant, and anticancer. Therefore, further efforts are needed to make peanut shells a helpful material.

Methods

In this study using the descriptive with design of research is pre-experimental. This research was carried out at the chemical laboratory of STIKes Mitra Keluarga in January – March 2023. The materials used were date palm seeds (phoenix dactylifera) type of Sukari, Peanut shells (Arachis hypogaea L.), 70% ethanol, 2,4,6-tripyridylstriazine (TPTZ) (Sigma), $FeCl_3$ (ferric chloride)(Brataco),and Vitamin C (L-Ascorbic Acid) (Sangon Biotech), Mg (Magnesium)

(Merck), HCl (hydrochloric acid), DMSO (Dymethyl sulfoxide), H_2SO_4 (Acetic acid), CH_3COONa (Sodium acetate), $FeSO_4$ (Brataco) and used several tools, namely test tubes, trays, test tube racks, plastic wrap, 60-80 mesh sieve, aluminum foil, knife, tissue, measuring cup,beaker glass,Spatula, Porcelain cup, Filter paper, Glass funnel, Stirring rod, Drop pipette, Vial bottle, Glass cuvette, Micropipette, Analytical balance, Microtip,Rotary evaporator(IKA), Oven, Vortex Mixer, UV-Vis Spectrophotometer (thermo).

Plant determination test

The dates seed and peanut shells used were subjected to a determination test at the National Research and Innovation Agency (BRIN), Bogor.

Preparation of Sample Extract

Date seeds are dried for 2 hours in an oven at 60°C. Furthermore, the date palm seeds are mashed using a blender until a fine powder is obtained. The fine powder of the date seeds was weighed as much as 250 grams (Siregar et al., 2018). Then maceration extraction was carried out soaked in 500 mL 70% ethanol. The yield was filtered using filter paper to separate the filtrate from the residue. The filtrate is concentrated usingrotary evaporatorsat 60°C at a speed of 90 rpm to obtain the ethanol extract of date palm seeds (Nazilah, 2019).

Peanuts were dried in an oven at 60°C for 3 days. After drying, the peanut shell simplicia is ground using a blender to make it into powder. Then the peanut shell powder was sieved using a 60 mesh sieve to obtain a fine peanut shell powder (Fadly *et al.*, 2022). Then maceration extraction was carried out by soaking peanut shell powder in 2 L of 70% ethanol and leaving it for 3 days. The juice is filtered and the residue is squeezed. The residue was re-macerated with the same solvent and left for 2 days. Then separate the filtrate and evaporate itrotary evaporatorsat a temperature of 60°C to obtain a thick ethanol extract of peanut shells (Kursia *et al.*, 2020).

Determination of Extract Water Content

The water content of the extract was tested using a moisturizer analysis tool by weighing 1 gram of extract of date palm seeds and peanut shells into a device that has been set at 105°C for 30 minutes (Susanto, 2021).

Phytochemical Screening

Phytochemical screening includes Flavonoid and phenol test. Flavonoid test on date seed extract and peanut shell extract was carried out by evaporating the extract until dry and then adding 2-3 drops of ethanol. After that, 0.2 gram of Mg powder and a few drops of 5M HCl were added. If a red, orange or purple color is formed, then there are flavonoid compounds (Ichsani, 2021). Phenol test on date seed extract and peanut shell extract was carried out by mixing the extract with 3-4 drops of 1% $FeCl_3$. The presence of phenol compounds can be seen from the formation of a blackish green color (Azizah & Widya Wati, 2018).

Preparation of Acetate Buffer

Acetate buffer with a pH of 3.6 was prepared from 0.155 grams of sodium acetate (CH_3COONa) added with 0.8 mL of concentrated acetic acid and dissolved with distilled water to exactly 50 mL in a measuring flask.

Preparation of 2,4,6-trippyridyl-striazine (TPTZ) Solution

TPTZ was weighed as 30 mg, dissolved in 40 mmol/L HCl to exactly 10 mL. A solution of 40 mmol/L HCl was prepared by dissolving 0.1656 mL of concentrated HCl in 50 mL of distilled water.

Preparation of FeCl3.6H2O Solution

FeCl3.6H2O was weighed as much as 0.27 grams, dissolved in distilled water in a measuring flask to exactly 50 mL.

Preparation of FRAP Reagent

FRAP reagent is prepared by mixing 25 mL of acetate buffer; 2.5 mL TPTZ solution; 2.5 mL FeCl3.6H2O solution. Then add distilled water to exactly 100 mL in the measuring flask.

Preparation and Testing of $FeSO_4.7H_2O$ Standard Solution

A total of 50 mg of $FeSO_4.7H_2O$ was dissolved in 50 mL of distilled water in a volumetric flask until a concentration of 1000 µg/mL was obtained. In a measuring flask 10 mL is made concentration of 40 – 140 µg/mL. From each concentration, 100 µL was taken and then put into a volumetric flask and 3 mL of FRAP reagent was added. After that, it was incubated for 10 minutes at 37°C. The absorbance of each concentration was read using a UV-Vis spectrophotometer with the maximum wavelength.

Maximum Wavelength Determination

The maximum wavelength was obtained by measuring the absorbance of the $FeSO_4.7H_2O$ standard with the highest concentration (140 µg/mL). 100 µL of the solution was taken, then 3 mL of FRAP reagent was added, then read at every wavelength of 580-610 nm.

Determination of Antioxidant Activity Values

A total of 10 mg of date seed samples was dissolved in distilled water to 10 mL (1000 µg/mL) in a volumetric flask. Then take a volume of 1 mL and then put it in a 10mL volumetric flask and dilute it with distilled water up to the boundary mark, so that a concentration of 100 µg/mL is obtained. A total of 20 mg of peanut shell sample was dissolved in distilled water to 10 mL (2000 µg/mL) in a volumetric flask. From these concentrations, 100 µL of each was taken plus 3 mL of FRAP reagent in a volumetric flask. After that, it was incubated at 37°C for 10 minutes. Then read the absorbance using a UV-Vis spectrophotometer with the maximum wavelength. Antioxidant activity was obtained from absorbance data against FeSO4.7H2O standard series and stated to be equivalent to ppm Fe2+. The percentage of FRAP activity is calculated according to the formula (Widowati et al., 2022):

$$\text{FRAP activity (\%)} = (A/B) \times 100 \qquad (1)$$

A : control absorbance, B : sample absorbance

Synergism Test

The synergism test was carried out by mixing date seed extract (100 µg/mL) and peanut shell extract (2000 µg/mL) in a ratio of 100: 100, then determining the synergistic extract by calculating using the following formula (Skroza et al., 2022).

$$\text{Difference (\%)} = \left[\frac{ab\ x\ 100}{(Individual\ a + individual\ b) \div n}\right] - 100 \qquad (2)$$

Differences: Effects resulting from a mixture of extracts a and b, Combination ab: Antioxidant activity (Fe^{2+} levels) in the mixture of extracts a and b, Individual a / b: Antioxidant activity (Fe^{2+} levels) in extracts a / b, n: The number of compounds in the mixture.

Results and Discussions

The test was carried out at the National Research and Innovation Agency (BRIN). The purpose of the determination test is to prevent sample errors and guarantee the correct identification of plants based on their taxonomy (Nurulita et al., 2019). The test results stated that the date seeds used in this study were date seeds with the Latin name Phoenix dactyliferaL., family Arecaceae. Meanwhile, the test results for peanut shells used in this research have Latin names Arachis hypogaeaL., Fabaceae family.

The results of thick date seeds extract are presented in Figure and the results of the characteristics of date seed extract are presented in Table 1.

Figure 1. Extract of Dates Seeds

Table 1. Results of the Characteristics of Date Seed Extract

Specification	Description
%yield	12.6%
Color	Brownish black
Smell	Typical of dates

The results of thick peanut shell extract are presented in Figure 2 and the results of the characteristics of peanut shell extract are presented in Table 2.

Figure 2. Peanut Shells Extract

Table 2. Results of Characteristics of Peanut Shells Extract

Specification	Description
%yield (Maceration 1)	1.25%
%yield (Maceration 2)	0.9%
Color	Chocolate
Smell	Typical of Peanut

The results obtained for the water content of date seed extract and peanut shell extract can be seen in table 3.

Table 3. Extract Water Content Test Results

Samples	Replication	Water Content (%)	Mean ± SD
Seed Dates Extract	1	3.68	3.74 ± 0.10
	2	3.68	
	3	3.86	
Peanut Shells Extract	1	6.73	6.70 ± 0.06
	2	6.73	
	3	6.63	

Phytochemical screening was carried out to qualitatively determine the content of active compounds in plants contained in date seed extract and peanut shell extract (Kusmiati et al., 2018). Phytochemical screening in this study was carried out on the flavonoid and phenol compounds. The flavonoid test was carried out by mixing the extract with 0.2 grams of Mg powder and several drops of 5M HCl. Samples containing flavonoid compounds obtained a color change to red, orange, or purple (Ichsani, 2021). Then the phenol test was carried out using FeCl reagent31%. The presence of phenolic compounds was indicated by the formation of a black color (Azizah *et al*, 2018). The phytochemical screening results obtained for date seed extract and peanut shell extract can be seen in table 4.

Table 4. Phytochemical Screening Results

	Flavonoid Test		Phenol Test	
Extract	**Before**	**After**	**Before**	**After**
Date Seeds	Yellow	(+) Orange	Yellow	(+) Black
Peanut Shells	Yellow	(+) Red	Yellow	(+) Black

Note : (+) = Compound detected, (-) = Compound not detected

Determine the maximum wavelength by reading the absorbance of FeSO standard standard$_4$.7H$_2$O with the highest concentration (140 µg/mL) in range 580 – 610 nm. The maximum wavelength obtained was 595 nm with an absorbance of 0.782. The maximum wavelength spectrum can be seen in Figure 3.

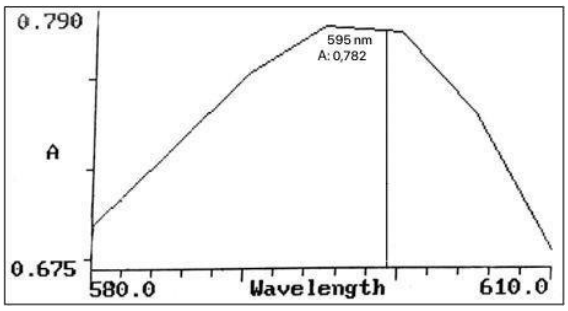

Figure 3. Maximum Wavelength Spectrum

The results of determining the standard curve absorbance were carried out in a series of solutions with a concentration of 50; 70 ; 100 ; 130 ; 140 µg/mL. The regression equation Y = 0.0032x + 0.1638 is obtained with a value of R^2 = 0.999, at a maximum wavelength of 595 nm, as shown in Figure 4. The standard curve concentration series absorbance table can be seen in table 5.

Table 5. Absorbance Data of FeSO$_4$.7H$_2$O Standard Curve

Concentration (µg/mL)	Absorbamce
50	0.328
70	0.384
100	0.482
130	0.585
140	0.611

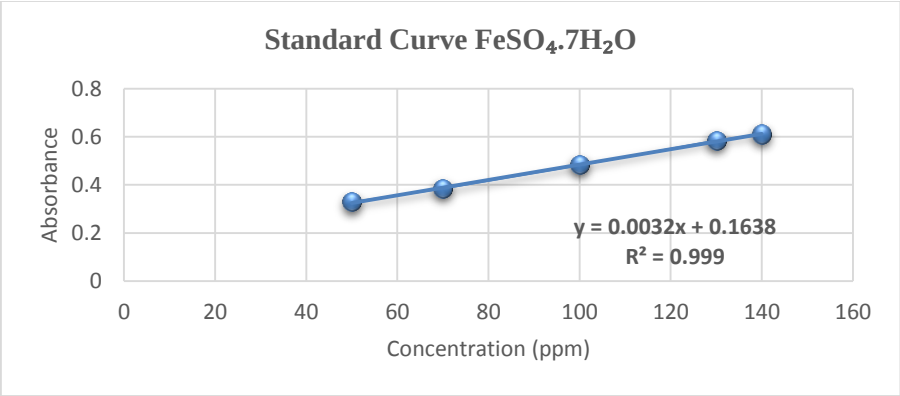

Figure 4. Standard Curve of FeSO$_4$.7H$_2$O

The control absorbance result was obtained at 0.611, while the sample absorbance was obtained by calculating the % FRAP activity to see how much antioxidant activity contained in date seed extract and peanut shell extract. The results of % FRAP Activity can be seen in table 6.

Table 6. FRAP Activity % Value

Formulas	Treatment		Fe^{2+} Rates (µg/mL)	Aktivity FRAP (%)	± SD
	Date Seeds Extract (%)	Peanut Shells Extract (%)			
F1	100	0	95,271	130,370	0,180
F2	60	40	100,062	126,240	0,313
F3	55	45	132,771	103,794	0,180

F4	50	50	107,771	120,118	0,180
F5	45	55	114,750	115,066	0,313
F6	40	60	80,375	145,131	0,313
F7	0	100	60,896	170,353	0,180

The synergism test was carried out in one step by mixing date seed extract and peanut shell extract in a ratio of 100:100. The synergism results of the two extracts can be seen in table 7.

Table 7. Synergism Test Values

Formulas	Treatment		Fe^{2+} Rates (µg/mL)	Difference (%)	± SD
	Date Seeds Extract (%)	Peanut Shells Extract (%)			
F1	100	0	95,271	-	0,180
F7	0	100	60,896	-	0,180
F8	100	100	139,438	78,575	0,313

The first stage in this research was the determination test for date palm and peanut plants which was carried out at the Bogorines BRIN Herbarium, Cibinong. This determination test aims to ensure the correct identity of the plants to be used in research, as well as to avoid errors in sampling for phytochemical analysis. The results of the determination test that has been carried out can be obtained with certainty that the date palm used in this study is the correct species Phoenix dactyliferaL. from the family Arecaceae. Then the peanut plants used in this research are the correct species Arachis hypogaea L. from the family Fabaceae.

Sample preparation of date seed extract and peanut shells was carried out by dried in an oven at 60°C for 2 hours for date seeds and 3 days for peanut shells. The drying process aims to reduce the water content in the date seeds and peanut shells which can inhibit the separation process and prevent the growth of bacteria and fungi. This drying process uses a temperature not exceeding 60°C because temperatures that are too high can damage the physiological properties of grains and thermolabile active compounds and can cause oil to be trapped inside the seed shell (Riyadi, 2018). Sample extraction was carried out using the maceration method. Maceration is the process of soaking samples with suitable solvents at room temperature so as to reduce the risk of damage to secondary metabolites (Ichsani, 2021). This method is safer because it can protect thermolabile active compounds such as flavonoids and phenols (Nazilah, 2019). The maceration process was carried out by soaking the samples in 70% ethanol solvent for 2x24 hours for date palm kernels and 3x24 hours for peanut shells and then re-macerated for 2x24 hours. The 70% ethanol solvent was chosen because it is polar, has a polarity index of 4.3 and has low toxicity.

Characteristic checks carried out on each extract include shape, color and odor as well as % yield. In the extract of date seeds, 30.4 grams were obtained from 250 grams of date seed powder, so the % yield obtained was 12.16%. Riyadi (2018) states that date seed extract has a yield value of 4.929%. Yield value calculated to determine the amount of extract obtained in each gram of date seed powder. Whereas in the first extract of peanut shell used for the qualitative test, 2.5 grams were obtained from 200 grams of peanut shell powder, so the % yield obtained was 1.25%. The condensed extract of the two peanut shells used for the antioxidant activity test was obtained as much as 1.8 grams from 200 grams of peanut shell powder so that the % yield obtained was 0.9%. Ichsani (2021) states that the % yield obtained from peanut shell extract is 28.553%.

The extract obtained is then tested for water content using a tool moisture analyzer which aims to provide a minimum limit for the amount of water content in the extract, the higher the water content, the easier it is for fungi and mold to grow so that it can reduce the biological activity of the extract during the storage period. The results of determining the water content of extracts of date palm seeds and peanut shells after 3x replication were obtained at 3.74 ± 0.10% and 6.70 ± 0.06%. This result meets the requirements set by BPOM, namely water content ≤ 10% to minimize mold growth so as to produce good storage and extract quality (Hanif *et al*., 2019).

Phytochemical screening was carried out to qualitatively determine the content of active compounds in plants (Nazilah, 2019). The results show of the phytochemical test that the ethanol extract of the two samples contained secondary metabolites, namely flavonoids and phenols. This is because the ethanol solvent extraction process can increase the permeability of the simplex cell wall to attract components of polar and semipolar compounds (Fikayuniar *et al*., 2022). Phytochemical screening of date seed extract obtained the same results as previous studies. Bouhlali *et al*

(2020) stated that the ethanol extract of date seeds contains flavonoid and phenol compounds. This can be influenced by environmental conditions where grown, harvest time, storage conditions, geographical origin, light, temperature, fertilizer and soil type and method of extraction. Then, phytochemical screening of peanut shell extract also obtained the same results as previous research. Franyoto *et al* (2019) stated that the ethanol extract of peanut shells contains flavonoids, tannins, saponins, alkaloids and terpenoids. Annadira (2021) stated that the ethanol extract of peanut shells also contains phenol compounds.

Testing the antioxidant activity of date seed extract and peanut shell extract using the FRAP method requires several stages, such as determining the maximum wavelength, determining the standard curve, and determining the antioxidant activity value of the sample. Determination of the maximum wavelength is carried out to determine the wavelength with the highest absorption. Determination of the maximum wavelength was carried out by measuring the absorbance of a standard solution of $FeSO_4.7H_2O$ with the highest concentration (140 µg/mL) in the range 580 – 610 nm. This is because $FeSO_4.7H_2O$ provides absorption in the wavelength range 580 – 610 nm. The wavelength measurement using a UV-Vis spectrophotometer gives the highest absorption at a wavelength of 595 nm with an absorbance of 0.782. The results obtained are the same as Nurhayati's *et a*l., (2022) obtained a maximum wavelength of 595 nm.

Making a standard curve with a standard solution of $FeSO_4.7H_2O$ was carried out with 5 series of concentrations. The choice of concentration is based on the Lambert-Beer law which states the absorption requirement is 0.2 – 0.8 to avoid photometric errors (Asmorowati & Lindawati, 2019). The standard curve absorbance measurement was carried out by repeating the test 3 times with the aim of obtaining more accurate data. The results of determining the $FeSO_4.7H_2O$ standard curve which can be seen in Figure 5.10 show that the concentration is directly proportional to the absorbance value. The greater the concentration of the standard standard solution $FeSO_4.7H_2O$, the higher the absorbance value produced (Rahayu *et al.*, 2021). When measuring the absorbance, the regression equation $FeSO_4.7H_2O$ was obtained, namely $y = 0.0032x + 0.1638$, where y is the absorbance and x is the concentration of antioxidant activity. The linearity value results are shown by the coefficient of determination (R^2) value of 0.999, where the condition for acceptance of a good coefficient of determination is ≥ 0.997. This shows that there is a linear relationship between the concentration of the $FeSO_4.7H_2O$ standard standard solution and the absorbance value. Linearity can be said to be good if it meets the acceptance requirements (Gayatri, 2021).

Determination of the value of antioxidant activity in this study using the FRAP method (Ferric Reducing Antiozidant Power). The principle of this method is the ability of the compound antioxidants in reducing the Fe^{3+}-TPTZ compound to Fe^{2+}-TPTZ (Wulandari, 2021). The more Fe^{3+} concentration reduced by sample becomes Fe^{2+} then the antioxidant activity of the sample also increases big (Pebriana *et al.*, 2019).

Determination of antioxidant activity in the form of absorbance. The absorbance was obtained when measuring the maximum wavelength of the $FeSO_4.7H_2O$ standard solution. The standard solution was reacted with FRAP reagent and then incubated at 37°C for 10 minutes. The aim of incubation is so that the antioxidant reaction process with FRAP reagent achieves a perfect reaction and is not influenced by stability factors. Antioxidant stability can be affected by several factors, namely light, oxygen, and pH. The value of the sample antioxidant activity is expressed in % FRAP activity with Fe^{2+} levels (µg/mL) using standard $FeSO_4.7H_2O$. The value of the antioxidant activity was determined from the two extract samples in 7 mixing stages (F1 – F7), where F1 only contained date seed extract and F7 only contained skin extract. peanuts, while F2 – F6 contains a mixture of extracts based on a predetermined formula. The results showed that F7 contained the highest FRAP activity value of 170.353%, which means that when the FRAP reagent was reacted with peanut shell extract it had a very strong antioxidant activity compared to date seed extract in F1 of 130.370%. Then when the extracts started to be mixed as in F2 – F6, the one with the highest FRAP activity value was F6 where date palm seeds were 40% and peanut shells were 60% which was 145.131%. This is directly proportional to the results of the activity of the peanut shell extract itself.

In this study, single extracts of date palm seeds and peanut shells had antioxidant activity. The ability as an antioxidant of these two plant extracts can still be improved, one way is by combining the extracts between the two. This is known as the synergistic effect. The synergism effect is the effect of two or more combinations of different types of antioxidants that have a greater effect than the effect of the individual antioxidants separately (Septiana *et al.*, 2020). Apart from producing a synergistic effect, the combination of extracts can also produce additive or antagonistic effects. In this study, the combination of date seed extract with peanut shell extract produced a synergistic effect with a % difference value of 78.575. The percentage value of this difference is used to determine antioxidant potential, namely having a synergistic effect if the value is positive (% difference > 0) or antagonistic if the value is negative (% difference <0).

Conclusions

Based on the results of quantitative tests using the FRAP method, samples of date seed extract have antioxidant activity with % FRAP activity of 130.370%. Then, based on the results of quantitative tests using the FRAP method, samples of peanut shell extract have antioxidant activity with % FRAP activity of 170.353%. The results obtained from the combination of date seed extract and peanut shell extract have a synergistic effect on increasing antioxidant activity with a % difference value of 78.575 because the % difference result is > 1.

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Effect of stevia sugar on innovation of guava, apple, lemon, and melon juice probiotic drink using *Lactobacillus casei* bacteria on amount Lactic acid bacteria

Mety Nur Ivani[1*], Reza Anindita[2], Maulin Inggriani[3], Maya Uzia Beandrade[4]

[1,2,3,4] Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Bekasi, Indonesia

[*]Correspondence author: metynurivani@gmail.com

Abstract

As time goes by, more and more attention is paid to functional drinks. Probiotic cultures provide several health benefits, namely maintaining a healthy gut microbiota, protection against gastrointestinal pathogens, boosting the immune system, reducing cholesterol levels, reducing blood pressure, and anticancer activity. One of the probiotic drinks to be tested in this study was made from guava, apple, lemon, and melon juice This research is a quantitative study with a laboratory experimental research design. In this study, *Lactobacillus casei* was used as a starter. The test material used was juice added with *L. casei* starter and stevia sugar with concentrations of 10%, 20%, and 30%. The average value of the total BAL of the Jamale juice probiotic drink without added sugar was 1,80 x 107 CFU/mL, added sugar 10% was 4,83 x 107 CFU/mL, added sugar was 20 % was 4,91 x 107 CFU/mL and the addition of 30% sugar was 6,05 x 107 CFU/mL. Jamale juice probiotic drink with 30% added sugar resulted in the best condition with a total number of lactic acid bacteria of 6,05 x 107 CFU/mL.

Keywords: *Lactobacillus casei;* pH; temperature; total lactic acid bacteria; zone of inhibition

Introduction

Functional drinks are receiving more and more attention as time goes by, mainly because people are becoming more interested in consuming a drink that has health benefits, including probiotic drinks. The ability to maintain a healthy gut microbiota, protection against gastrointestinal pathogens, immune system enhancement, cholesterol reduction and Blood pressure, and anticancer activity are some of the benefits of the drink probiotics (Dewi *et al*., 2021). Currently, there is much focus on adding probiotics to non-dairy drinks because few people choose to avoid the product milk due to lactose intolerance, milk allergy, or just a fundamental dislike. Required product innovations can open opportunities to combine the benefits of culture probiotics with bioactive molecules.

One of the benefits of probiotic drinks is that they are beneficial for health. Probiotic drinks have a very sour taste and intense aroma, so many people are not interested (Hidayah, 2019). Study this aims to overcome this by using fruit juice as an ingredient, the basis for making probiotic drinks, so that they are tastier to the general public and increase their appeal. Probiotics supplement live microbial brew and produce a variety of beneficial biomolecules, including bacteriocins (Al-Dhab *et al*., 2020). One of the probiotic drinks you want to develop in this research were red guava, melon, apple and fruit juices with *Lactobacillus casei* starter.

According to Winarni *et al*. (2020), giving juice drinks probiotic red guava is more effective than orange juice in increasing haemoglobin levels to prevent anaemia. Yusmarini *et al.* (2021) proposed using melon fruit as an essential ingredient for making drinks. Probiotics are known to be able to meet the nutritional needs of the body. Setyiyaningsih *et al.* (2020) reported that administering a Lemon-based probiotic drink could increase haemoglobin levels by 1.1 g/dl. Hidayah (2019) states that Adding apple juice to probiotic drinks can increase levels of vitamin C. Rosiana and Khoiriyah (2018) prove that making probiotics with apple juice and honey has activity antioxidants of 27.10% to 32.83%. From various previous studies, it is known that red guava, lemon, melon and apple have the potential as ingredients the basis for making juice probiotic drinks with *L.casei* starter as drink health.

Previous research that has been done regarding the feasibility of *L.casei* as the starter in the production of probiotic drinks, namely the research of Boro and Fidyasari (2017), stated that the results of the fermentation of yellow soursop juice were supplemented the *L.casei* line from the Yakult line showed no significant difference ($P<0.05$). Harahap *et al.* (2018) suggested that *L.casei* significantly influenced total lactic acid bacteria, total acidity, sucrose and ash but not on pH when preparing fermented beverages from tomato fruit juice. Nurlilayanti *et al*. (2019) state that *L. casei* bacteria produce cytokines, lymphocytes, antibodies, monocytes, and macrophages that can phagocytose harmful bacteria. *L. casei* can stimulate local and systemic immune responses. Various studies show that *L. casei* can be used as a starter in fermenting juice drinks, such as fruit.

Based on the background above and several previous studies that have been carried out using only one to two types of fruit, researchers are interested in making a probiotic drink with more types of fruit, namely from juice (Red guava, Melon, Apple and Lemon) with *L.casei* starter as a drink health. The results of this research are expected to

provide information regarding total Lactic Acid Bacteria from the probiotic drink Jamale juice with *L.casei* starter as a health drink.

Methods

This research is quantitative experimental research. This research was conducted at the STIKes Mitra Keluarga Pharmacology Laboratory, Bekasi, West Java, from January to March 2023. The materials used in this research are Red Guava fruit, Melon, Apple, Lemon and purchased pure culture of *Lactobacillus. casei* bacteria at PT. Agritama Sinergi Inovasi (AGAVI) and bacterial cultures of *Escherichia coli, Staphylococcus aureus,* and *Salmonella typhi* taken from the Microbiology Laboratory Family Partner STIKes.

This research begins with sterilizing tools and materials, which are then made by DeMan Rogosa Sharp Broth (MRSB) and DeMan Rogosa Sharp Agar media (MRSA). Making MRSB is used to multiply bacteria by means weighing 19.6 g of MRSB, which was then dissolved in 700 ml of distilled water in an Erlenmeyer, covered with cotton and gauze, heated on a hotplate, and then sterilized by autoclave. MRSA preparations were used to count total colonies BAL by weighing 37,512 g MRSA 4 g back to agar, which was then dissolved with 720 ml of distilled water in an Erlenmeyer, covered with cotton and gauze, heated above a hotplate then sterilized by autoclave.

The next stage is the multiplication of L.casei bacteria by taking the pure culture of the bacteria as much as one aseptic ossicle needle, then inserting it into a test tube containing 5 ml MRSB, incubating at 37°C for 18 hours. The next stage is making a starter by inoculating 100 ml MRSB media with 2% L. casei culture incubation for 24 hours at 37°C. The next stage is making the Jamale juice probiotic drink with 75 ml of MRSB, and 25 ml of Jamale juice added with 2% L.casei substrate from a starter solution volume of 100 ml, incubating for 24 hours at 37°C. Results This is considered incubation 1. In the next stage, 50 ml of MRSB is made, and 50 ml of Jamale juice in the sample bottle included 2% L.casei taken sequentially from incubation process 1 sequentially. These results are considered as incubation 2. Do the same for incubation 3 (75 ml MRSB and 25 ml juice) and 4 (100 ml juice). The following is the juice formulation that you can see in Table 1.

Table 1. Juice Probiotic Drink Formula

Material	Formula			
	F1	F2	F3	F4
Red guava	40 g	40 g	40 g	40 g
Apple	20 g	20 g	20 g	20 g
Melon	20 g	20 g	20 g	20 g
Lemon juice	20 g	20 g	20 g	20 g
Stevia sugar	0 g	10 g	20 g	30 g
Water	100 ml	100 ml	100 ml	100 ml

Results and Discussions

This test aims to determine the amount of LAB contained in juice probiotic drink, which is a sign of the success or failure of the process ongoing fermentation. The method used in the total BAL test with using the Total Plate Count (TPC) method. Table 2. the results of this research shows the average value of total lactic acid bacteria.

Table 2. Average Value of the Number of LAB in Jamale Juice Probiotic Drinks

Treatment	CFU/ml ($\bar{x}$) ± SD
No sugar	1,80 x 10^{7a} ± 0,72
Addition of 10% stevia sugar	4,83 x 10^{7a} ± 0,38
Addition of 20% stevia sugar	4,91 x 10^{7a} ± 2,47
Addition of 30% stevia sugar	6,05 x 10^{7a} ± 3,34

Note: The data presented is the average value ($\bar{x}$) ± standard deviation (SD). The mean followed by superscript (a) in the same column shows that the difference is not significant using the One Way ANNOVA test.

From these results, it can be seen that the total LAB results in probiotic juice drinks Jamale meets the criteria for probiotic drinks according to National Standards Indonesia (SNI) with a minimum total BAL criteria of (6 log CFU/mL) (Tampinongkol *et al*., 2016). The results in the table above show that the total average value of The LAB in

the Jamale juice probiotic drink ranges from 1.80 x – 6.05 x CFU/mL, so it can be seen that the average value already meets the criteria according to SNI 7552:2009. The lowest average total LAB was found in Jamale Juice with a 10% stevia sugar concentration of 1.80 x CFU/mL, and the highest average total LAB was found in Jamale Juice with a 30% stevia sugar concentration of 6.05 x CFU/mL.

Probiotics juice, with a stevia sugar concentration of 30%, has an average total LAB value that is high due to adding more stevia sugar than the other concentrations. The more sugar added, the higher the total LAB value produced, where sucrose is a carbon source of LAB which will degrade various types of sugar into lactic acid so that LAB production will increase (Cinderela *et al*., 2022). Based on these results, it can be concluded that probiotic juice drinks Jamale that have been made meet the total BAL value standards. The highest total BAL value found in Jamale Juice with the addition of 30% stevia sugar concentration proves that the growth of LAB colonies is influenced by the sugar content contained in the sample. This is supported by research conducted by Cinderela *et al*. (2022), which states that glucose is an important nutrient for growth LAB is a source of energy and can trigger rapid growth of LAB colonies in bulk. The high CFU value in the probiotic drink sample juice with the highest addition of sugar is 30% due to the large amount of glucose in the sample. The more lactic acid produced by BAL by means

Glycolysis is breaking down glucose into lactic acid. Fruits are also used can affect total LAB production because the carbohydrates contained in The fruit used is supported by research by Azizah *et al*. (2019), who stated that Lactic Acid Bacteria (LAB) can hydrolyze carbohydrates into lactic acid and can increase the ability of bacteria to break down lactose.

Based on the number of replications, three replications were carried out. It can be seen that in the last replication, there was a decrease in the total value of BAL because The length of storage time causes the stevia sugar content in the sample It has been broken down into lactic acid by LAB, so probiotic drinks are not It is recommended to be stored for a long time and must be consumed immediately. As for the results, the one-way ANOVA test in this study was the total Lactic Acid Bacteria (LAB) test of 0.407 (P>0.05). Results indicate that increasing sugar concentration does not significantly affect the total BAL produced (P>0.05). According to Apriliyanto *et al*. (2020), the concentration of sugar added to the product does not affect the amount of lactic acid. This is caused by the ability of LAB to utilize the glucose to become lactic acid.

The results of the research that has been done are supported by the research that has been carried out by Rizal *et al*. (2016) regarding the study of pineapple juice lactic fermented drinks with a fruit and water composition ratio of 4:1 using a starter *Lactobacillus casei*. The best treatment from research is to produce a total value of BAL 1.1 x CFU/mL, presumably because at the time of incubation, there was a process of glucose breakdown by LAB, which takes longer and is optimal so that the lactic acid produced increases tall. Research conducted by Diza *et al*. (2016) made the drink probiotics with yam-based ingredients using a combination of *Lactobacillus* starter *acidophillus, Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Producing a total BAL value of 2.38 x in the 0th week, but in the fourth week, there was a decrease of 2.6 log cycles, presumably due to the use of mixed cultures causing there is competition between bacteria in fulfilling nutrition so that it can be concluded that the storage of fermented beverages for quite a long time will reduce the total LAB value contained therein due to the addition of sugar as LAB nutrition decreases because the LAB consumes it used.

Conclusions

Test the total plate number on the probiotic drink juice using a starter *L.casei* with a sugar concentration of 0%, 10%, 20% and 30% is declared to meet the criteria probiotic drinks according to SNI 7552:2009 with a minimum BAL of (6 log CFU/mL) namely probiotics juice without added sugar of 1.80 x CFU/mL, probiotics juice with the addition of 10% sugar of 4.83 x CFU/mL, probiotics juice with the addition of 20% of 4.91 x CFU/mL and probiotics juice with the addition of 30% sugar of 6.05 x CFU/mL.

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Formulation test of facial serum mangosteen rind extract (*Garcinia Mangostana*) and niacinamide

Mia Marlina[1], Maya Uzia Beandrade[2*], Dede Dwi Nathalia[3], Reza Anindita[4]
[1,2,3,4] Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga Bekasi, Indonesia
*Correspondence author : maya.uzia@stikesmitrakeluarga.ac.id

Abstract

Facial serum can be described as a product that contains active ingredients to treat skin and fade acne scars. Mangosteen peel has benefits as an antibacterial. The ingredients contained in the mangosteen rind are flavonoids, alkaloids, and phenols. The research design used was pre-experimental, using mangosteen rind samples. The physical stability test was carried out by organoleptic, homogeneity, pH, and viscosity tests for 14 days. Data analysis was performed with a descriptive test. The results of the phytochemical screening test on the flavonoid, phenol, and alkaloid tests obtained positive results. Organoleptic test results during 14 days of storage did not change color and smell. pH test results on F1 6.33- 6.41; F2 5.85-6.06 ; and on F3 5.48-5.69. Homogeneity test results on the three homogeneous formulas, and the F1 viscosity test 10,333- 22,500 cP; F2 11.000-21.667 cP; and F3 10.833-21.333 cP. It can be found that the decrease in the pH value was found in F1 (1%) by 0.15%, F2 (3%) by 3.4% and F3 (5%) by 3%. The decrease in viscosity in F1 (1%) was 54%, F2 (3%) was 49.22%, and F3 (5%) was 49.21%. So, it is necessary to add observation time in order to obtain information on the physical stability of a good serum preparation formula.

Keywords: *Mangosteen Rind Extract; Niacinamide ; Facial Serum ; Physical Stability Test*

Introduction

Now, skin care products and cosmetics are the most important part of many people's lives. Facial care is used to maintain appearance and increase self-confidence (Pratiwi *et al*., 2021). As time goes by, many people use dangerous cosmetics such as hydroquinone and mercury to get fast results (Kurniawan & Nugraha, 2022). However, there is a demand for plant extracts that are effective in skin care and treating skin problems.

One of the microorganisms that causes skin problems is bacteria. Several bacteria can occur on facial skin, namely *P. vulgaris, P. acnes, S. epidermidis,* and *S. aureus* (Gidde, 2021). These bacteria can cause blockages, inflammation and acne on the skin or acne vulgaris (Budiman *et al*., 2018). Acne Vulgaris, or acne, is a disease that results from excessive secretion of oil glands on the face (Duru & Örsal, 2021). Acne vulgaris can be characterized by the presence of comedones, papules and pustules (Tayel *et al*., 2020). The prevalence of acne vulgaris in the world is 9.4%, and it is the most common disease throughout the world (Layton *et al.*, 2021). The prevalence of acne vulgaris in Southeast Asia is around 40-80% of the population, and the prevalence of acne vulgaris in Indonesia is 80-85% in adolescents aged 15-18 years (Pramuningtyas and Oktafiani., 2022).

The problem of acne vulgaris can be caused by hormones, sun exposure, and using inappropriate skincare on a person's facial skin. Apart from that, it can also be caused by age and sleep quality (Yang *et al*., 2020). The impact of acne vulgaris itself is that it can cause a person to lack self-confidence, stress, depression, cause feelings of embarrassment, and can also cause anxiety or sadness (Layton *et al.*, 2021). Prevention of acne vulgaris can be carried out according to different levels of severity, systemic prevention, and topical prevention, such as retinoids. Acne treatment and skin care can use moisturizers that have been successful in reducing skin irritation. Acne vulgaris can be prevented by using cosmetics that contain active ingredients such as salicylic acid, niacinamide, retinoids, and alpha-hydroxy acids (AHA) (Dréno *et al*., 2020). Prevention of acne vulgaris can also be prevented by using cosmetic products that contain natural extracts, one of which is mangosteen rind extract. Mangosteen peel has antibacterial, antioxidant and anti-cancer benefits. The compounds contained in mangosteen peel are flavonoids, phenols, and alkaloids (Nurdianti *et al*., 2022). Based on previous research using mangosteen rind extract at concentrations of 0.5%, 2%, 3.5%, and 5% and without solvents, however, in this research, a product has never been developed to produce facial serum with 1%, 3% mangosteen rind extract. % and 5% and 2% niacinamide, so researchers are interested in formulating innovative facial serum products from ethanol extract of mangosteen peel and niacinamide. The four tests carried out by researchers included organoleptic tests, pH, homogeneity and viscosity tests. It is hoped that the results of this research can become a reference for further research regarding the antibacterial activity of innovative facial serum products containing ethanol extract of mangosteen peel (Garcinia mangostana) and niacinamide.

Methods

Sample Preparation

Mangosteen rind samples were taken from plantations in Yogyakarta. Determination of natural material sources of mangosteen rind was carried out at LIPI Bogor. The ethanol extract of mangosteen rind is made at Palapa Muda Perkasa, Depok. In this study, the maceration method was used, beginning with the manufacture of mangosteen rind simplicia powder, then soaked in 96% ethanol solvent for 3x24 hours. After three days, the sample was filtered, and then the filtrate was taken and concentrated with a rotary evaporator at ± 40°C to obtain a thick extract of mangosteen rind (Chairunnisa *et al*., 2019).

Making Facial Serum Innovations

Weigh the ingredients, then heat 100 mL of distilled water to a temperature of 90oC. Put the distilled water into the mortar then add 2 grams of CMC Na into the mortar little by little, grind until a serum base is formed, add 2 grams of niacinamide into the mortar until homogeneous, then add 1 gram, 3 grams and 5 grams of mangosteen rind ethanol extract to the mortar. different then grind until homogeneous then add distilled water ad 100 mL, then mix again using a mixer after that put half of the preparation into a plastic pot and put the rest into a serum bottle.

Table 1. Formulation of Mangosteen Peel Extract Facial Serum

Material	F1	F2	F3
Mangosteen Peel Extract	1%	3%	5%
Niaciamide	2%	2%	2%
CMC Na	2%	2%	2%
Aquadest ad	100 mL	100 mL	100 mL

Physical Stability Test

The physical stability tests in this research include organoleptic, homogeneity, pH and viscosity tests, which refer to the research of Ariyanti *et al*. (2020), Murshid (2017) and Yuniarsih and Sari (2021). Organoleptic tests were carried out independently by looking at the color and smell of F1, F2 and F3 facial serum preparations in plastic pots. The homogeneity test is carried out by taking enough serum and placing it on the watch glass. After that, the serum is touched and rubbed. Coarse particles must not be present on the watch glass. The pH test is carried out by inserting a pH meter into the facial serum preparation in plastic pots F1, F2 and F3. Looking at the pH results of the facial serum preparation shows an acidic or alkaline pH. The viscosity test was carried out by inserting the spindle into a plastic pot containing facial serum samples F1, F2 and F3, then seeing that the viscosity results met the range of 3,000-50,000 cP.

Phytochemical Screening

Phytochemical screening in this study was carried out based on a modified procedure from Anggriani *et al.* (2017), Reiza *et al*. (2019) and Putri *et al.* (2019). The flavonoid test was carried out by taking mangosteen rind extract and putting it in a test tube, adding pieces of Mg band and adding three drops of concentrated HCl. Orange, red or blue color indicates positive flavonoids. The alkaloid test was carried out with 0.5 g of mangosteen rind extract put into a test tube added 1 mL of 2N HCl shaken until homogeneous, then added Wagner, Mayer, and Dragendorf reagents in each test tube as much as 2-3 drops. The presence of alkaloids in Wagner's reagent shows a brownish-yellow precipitate. In Mayer's reagent, a white or yellow precipitate is indicated. The Dragendorf reagent shows a yellow or orange precipitate. A phenol test was carried out with 0.5 grams of ethanol extract of mangosteen rind in a test tube, added with $FeCl_3$. A change in color to green, red, purple, blue, or solid black indicates phenol positivity.

Results and Discussions

Organoleptic Test

Examination of organoleptic tests of facial serum on F1, F2, and F3 includes odor and color. Organoleptic testing was carried out for 14 days and observed changes in color and odor in the facial serum preparation. Organoleptic test results can be seen in **table 2.**

Table 2. Serum Organoleptic Test

Formula	Organoleptic Test Results	
	Day-1	Day-14
F1 (1%)	Color : Light Yellow Smell: Typical aromatic Mangosteen	Color: Light Yellow Smell: Typical aromatic Mangosteen
F2 (3%)	Color: Light Brown Smell: Typical aromatic Mangosteen	Warna : Coklat Muda Smell: Typical aromatic Mangosteen
F3 (5%)	Color : Dark Brown Smell: Typical aromatic Mangosteen	Color : Dark Brown Smell: Typical aromatic Mangosteen

Homogeneity Test

The F1, F2, and F3 homogeneity tests were carried out by applying the serum preparation to a watch glass to see whether the facial serum preparation was mixed evenly. The homogeneity test results can be seen in **table 3**

Table 3. Serum Homogeneity Test

Formula	Homogeneity Test Results	
	Day-1	Day-14
F1 (1%)	Homogeneous	Homogeneous
	There are no coarse particles	There are no coarse particles
F2 (3%)	Homogeneous	Homogeneous
	There are no coarse particles	There are no coarse particles
F3 (5%)	Homogeneous	Homogeneous
	There are no coarse particles	There are no coarse particles

pH Test

This test is carried out to see whether the pH value of facial serum in F1, F2 and F3 is acidic, alkaline or neutral. The pH stability test on F1, F2, and F3 was carried out during 14 days of storage. The results of the pH test in this study can be seen **in table 4**

Table 4. Average Serum pH Value with n=3

Day	F1 (1%)	F2 (3%)	F3 (5%)
1	6,34 ± 0,10	6,06 ± 0,00	5,65 ± 0,08
3	6,40 ± 0,02	5,91 ± 0,05	5,58 ± 0,03
6	6,41 ± 0,01	6,04 ± 0,02	5,69 ± 0,02
9	6,35 ± 0,06	5,86 ± 0,10	5,62 ± 0,04
14	6,33 ± 0,13	5,85 ± 0,02	5,48 ± 0,017

Viscosity Test

The viscosity test was carried out to determine the viscosity value of facial serum preparations on F1, F2, and F3. The viscosity stability test of the three formulas was carried out for 14 days of storage. The results of the serum viscosity test in this study can be seen in **table 5**

Table 5 Average Serum Viscosity Values with n=3

Day	F1 (1%)	F2 (3%)	F3 (5%)
1	22.500 ± 1322,88	21.666 ± 2081,67	21.333 ± 3403,43
3	21.166 ± 1892,97	18.000 ± 500,00	20.666 ± 2362,91
6	16.833 ± 577,35	13.333 ± 288,68	13.000 ± 0,00
9	11.500 ± 1000,00	11.333 ± 1443,38	11.166 ± 763,76
14	10.333 ± 577,35	11.000 ± 0,00	10.833 ± 577,35

The formula composition (F) of this research serum preparation includes ethanol extract of mangosteen peel with concentrations of 1%, 3% and 5%, respectively, calcium carboxymethylcellulose (CMC Na) 2%, niacinamide 2% and distilled water ad 100 mL. In F1, with an extract concentration of 1%, the color is light yellow. Formula 2, with an extract concentration of 3%, is light brown, and in Formula 3, with an extract concentration of 5%, the color is dark brown. There is a difference in color between the three formulations due to the use of the ethanol extract concentration of mangosteen peel. The greater the concentration of extract used, the darker the brown color obtained.

In the physical stability test carried out during 14 days of storage, there was no change in shape. There was only a change in color, which was caused by the difference in concentration in the three formulations. This is because when storing facial serum preparations are stored in a tightly closed container and protected from light. The results of the homogeneity test showed that the serum preparation remained homogeneous during the 14-day storage period because the watch glass did not show any coarse grains or smooth surfaces. This is because, during the formulation of the active

ingredient, serum preparations and other additional ingredients have been mixed perfectly. Therefore, the results obtained are homogeneous serum preparations in the three formulations.

Based on the pH test of the facial serum preparation from the ethanol extract of mangosteen rind, which was carried out every three days during 14 days of storage at room temperature, it still falls within the permissible pH requirements of 4.5-6.5 (Fitria *et al.*, 2022). The purpose of testing the pH of the preparation was carried out several times within a predetermined time range, namely to determine the pH stability of the resulting facial serum preparation (Erwiyani *et al*., 2018). The pH difference is caused by differences in the concentration of the extract used in facial serum preparations. The results of pH measurements for 14 days showed that the pH of all formulations rose and fell but was still included in the skin pH limit of 4.5-6.5. Theenvironmental factors and poor storage could cause the decrease and increase in pH of the preparations during storage.

Based on the results of the viscosity test, the serum preparation from ethanol extract of mangosteen peel, which was stored for 14 days at room temperature, still met the viscosity range of a preparation. Based on the viscosity results obtained by the three facial serum preparation formulations when the stability test was carried out at room temperature, the viscosity value experienced an increase and decrease when stored for 14 days, but the viscosity value in the three preparation formulas still fell within the requirements, namely 3,000-50,000 cP. The viscosity value obtained in this study decreased during 14 days of storage at room temperature. Several factors can cause a decrease in viscosity values, one of which is stirring or mixing the preparation. Stirring for too long can damage the gel preparation and cause a decrease in viscosity (Rahayu *et al.*, 2023). Another factor that can influence changes in viscosity values is storage at room temperature, which will cause the growth of bacteria in the gel formulation, which can cause an acidic atmosphere in facial serum preparations. The viscosity can change (Mardiana *et a*l., 2020).

Research on phytochemical testing obtained positive results in the phenol, flavonoid, and alkaloid tests with dragendorf, Wagner, and mayer reagents obtained positive results. The flavonoid test was carried out by placing the extract in a test tube and then adding magnesium ribbon and concentrated HCl. Concentrated HCl acts on the benzopyrone nucleus in the flavonoid structure, which is cleaved by concentrated HCl to give red or orange flavilium salts. The results of the flavonoid test in this study were positive because there was an orange color in the sample. An orange solution is formed due to the reduction reaction between Mg and HCl, resulting in an orange solution.

In the alkaloid test, the ethanol extract of mangosteen peel was mixed with HCl, Mayer, Wagner and Dragendorf reagents. The alkaloid test begins with the addition of HCl because HCl can help increase the solubility of alkaloids. After all, alkaloid compounds can react with HCl to form salts that dissolve easily in water, while the Mayer, Dragendorf and Wagner reagents function to identify alkaloid compounds. The alkaloid test with Mayer's reagent obtained a positive result, forming a white precipitate. The white color is formed because it comes from a potassium-alkaloid complex compound. The alkaloid test with the Wagner reagent obtained a positive result, which formed a brownish-yellow precipitate. The formation of a brownish-yellow precipitate is due to the metal ion K+ binding to nitrogen in the alkaloids (Parbuntari *et al*., 2018).

Conclusions

Stability tests of facial serum preparations at F1 concentration (1%), F2 (3%), and F3 (5%) were carried out for 30 days, but the research was only carried out for 14 days. Based on the results of the viscosity test, there was a percentage reduction in F1 (1%) of 54%; F2 (3%) was 49.22% and F3 (5%) was 49.21%. Thus, it is necessary to increase the observation time so that information on the physical stability of a good serum preparation formula is obtained.

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Acute toxicity test of probiotic red guava, apple, lemon, melon juice with bacteria starter *Lactobacillus acidophilus* on male Balb/c. Mice

Reza Anindita[1*], Nurin Natasha[2], Nofria Rizkia Amalia Harahap[3], and Ariska Deffy Anggarany[4]
[1,2,3,4] Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga Bekasi, Indonesia
*Correspondence author : rezaaninditaa@gmail.com

Abstract

Introduction: Before a new product candidate for a functional drink can be produced and distributed as a supplement that is beneficial to the body, it is necessary to test its safety for consumption by humans, which includes the selection stages, namely preclinical testing, one of which is an acute in vivo toxicity test. Purpose: This study was to determine whether or not a toxic effect occurred after the administration of probiotic drinks. In doses of 5, 50, 300, and 2000 mg/KgBB. **Methods**: The study used an experimental design with a quantitative research type using the oxidation method, namely the Fixed Dose Method. Fifteen male mice (Balb/c) were used as test animals divided into 5 groups. Probiotic drinks were administered orally at 5, 50, 300, and 2000 mg/KgBW doses, and distilled water was used as the control group. The test animals were observed individually for 14 days of observation. The parameters observed were symptoms of toxicity and the number of deaths that occurred. **Results**: The results of the *Kruskal Wallis* analysis showed no significant difference in the number of deaths caused by the administration of probiotics. Toxicity symptoms occurred in the group treated with distilled aqua. (Toxic symptoms include hair loss, trembling, and walking on the stomach. **Conclusion**: From this study, giving juice probiotics with *L.acidophillus* starter and doses of 5, 50, 300, and 2000 mg/KgBW did not cause death and symptoms of toxicity were changed in male balb/c mice observed for 14 days.

Keywords : *lemons;Apple; melon; red guava; Lactobacilus acidophillus; Probiotics, acute; toxicity*

Introduction

Along with the development of technological and information advances. Many people, especially in Indonesia, are starting to pay attention to the type of food they consume. In this regard, the modern food industry is starting to compete to produce various types of functional food, namely food that provides nutrition and can also make the body healthy. One type of functional food widely produced contains probiotics (Helmalia *et al*., 2019). Probiotic drinks can affect health if contains some live cells of 200 µl (BL (1 x 107 CFU/ml), with a total consumption of probiotic drinks of 100-150 mL/day or 3-4 times a week (Wibawa, 2016). However, the current market production of probiotic drinks is still dominated by probiotic drinks that use milk-based ingredients such as yoghurt, acidophilus milk, Bulgarian milk, kefir, raisins, prima, skyr, taetee, Leben from Egypt, dahi from India, hamao from Central Asia, and bayakult from Japan, so the price relatively more expensive (Rizal *et al*., 2016). Most consumers allergic to milk find it challenging to find healthier alternatives at more affordable prices. In addition, the fat content in cow's milk is also higher (4.52 g/100 g) compared to camel milk (2.69 g/100 g) (Mojikon *et al*., 2022). Therefore, research needs to be conducted to develop the use of fruit as an essential ingredient in making probiotic drinks to meet market demand as an alternative to milk-based products. However, before being sold commercially, empirical tests need to be carried out to support scientific evidence so that probiotic drinks based on fruit juice can be developed and accepted for health purposes. Data can be evidence from systematically obtained study results that are safe for human consumption. Functional food products with new formulations need to undergo safety testing, which includes selection stages, namely preclinical testing, standardization and clinical testing, before being produced and distributed to consumers as supplements that are beneficial for the body (Rizal *et al*., 2016).

Based on this background, it is crucial to carry out toxicity tests to ensure the safety of consumption of pharmaceutical preparations, one of which is functional drinks (Sandhiutami *et al*., 2022). This test is carried out to examine the pharmacological activity of the compound after administration of a single dose or repeated doses, which can occur within a short time, namely less than 24 hours after exposure (Jumain *et al*., 2018). As for the acute toxicity test in the study this was done by administering probiotics from red guava, lemon, melon, and apple juice at 5, 50 - 300, and 2000 mg/KgBW to male balb/C strain mice. This research is expected to be able to provide information to consumers regarding the safety of consumption from the results of preclinical beverage test studies probiotics from red guava, lemon, melon, and apple juice are good for health.

Methods

This research uses an experimental design. This research design was carried out by providing treatment or intervention to test animals using controls. Method used is a Fixed-Dose Method to test the effect of acute toxicity of the innovative probiotic drink Guava, Lemon, Melon and Apple juice with the starter bacteria Lactobacillus acidophilus using white male mice Strain Balb/c with various formulas F1, F2, F3, F4 **(table 1).** The sample used was the probiotic drink juice Red Guava, Lemon, Melon, and Apple with the starter bacteria *Lactobacillus acidophilus*. The test animals were 15 Balb/C strain mice aged 8-12 weeks. Ethical permission was issued for this study by the Muhammadiyah University Health research ethics commission Purwokerto with Ethical clearance number KEPK/UMP/07/II/2023.

Tabel 1. Fixed Dose per 10 mL Probiotik

Fruits	Formula I (5 mg/KgBB/ 27,3 mg)	Formula II (50 mg/KgBB/ 273 mg)	Formula III (300 mg/KgBB/ 1.638 mg)	Formula IV (2000 mg/KgBB/ 10.920 mg)
Apple	6,5 mg	65 mg	390 mg	2700 mg
Red guava	6,5 mg	65 mg	390 mg	2700 mg
Melon	6,5 mg	65 mg	390 mg	2700 mg
Lemon	6,5 mg	65 mg	390 mg	2700 mg
Sugar	1,3 mg	13 mg	78 mg	120 mg

This research uses the *Fixed Dose Method*. Administration of test preparations should be at most 24 hours. The feed may be given again 1-2 hours after treatment. Mice were divided into 5 treatment groups, where each group consisted of 3 male mice that were determined randomly. Group 1 was the control group with distilled water, and groups 2-5 were the treatment group, so 15 mice were used. The oral route of administration is administered with a syringe compatible with a needle/sonde. Design the trial of administering the probiotic drink can be seen in **Table 2**

Tabel 2. Experimental design

Groups	Treatments
Negative control	Aquades ad libitum was 0.5 mL
Treatment group (F1)	Probiotic juice with dose of 5 mg/kgBB is 0.5 mL
Treatment group (F2)	Juice Probiotic with dose of 50 mg/kgBB is 0.5 mL
Treatment group (F3)	Juice Probiotic with dose of 300 mg/kgBB is 0.5 mL
Treatment group (F4)	juice probiotic drink with dose of 2000 mg/kgBB is 0.5 mL

Observations were made after giving the probiotic juice drink starts at 09.00 am until 16.00. Observations were made in the first 30 minutes after administration of test preparations, periodically every 3 hours for 8 hours a day and every day for 14 days to see the effects the postponed. Observe symptoms of toxicity that may arise, among others namely seizures, body tremors, decreased movement activity, loss gripping power, grooming, walking using the stomach, walking withdrawal, diarrhoea and salivation after administering the test preparation compared to controls (Jumain *et al*., 2018). Data on symptoms of toxicity (convulsions, tremors, walking backwards, walking using the stomach, body shaking, diarrhea and salivation) was carried out using descriptive tests.

Results and Discussions

The results of the average number of deaths that occurred in acute toxicity testing regarding the effects of administering the probiotic juice with the starter bacteria *Lactobacillus acidophillus* to the group without treatment observed for 14 days are shown in **Table 3.**

Table 3. The average number of deaths of test animals

Treatment	Repetition	Number of deaths
Negative control	3	0
Dose 5 mg/KgBW	3	0
Dose 50 mg/KgBW	3	0
Dose 300 mg/KgBW	3	0
Dose 2000 mg/KgBW	3	0

Table 3. shows the results of the average number of deaths after being treated with the test substance aqua distillate, the probiotic juice at doses of 5 mg/KgBB, 50 mg/KgBB, 300 mg/KgBB and 2000 mg/KgBB. These results show that no death occurred in all treatment groups. The results of the group treated with probiotics juice at doses of 5 mg/KgBW, 50 mg/KgBW, 300 mg/KgBW and 2000 mg/KgBW showed that no deaths occurred in male balb/C mice observed for 14 days. These results indicate that administering the japelemo probiotic drink at a dose 5 mg/KgBW, 50 mg/KgBW, 300 mg/KgBW, and 2000 mg/KgBW did not affect the mortality in male balb/C strain mice observed for 14 days.

The cause of death is thought to be because the mice experienced a stressful condition during observation, characterized by symptoms of decreased body weight and hair loss. The group given the Japlemo probiotic drink showed normal clinical symptoms. It is known that the gut microbiota contained in the Japlelemo probiotic drink can affect the CNS through the immune system and ENS. The enteric nervous system (ENS) can regulate gastrointestinal behaviour independently of the central nervous system (CNS). An intact ENS is crucial for life and is often associated with digestive disorders. This is related to stress disorders in stressful conditions. Probiotics can balance intestinal microbiota, increase immunity, improve digestive function, fight the overgrowth of harmful bacteria, and help achieve mental health and mood stability (psychobiotics) as well as fight psychological conditions, such as anxiety and depression, so that mice treated with the probiotic drink Japelemo show normal behaviour and stress levels tend to be low so that death due to stress can be minimized (Rao *et al.*, 2016).

These results are supported by research by Diah *et al*. (2022), who found that consumption of probiotics causes lower plasma cortisol, which can be interpreted as a measure indicating a decrease in anxiety during stressful situations. These results were also proven in weaning mice that experienced septic peritonitis given treatment using probiotics with different strains, *Lactobacillus rhamnosus* and bifidobacterium, providing a significantly increased survival benefit of 7 days survival compared to septic mice that were not given probiotic therapy (Khailova *et al.*, 2017).

Symptoms of toxicity are the first indication that can be seen directly when a toxic effect occurs after administering the test substance. **Table 4** shows the results of observations of symptoms of toxicity in the treatment group given aqua distillate as a negative control and the probiotic drink japelemo at doses of 5, 50, 300, and 2000 mg/KgBW from the start of treatment until 14 days of observation.

Table 4 Results of behavioural observations of test animals

Dosage		Symptoms of Toxicity for 14 days						
		Seizures	Trembling	Walking backwards	Running with stomach	Salivation	Grooming	Diarrhoea
Control	P1	-	-	-	-	-	-	-
Negative	P2	-	-	-	-	-	-	-
	P3	-	-	-	-	-	-	-
	P1	-	-	-	-	-	-	-
Dose 5	P2	-	-	-	-	-	-	-
mg/KgBB	P3	-	-	-	-	-	-	-
Dose 50	P1	-	-	-	-	-	-	-
mg/KgBB	P2	-	-	-	-	-	-	-
	P3	-	-	-	-	-	-	-
Dose 300	P1	-	-	-	-	-	-	-
mg/KgBB	P2	-	-	-	-	-	-	-

Table 4. The treatment group given the test ingredient probiotic juice at 5, 50, 300, and 2000 mg/KgBB showed normal symptoms. The symptoms of toxicity observed did not indicate any toxic symptoms due to the administration of probiotic juice. Behavioural testing aims to see whether there are symptoms of toxicity and the influence of changes in clinical symptoms in test animals after administering the probiotic drink Japelemo. Behavioural test observations include seizures, tremors, salivation, walking on the stomach, walking backwards, and grooming or loss of test feathers, which were observed for 14 days. These toxic symptoms are observed through visualization or with the naked eye and

tend to be subjective to see whether or not toxic symptoms are caused to find out the symptoms that are caused. They must be compared with the habits of the test animals during the acclimatization period or with the negative control group for each test group. External factors greatly influence changes in the behaviour of test animals, including temperature, humidity, lighting and everything about the cage where they live (Abdillah *et al.*, 2020). In this study, cages were placed in a particular room for test animals to avoid noise, which could trigger stress in the test animals. Indoor cages are supported by adequate ventilation and air circulation equipment to provide adequate air quality and oxygen supply (Mutiarahmi *et al.*, 2021). These results are similar to research by Mojikon et al. (2022), which revealed that mice given probiotics could reduce depression compared to mice not given probiotics. These results are also supported by Mcginn's (2019) research on rhesus macaque monkeys with Self-injurious behaviour (SIB) given orange-flavoured chewable tablets containing the probiotic Lactobacillus reuteri can reduce biting behaviour and improve the monkey's sleep quality.

Conclusions

The administration of japelemo probiotic drinks at doses of 5, 50, 300, and 2000 mg/KgBB did not cause any toxicity symptoms that appeared after oral administration of japelemo probiotic drinks observed during 14 days of observation of test animals with the administration of test materials showed normal and healthy behaviour during observation.

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Synergism test on dates (*phoenix dactylifera* l.) Fruit extract and soybean (*glycine max* (l.) Merr.)) Nuts extract on antioxidant activity

Sinta Nuriyah Awaliah[1], Intan Kurnia Putri[2*], Melania Perwitasari[3], Maya Uzia Beandrade[4]

[1,2,3,4] Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga Bekasi, Indonesia
*Correspondence author : intan.kurnia.p@gmail.com

Abstract

The exposure of sunlight containing UV radiation may trigger the formation of free radicals, namely ROS (Reactive Oxygen Species) which lead to various skin problems. Antioxidants play important roles in protecting the skin by stabilizing free radicals which cause skin damage. Dates (*Phoenix dactylifera* L.) and soybeans (*Glycine max* (L.) Merr.)) were reported to contain several natural antioxidants such as polyphenols, flavonoids, and isoflavones. The samples used in this study were ethanolic extracts of dates and soybeans. The Ferric Reducing Antioxidant Power (FRAP) method using a UV-Vis spectrophotometer was applied to evaluate the antioxidant activity. The results of the phytochemical screening showed that both samples contained phenolic compounds marked by a change in the color of the extract to a black color and flavonoid compounds indicated by the presence of an orange color. The results of antioxidant activity expressed in % FRAP activity were 160.508% for date fruit extract and 123.851% for soybean extract. The combination of these two extracts was indicated to be synergistic according to the calculation of the difference percentage, which was 60.273%. Date fruit extract has higher antioxidant activity than soybean extract and the combination of the two has a synergistic effect.

Keywords: Dates (*Phoenix dactylifera* L.); Soybean (*Glycine max* (L.) Merr.); FRAP; UV rays; Synergism

Introduction

Ultraviolet (UV) light is a type of light that comes from the sun. Radiation from UV light cannot be seen but can be felt by humans (Liana *et al*., 2021). Too frequent exposure to sun-containing UV rays will cause skin damage, such as erythema, pigmentation, photosensitivity, and premature aging (Rahmawati *et al*., 2018). This skin damage is caused by ROS namely, environmental exposure that triggers the formation of free radicals (Andarina & Djauhari, 2017).

Antioxidants are an alternative to inhibit the oxidation process and protect cells from damage caused by free radicals, even if only in small concentrations (Martemucci *et al*., 2022). Several natural components that act as antioxidants to protect the skin include polyphenols, monoterpenes, flavonoids, carotenoids, organosulfides, indole, chromanol, and chromenol. This compound has been proven to stimulate immune responses, be anti-inflammatory, modulate antioxidants, detoxify cells and tissues, change gene expression, and protect skin from aging (Pourzand *et al*., 2022).

Combining the two extracts containing antioxidants can produce synergistic, additive, or antagonistic interactions (Marianne *et al*., 2018). *Synergistic* is defined as the combined effect of two or more compounds that is greater than the sum of the individual effects. An additive effect is generally considered the combined effect of two or more compounds equal to the sum of the individual effects. In contrast, an antagonistic effect is observed when the effect of two or more substances in combination is smaller than the sum of the individual effects of the substance (Basavegowda & Baek, 2021).

The aim of carrying out this research is so that researchers can determine the antioxidant activity of date extracts, soybeans, and their combination, as well as the synergistic effect produced by the FRAP method, and provide recommendations regarding the selection of natural antioxidant sources that can be used as an alternative to the use of synthetic antioxidants, as well as ingredients. Raw material for entrepreneurial innovation that is useful as an antioxidant.

Methods

Plant determination test

The dates used were subjected to a determination test at the National Research and Innovation Agency (BRIN), Bogor. Meanwhile, the soybeans used were subjected to a determination test at the Department of Biology, University of Indonesia, Faculty of Mathematics and Natural Sciences, Depok, West Java.

Preparation of Sample Extract

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Sukkari dates are washed and separated from the seeds, then dried in an oven at 60°C for 24 hours. Dried simplicia powder of dates was weighed as much as 500 mg and macerated for 2 x 24 hours using 70% ethanol with a ratio of 1:2. The maceration results were filtered and concentrated using a rotary evaporator at 40°C with 80 rpm. Soybeans are washed and separated from the epidermis, then dried in an oven at 60°C. 250 mg of dried fruit simplicia powder was weighed and macerated for 2 x 24 hours using 70% ethanol with a ratio of 1:10. The maceration results were filtered and concentrated using a rotary evaporator at 40°C with 80 rpm.

Phytochemical Screening

The flavonoid test of extracts of dates and soybeans was carried out by mixing the extract with 5 mL of concentrated HCl and adding Mg powder. The presence of flavonoids can be seen in the formation of red, yellow, or orange colors (Nur, 2022). The phenol test for extracts of dates and soybeans was carried out by mixing the extract with 3-4 drops of 1% FeCl. The presence of phenolic compounds can be seen from the formation of a bluish-black color (Azizah & Wati, 2018).

Preparation of Acetate Buffer

Acetate buffer with a pH of 3.6 is made from 0.155 grams of sodium acetate trihydrate ($CH_3COONa.3H_2O$) added with 0.8 mL of concentrated acetic acid and dissolved with Aquadest to exactly 50 mL in a measuring flask.

Preparation of 2,4,6-tripiridyl-striazine (TPTZ) Solution

TPTZ was weighed at 30 mg and dissolved in 40 mmol/L HCl to exactly 10 mL. A 40 mmol/L HCl solution dissolves 0.1656 mL of concentrated HCl in 50 mL Aquadest.

Preparation of $FeCl_3.6H_2O$ Solution

A total of 0.27 mg $FeCl_3.6H_2O$ was dissolved with acetate buffer in a volumetric flask to exactly 50 mL.

Preparation of FRAP Reagent

The FRAP reagent is prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of FeCl3.6H2O solution. Then, add distilled water to precisely 100 mL in the measuring flask.

Preparation and Testing of $FeSO_4.7H_2O$ Standard Solution

50 mg of FeSO4.7H2O was dissolved in 50 mL of distilled water in a volumetric flask until a concentration of 1000 ppm was obtained. A concentration series of 50, 70, 100, 130, and 140 ppm was prepared in a 10 mL volumetric flask. From each concentration, 100 µl was taken, and 3 mL of FRAP reagent was added in a 5 mL measuring flask, homogenized, and stored in a brown vial. The solution was incubated at 37°C for 10 minutes. The absorbance of each concentration was read using a spectrophotometer with the maximum wavelength of the optimization results.

Maximum Wavelength Determination

100 µl of FeSO4.7H2O solution with a concentration of 140 ppm was added with 3 mL of FRAP reagent in a 5 mL measuring flask, homogenized. Furthermore, stored in a brown vial. The solution was incubated at 37°C for 10 minutes. Scan the solution at a wavelength of 580-610 nm.

Determination of Antioxidant Activity

100 µl of sample solution was added to 3 mL of FRAP reagent in a measuring flask 5 mL, homogenized, and stored in a brown vial. The solution was incubated at 37°C for 10 minutes. Then, 1 mL of the solution was taken and diluted to a volume of 10 mL in a measuring flask. Each sample was read for absorbance at the maximum wavelength. Antioxidant activity was obtained from absorbance data against the standard series FeSO4.7H2O and was expressed as equivalent to ppm Fe2+. The percentage of FRAP activity is calculated based on a formula.

FRAP activity (%) = (A/B) x 100 … (1)

A: control absorbance, B: sample absorbance

Antioxidant Activity Synergism Test

A total of 10 mg of date fruit sample was dissolved in distilled water to 10 mL (1000 µg/mL). A total of 20 mg of soybean sample was dissolved in distilled water to 10 mL (2000 µg/mL). From this concentration, 5 mL of each was taken and homogenized in a 10 mL volumetric flask. Take 0.1 mL and add 3 mL of FRAP reagent. Next, the absorbance was read at the maximum wavelength using a UV-Vis spectrophotometer. The synergism test is determined by calculating the % difference using the formula.

Difference (%)= [(Combination ab x 100)/((Individual a + Individual b)÷n)]-100 … (2)

Differences : Effects resulting from a mixture of extracts a and b,
Combination ab : Antioxidant activity (Fe2+ levels) in a mixture of extracts a and b,

ISSN 3031-0911

Individual a/b : Antioxidant activity (Fe2+ levels) + in extracts a/b,
N : Number of compounds in the mix.

Results and Discussions

The purpose of the determination test is to prevent sample errors and guarantee the correct identification of plants based on their taxonomy (Nurulita *et al.*, 2019). Test results show that this research used sukkari date fruit with the Latin name *Phoenix dactylifera* L of the Arecaceae family. Meanwhile, soybeans have the Latin name *Glycine max* (L.) Merr., Fabaceae family. The result of date fruit extract is a thick extract, brownish-yellow in color, and has a distinctive smell of dates. The result of soybean extract is a thick extract, brownish-yellow in color and odorless. The result yields were 20.92% and 8.4% respectively. The results of the acquisition of water content from date fruit extract and soybean extract were 7.17% and 0.46%, respectively.

Table 1. Results of Phytochemical Screening

	Flavonoid Test		Phenol Test	
Extract	**Before treatment**	**After treatment**	**Before treatment**	**After treatment**
Dates	Yellow clear	+ (Orange)	Orange	+ (Blackish brown)
Soybean	Yellow	+ (Orange)	Brownish yellow	+ (Blackish Green)

The phytochemical screening carried out on date fruit extract and soybean extract were the flavonoid test and the phenol test. In the flavonoid test, both samples produced an orange color. While the phenol test produced blackish brown and blackish-green. This shows that the positive sample contains flavonoid and phenol compounds.

Determining the maximum wavelength is done by looking at the absorbance standard curve of $FeSO_4.7H_2O$ at the highest concentration of 140 µg/mL in the range of 580-610 nm. The absorbance obtained is 0.782 at a maximum wavelength of 595 nm. The maximum wavelength spectrum is shown in **figure 1.**

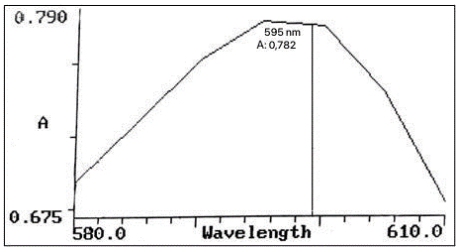

Figure 1. Maximum wavelength spectrum

Determination of standard curve absorbance in series solutions with concentrations of 50 µg/mL, 70 µg/mL, 100 µg/mL, 130 µg/mL, and 140 µg/mL resulted in the regression equation Y=0.0032x + 0.1638 with a value r2=0.999. The absorbance data obtained was used to calculate the % difference in the synergism test. The standard curve graph for $FeSO_4.7H_2O$ is shown in **figure 2.**

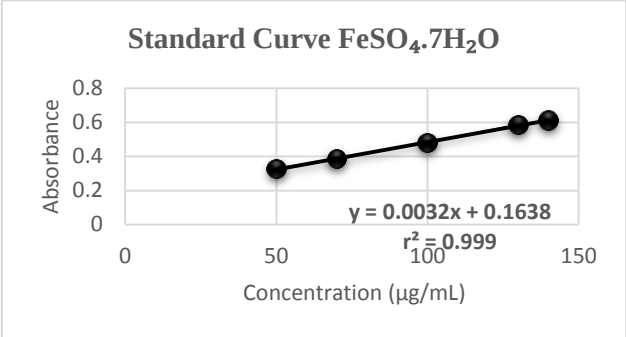

Figure 2. Standard curve graph

ISSN 3031-0911

Antioxidant activity in single extracts of dates and single extracts of soybeans (F1 and F9) as well as their combination (F2-F9) is expressed in % FRAP activity and Fe^{2+} content. The antioxidant activity of a single extract of dates obtained a Fe^{2+} level of 67.771 with a FRAP activity of 160.508%. While the activity of soybean extract obtained Fe^{2+} levels of 102.979 with FRAP activity of 123.851%.

Table 2. FRAP Activity % Value

Formulas	Treatment		Fe^{2+} level (µg/mL)	FRAP Activity (%)
	Date Fruit Extract (%)	Soybean Extract (%)		
F1	100	0	67,771	160,508
F2	80	20	56,938	176,590
F3	60	40	54,958	179,882
F4	55	45	82,979	142,314
F5	50	50	71,208	156,000
F6	45	55	73,188	153,518
F7	40	60	75,479	150,740
F8	20	80	60,271	171,308
F9	0	100	102,979	123,851
F10	100	100	136,833	101,551

The synergism test was carried out by mixing date extract and soybean extract in a ratio of 100:100. In calculating the % difference to determine the synergistic effect of the antioxidant activity of the samples, data is needed regarding the levels of antioxidant activity in a single sample extract. The sample synergism test value obtained a difference of 60.273%.

Table 3. synergism test

Formulas	Treatment		Fe^{2+} level (µg/mL)	Difference (%)
	Date Fruit Extract (%)	Soybean Extract (%)		
F1	100	0	67,771	-
F9	0	100	102,979	-
F10	100	100	136,833	60,273

In general, the weight of dates is 2-60 grams and 3-7 cm long (Azkiyah & Rahimah, 2022). The type of date studied was the Sukkari date, which has a light brown color and ripe yellow at the ends (Fandi, 2020). In general, soybean seeds have an oval, round, or flat shape, measuring 6-30 g per 100 seeds. Soybean seeds, which are wrapped in a seed coat, have two pieces called lesta (Stefia, 2017). Soybeans of the Anjasmoro variety were chosen as the research sample. Anjasmoro soybeans have large seeds (14.8-15.3 g/100 seeds), have the potential to obtain high yields of 2.3 tons/ha, and are often used as an essential ingredient in making tempe and tofu (Tambunan *et al*., 2020).

The assessment of a sample using human senses as the main tool is called organoleptic testing (Gusnadi et al., 2021). The focus of testing is color, shape, taste, and smell (Handoyo & Pranoto, 2020). Sukkari date pulp extract is a thick extract, brownish yellow, and has a distinctive date smell. This follows research conducted by Rani (2021), with the same sample forming a black-brown viscous extract with a distinctive smell of dates. Another study conducted by Azkiyah & Rahimah (2022) produced a thick extract, dark brown, and had a sweet smell typical of dates and a sweet taste. The color difference in the extract depends on the color of the date fruit powder obtained.

The results of the organoleptic test carried out on soybean extract were in the form of a thick extract, brownish yellow, and odorless. The yield value obtained indicates the number of components of the bioactive compounds contained in the sample extract (Senduk *et al*., 2020). From the thick extract of dates, the yield was 20.92%. Research conducted by Rani (2021) obtained a percent yield of date fruit extract of 8.82%. The results obtained are lower because of the difference in the solvent used, namely methanol, in the extraction process. Apart from that, there is a difference in the water content of the date extract, which is less, namely 5.88%, caused by the drying process using a high temperature, namely 80℃ for 10 hours. The total soybean thick extract obtained a percent yield value of 8.4%. The yield results obtained are in line with the research of Hasanah *et al*. (2019), namely 8.1% in soybean extract with the anjasmoro variety. The water content test obtained has met the quality requirements from The Indonesian Food and Drug Authority (BPOM), namely <10% (Wijaya, 2022). The high water content in simplicia causes damage to the compounds contained in the simplicia because it becomes a medium for the growth of fungi and bacteria (Santoso *et al*., 2022).

ISSN 3031-0911

Phytochemical screening is carried out using specific reagents and observing the color changes that occur (Vifta & Advistasari, 2018). Phytochemical screening of date and soybean fruit extracts was conducted, namely testing for flavonoid and phenolic compounds. Research with samples of date fruit extract and soybean extract resulted in a change in color to orange and positively contained flavonoid compounds. Research by Ramayani *et al.* (2021) showed a color change to orange after the sample was reacted with concentrated HCl and Mg powder so that it positively contained flavonoid compounds. The results obtained from the phenol test of date and soybean extracts were blackish brown and blackish green. This is in line with Nazilah's (2019), with ajwa date fruit extract producing a black-green color, and Rani's research (2021) using sukkari date fruit extract producing a black color after being reacted with $FeCl_3$.

Flavonoid and phenolic compounds are the largest group of phenolics in plants that are beneficial to health as antioxidants (Siddeeg *et al*., 2019). The hydroxy groups on the aromatic rings of flavonoids and phenolic compounds are easily oxidized so that they can provide their hydrogen atoms to free radicals (Dhurhania & Novianto, 2019). The ability of phenolic compounds to donate their hydrogen atoms can neutralize free radicals so that the chain radical reactions that are occurring can be stopped. Meanwhile, hydrogen atom donors in flavonoids play a role in inhibiting the formation of free radicals and preventing oxidation processes by chelating metal ions (Liza *et al*., 2020).

The determination of the maximum wavelength is carried out to determine the absorption area that can be generated in the form of absorbance values from the standard solution using a UV-Vis spectrophotometer (Sukmawati, 2018). The most excellent sensitivity and the best absorption of the test solution will occur at the maximum wavelength, resulting in optimal sample absorbance measurements (Wahid *et al*., 2017). This study used the 580-610 nm range for standard $FeSO_4.7H_2O$ at a concentration of 140 µg/mL to determine the maximum wavelength (Nurulita *et al.*, 2019). The highest absorbance value was 0.782, obtained at a wavelength of 595 nm. This follows research by Nurhayati *et al.* (2022) in determining the maximum λ using the standard $FeSO_4.7H_2O$ obtained a maximum wavelength of 595 nm.

The sample concentration can be determined by looking at the relationship between the absorbance value of the standard curve solution and its concentration in determining the standard curve (Suharyanto & Prima, 2020). The standard in this study used $FeSO_4.7H_2O$. This is related to the FRAP method reaction, which has a working principle, namely that antioxidant compounds are able to reduce Fe3+ ions to Fe2+ ions. $FeSO_4.7H_2O$ has Fe2+ ions, which can be used as an antioxidant standard. The determination of the standard curve was made in series solutions with concentrations of 50 µg/mL, 70 µg/mL, 100 µg/mL, 130 µg/mL, and 140 µg/mL. This is in accordance with research by Rumpf *et al.* (2023), which explains the standard curve range used, namely 40-190 µg/mL. A series of standard curves were created, and then the absorbance was read using a UV-Vis spectrophotometer. The absorbance data obtained is processed into a linear regression equation. The linear regression equation obtained is Y = 0.0032x + 0.1638, with a value of r2 = 0.999. The r2 value or correlation coefficient close to 1 shows that this regression equation is linear, so absorbance and concentration have a very strong correlation (Asmorowati & Lindawati, 2019).

Measurement of antioxidant activity in samples used a concentration of 1000 µg/mL for ethanol extract of dates and 2000 µg/mL for soybean extract. The sample absorbance value must enter the standard curve absorbance range, 0.328-0.611. Both extracts were formulated (F1-F10). Based on the FRAP activity values presented in Table 2, it is known that the single extracts tested, namely F1 and F9, obtained different FRAP activity values. F1, which is a date fruit extract, has higher FRAP activity than soybean extract, with an FRAP activity value of 160.508, while F9, which is a single extract from soybeans, has an FRAP activity value of 123.851. This follows research conducted by Nafisah (2019) by determining antioxidant activity using the DPPH method in ajwa date extract, obtaining an IC50 value of 9.13 ppm and belonging to the extreme category. Meanwhile, the antioxidant activity of the anjasmoro variety soybean extract carried out by Asshidiqy *et al*. (2020) obtained an IC50 of 65.07 ppm and is included in the potent antioxidant category.

Apart from testing a single extract, mixing of the two extracts was carried out, namely at F2-F8. The highest FRAP activity was shown in a mixture of 60% date fruit extract and 40% soybean extract (F3), with an FRAP activity value of 179.882. Although the F3 mixture has a more excellent composition of date fruit extract than soybean extract, it cannot be said that this date fruit extract has a more significant role in its antioxidant activity. This can be seen from the acquisition of FRAP activity in F8 with a composition of 20% date fruit extract and 80% soybean extract, which had a higher FRAP activity value of 171.308 compared to F4, F5, and F6, which had a larger composition of date fruit extract. This is because there is a very complex interaction between each extract, so it cannot be determined which extract plays the most dominant role in providing antioxidant activity. In addition to determining antioxidant activity in single and mixed extracts, antioxidant activity was also determined in samples with a ratio of 100:100. This is done to see the antioxidant activity of the combination of samples with the same composition. Then the synergism test will be calculated.

ISSN 3031-0911

The synergistic effect, namely the antioxidant effect of two or more mixtures of different types of antioxidants, is greater than the effect of the individual antioxidants separately. Based on the calculations, it was found that the combination of the two extracts obtained synergistic results with a difference value of 60.273%. This percent difference value is used to determine antioxidant potential, namely having a synergistic effect if the value is positive (% difference > 0) or antagonistic if the value is negative (% difference < 0) (Skroza *et al*., 2022).

Research conducted by Vifta *et al.* (2019) showed that there was a synergistic effect on antioxidant activity using the ABTS method, which was characterized by obtaining a better IC50 from the combination of parijito fruit extract and red ginger rhizome compared to the IC50 of the single extract. Research testing the effect of synergism was previously carried out by Rani (2021) using the DPPH method on date pulp extract and raw tempeh extract. However, there is a difference in determining the effect of the synergism, namely by looking at the gain in % inhibition resulting from the combination of samples. The results of statistical tests show that the combination of samples with a ratio of 50:50 affects increasing antioxidant activity. Other synergistic effects can be determined by looking at the Combination Index (CI) value in the IC50 sample. This was implemented by Septiana *et al*. (2020), who used the DPPH method on a combination of the ethanol extract of jarong leaves and center stems. The CI value obtained was 0.7723-0.9607 and showed that the combination of the two sample extracts had a mild-to-moderate to almost additive synergistic effect. The interpretation of the CI value is <0.1, powerful synergy; 0.1-0.3 strong synergist; 0.3-0.7 synergistic; 0.7-0.9 mild–moderate synergist; 0.9-1.1 is close to additive; 1.1-1.45 mild–moderate antagonist; 1.45-3.3 antagonist; >3.3 strong–potent antagonist.

Conclusions

Date fruit extract has higher antioxidant activity than soybean extract, and combining the two has a synergistic effect. Researchers suggest that this research be continued by calculating the SPF value, testing for antibacterial activity, and conducting preclinical tests with animal mice on cosmetic preparations made from a combination of date fruit extract and soybean extract because it can produce higher antioxidant activity.

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Determination of Hydroquinone Content in Whitening Toner Sold in Online Market Place X by Reverse Phase High Performance Liquid Chromatography (HPLC)

Stevani Hana Marumata[1*], Melania Perwitasari[2], Intan Kurnia Putri[3], Maya Uzia Beandrade[4]
[1,2,3,4] Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Bekasi, Indonesia

[*]Correspondence author: vanymarumata@gmail.com

Abstract

Hydroquinone is often misused in cosmetic formulations as a whitener. According to the Badan Pengawas Obat dan Makanan/BPOM (Food and Drug Administration of Indonesia) regulation No. 23 of 2019, the content of hazardous substances and dyes such as hydroquinone is prohibited for use in cosmetics except for artificial nail preparations. This study aimed to determine the hydroquinone content in facial whitening toners sold in the X online marketplace using high-performance liquid chromatography (HPLC). This research is descriptive and non-experimental, and 15 toner samples were taken at online marketplace X with a purposive sampling method. Qualitative analysis was carried out by organoleptic and colour tests. Quantitative analysis on whitening toner was performed using the reverse phase HPLC method. The detection wavelength of 295 nm was applied, and a linear regression formula of y = 42.799x – 8.2248 with r = 0.9996 was obtained. Analytical method validation has been successfully executed and resulted in the resolution value of Rs 4.042, recovery percentage of 96.99%, RSD value of 0.801%, LOD and LOQ values of 0.7 ppm and 33.45 ppm, respectively. Hence, it can be stated that the RP-HPLC method was specific, accurate, and sensitive. The quantitative analysis test results showed that 8 out of 15 samples contained hydroquinone with an average concentration in sample T2 obtained 0.36499%, T4 0.35248%, T6 0.01253%, T7 0.35271%, T8 0.00371%, T9 0.00516%, T12 0.00272% and T13 0.00272%. According to the research data, it can be concluded that the hydroquinone content used in the whitening toner samples does not meet the BPOM requirements.

Keywords: *Bleach Toner ; HPLC ; Hydroquinone ; Validation*

Introduction

Based on research conducted by BPOM in 2019, it was found that the prevalence of 29% of cosmetic products contained the dangerous ingredient hydroquinone. From June 2020 to September 2021, a 25% prevalence of cosmetic products containing the hazardous ingredient hydroquinone was found. Based on this data, it has been proven that hydroquinone use in skin whitening cosmetics is still widely practised and widely circulated in society even though there are regulations prohibiting hydroquinone (BPOM, 2022). In the 2019 Food and Drug Supervisory Agency (BPOM) regulations regarding cosmetics containing dangerous substances and prohibited dyes, 0% hydroquinone is used in cosmetic preparations. Hydroquinone can only be used for nails at a level of 0.02% and hair dye at a maximum level of 0.3% because it can cause harmful health. This is because the side effects of using hydroquinone on the skin are irritation, the skin becoming red/erythema and a burning feeling. This effect will occur if hydroquinone is used in high concentrations. Hydroquinone concentrations above 0% for extended or continuous use will cause contact leukoderma and exogenous ochronosis. In addition, there is evidence that hydroquinone can cause cancer in mice after oral administration and can cause ochronosis (dark skin and black spots) when applied topically to the skin (Ruiyi *et al*., 2018).

Based on previous research, checking hydroquinone levels in cosmetic products is predominantly done on whitening creams and lotions. However, it has never been carried out on toners in the Bekasi district and Bekasi city. So, the researchers were interested in analyzing hydroquinone levels using the reverse phase High-Performance Liquid Chromatography (HPLC) method and bleach toner samples circulating in online marketplace X in the Bekasi district and Bekasi city. This research is expected to provide information regarding the presence or absence of hydroquinone content in whitening toner products.

Methods

Design

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This study uses a descriptive, non-experimental research design using purposive sampling. This research was conducted at the Pharmaceutical Chemistry Laboratory, Mitra Keluarga Bekasi College of Health Sciences in January-April 2023 with a population of all cosmetic shops in Bekasi city and Bekasi district in the X online marketplace shop.

Qualitative Analysis

Samples consisting of 15 toner products sold in the online marketplace X in the Bekasi city and Bekasi district were subjected to organoleptic testing, namely testing the colour, texture and aroma of the toner (Sari *et al*., 2021). Each toner sample was measured as much as 5 mL and then stored in a test tube. Then, 4 drops of Benedict were added, and colour changes were observed. Suppose there is a change in colour to brick red. The positive sample containing hydroquinone compounds will be followed up with a quantitative test using Reversed-Phase High-Performance Liquid Chromatography (HPLC).

Quantitative Analysis

The first preparation of the mobile phase was prepared with a mixture of 100 mL of methanol and distilled water (55:45), then filtered using a 0.45 µm millipore filter membrane and de-aired with an ultrasonicator for 10 minutes. Make a standard solution of 100 ppm, namely 5 mg of pure hydroquinone, dissolve it in 50 mL of mobile phase, then shake the solution until homogeneous. Make a standard calibration curve for hydroquinone, namely taking 3 mL, 3.5 mL, 4.5 mL, 6 mL and 8 mL of 100 ppm hydroquinone standard solution and dissolving in 25 mL of mobile phase and then shaking until homogeneous and concentrations of 12 ppm, 14 ppm, 18 ppm, 24 ppm and 32 ppm were obtained. Determination of the maximum wavelength is carried out by scanning a 20 ppm hydroquinone standard liquid, which is included in a cuvette using a blank solution, namely methanol p.a and the absorbance is measured at a wavelength of 200-400 nm so that the spectrophotometer will display maximum wavelength data by producing the very maximum absorbance value (Alqarni *et al*., 2021).

Validation Method

The specificity test was carried out by putting 1 mL of sample into a 10 mL volumetric flask, adding 1.4 mL of a 100 ppm standard solution, and entering the mobile phase up to the mark to make a volume with a final concentration of 14 ppm then shaking until homogeneous. Filter the solution with a 0.45 µm filter membrane into the vial and then in the sonicator for 10 minutes to remove bubbles and inject it into the HPLC instrument. Note each peak's resolution value (Rs) to see perfect separation. The accuracy test was carried out by inserting 1 mL of sample into 3 10 mL volumetric flasks and adding the mobile phase up to the boundary mark to make replications 1, 2 and 3. 1 mL of sample was taken and transferred into 9 10 mL volumetric flasks containing 1.4 mL; 1.8 mL and 2.4 mL of 100 ppm hydroquinone standard solution. A mobile phase was added to make final concentrations of 14 ppm, 18 ppm and 24 ppm. To get the % recovery, calculate the concentration using HPLC (Rahmayuni et al., 2018). The linearity test was obtained at five hydroquinone concentration levels (12 ppm, 14 ppm, 18 ppm, 24 ppm and 32 ppm), and then the area was plotted in the linear regression equation. The precision test was carried out by preparing a hydroquinone solution with a concentration of 18 ppm, measuring the area at the hydroquinone's retention time, and repeating the measurement 6 times. The standard deviation value and the coefficient of variation can be determined from the data obtained. The range test is determined from the lowest and highest concentrations of the hydroquinone standard series, which still meet the parameters of linearity, accuracy and precision (Saraswati, 2022). The limit of detection (LOD) and limit of quantification (LOQ) use a sample of the linearity parameters and are then calculated using the formula. This value is calculated from the standard deviation (SD) of the response and the slope of the curve (S) using the equation: LOD = 3.3 (SD/S) and LOQ = 10 (SD/S), where SD is the standard deviation of the detector response and S: the slope of the calibration curve. The system suitability test was carried out with a standard mixed solution (18 ppm) injected as much as 20 µl into the HPLC with the selected analysis conditions. Six replications were carried out to ensure a constant peak area. The injection results were recorded, and the coefficient of variation (%CV) was calculated. The % CV value must be $\leq$ 2%. The system suitability test must meet resolution parameters, precision system determination, asymmetric factors, column efficiency, and capacity (Rahmayuni *et al.*, 2018).

Analysis of Hydroquinone Levels

The toner samples used were from various brands and purchased from the online marketplace X. A liquid sample (toner) was measured 1 mL in a beaker glass, and 10 mL of mobile phase was added and mixed until homogeneous. The solution is then filtered using a filter membrane (Alqarni *et al*., 2021). Hydroquinone levels, which met the validation

ISSN 3031-0911

parameters, were determined using HPLC. The HPLC system was set at a 1 mL/minute flow rate. The mobile phase used 55 mL methanol and 45 mL distilled water, and the stationary phase used a C18 analysis column. A total of 20 µL of sample was put in a vial, then analyzed for levels using HPLC. The results of the HPLC area obtained at each concentration are plotted into linear regression (Zukepli *et al*., 2015).

Results and Discussions

Qualitative Analysis

Organoleptic tests can be carried out using sensory organs such as smell, appearance, taste and texture. Organoleptic tests determine the odour, appearance, aroma and texture of samples. From the results of **Table 1**, as many as 7 samples (47%) had a pungent odour, 5 samples (33%) had a fragrant aroma, and 3 samples (20%) had no aroma. This test is carried out to determine the presence of hydroxyl groups responsible for hydroquinone's oxidation-reduction reaction, which reacts with Benedict's reagent to form a brick-red colour. From Table 1, there are 2 samples (13%) which changed colour to brick red, which is thought to contain hydroquinone, 6 samples (40%) which specifically changed from their original colour, and 7 samples (47%) which changed due to the influence of the colour of the reagent benedict.

Table 1. Color Test Results for Bleach Toner Samples with Benedict's Reagent

Sample Code	Reagents	Before Testing	After Testing	Result
Control (+)		Colorless	Brick Red	+
Control (-)		Light Yellow	Light blue	-
T1		Bright yellow	Light blue	-
T2		Yellow	Yellow Greenish	+
T3		Light Brown	Green	-
T4		Dark Brown	Yellow Greenish	+
T5		Pink	Light Brown	-
T6		Light Brown	Brick Red	+
T7	Benedict	Dark Yellow	Brick Red	+
T8		Light Blue	Greenish Brown	+
T9		Pink	Yellow Greenish	+
T10		Light Yellow	Light Blue	-
T11		Light brown	Light Purple	-
T12		White	Yellow Purplish	+
T13		Light Brown	Dark Brown	+
T14		Colorless	Light Blue	-
T15		Colorless	Yellow Greenish	-

Quantitative Analysis

Determination of the Maximum Wavelength of Hydroquinone

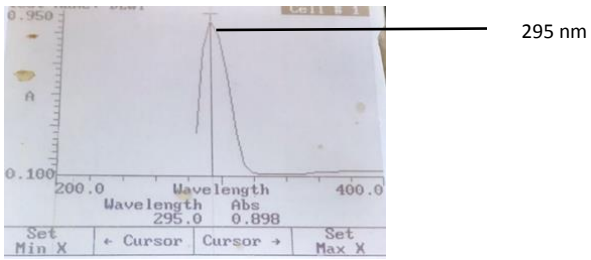


Figure 1. Maximum Wavelength of Hydroquinone with Methanol Solvent

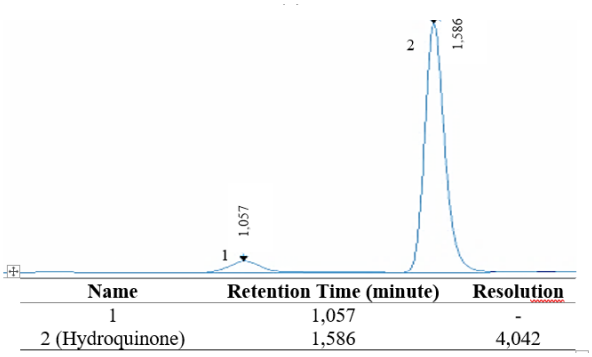
Based on **Figure 1**, the maximum absorbance of hydroquinone is 0.898 with a wavelength of 295 nm obtained from the UV-Vis Spectrophotometer instrument.

ISSN 3031-0911

Validation Method

Specificity

The results obtained from the specificity test are separation or Rs of 4.042 with the chromatogram can be seen in the **Figure 2.**



Name	Retention Time (minute)	Resolution
1	1,057	-
2 (Hydroquinone)	1,586	4,042

Figure 2. Hydroquinone Specificity Test Value with Reversed Phase HPLC.

Accuracy

The results of accuracy testing with three concentrations have an average recovery value of 96.99% which can be seen in **table 2.**

Table 2. Accuracy Test Values for Hydroquinone with Reversed Phase HPLC

Concentration (ppm)	Rep	Concentration (ppm)			% *Recovery*	Average ± SD
		Sample	Addition Sample	Measurable Standard		
14	1	12,62	25,38	12,76	91,16	91,54 ± 0,486
	2	12,68	25,47	12,79	91,37	
	3	12,28	25,17	12,89	92,09	
18	1	12,62	29,84	17,23	95,70	102,76 ± 1,341
	2	12,68	29,98	17,30	96,12	
	3	12,28	29,96	17,68	98,23	
24	1	12,62	37,16	24,54	102,24	96,70 ± 0,953
	2	12,68	37,20	24,52	102,17	
	3	12,28	37,20	24,93	103,86	

Linearity

The results of determining the standard curve linear regression show the relationship between area size as a response and concentration, which can be seen in **table 3**

Table 3. Hydroquinone Linearity Test Results with Reversed Phase HPLC

Replication 1		Replication 2		Replication 3	
Concentration (ppm)	Area	Concentration (ppm)	Area	Concentration (ppm)	Area
12	509,3	12	486,9	12	504,4
14	611,1	14	609,5	14	588,3
18	781,9	18	782,7	18	773,8
24	1078,3	24	1068,1	24	1007
32	1383,2	32	1391	32	1365,3
A = -6,543		A = -29,845		A = -8,2248	
B = 43,965		B = 44874		B = 42,799	
r = 0,9986		r = 0,9987		r = 0,9996	

Precision

The results of precision testing can be seen in table 4 with the average results from 6 replications with a concentration of 18 ppm of RSD = 0.801%

ISSN 3031-0911

Table 4. Precision Test Results for Hydroquinone with Reversed Phase HPLC

Replication	Retention Time	Area
1	1,59	781,900
2	1,58	782,700
3	1,59	773,800
4	1,59	791,600
5	1,59	789,000
6	1,59	781,600
Average	1,586833	783,433
SD	0,003545	6,273
%RSD	0,223398	0,801

LOD (Limit Of Detection) and LOQ (Limit Of Quantitative)

The results of LOD and LOQ testing can be seen in table 5 LOD obtained 0.7 ppm and LOQ obtained 33.45 ppm.

Table 5. LOD and LOQ Test Results for Hydroquinone with Reversed Phase HPLC

No	Concentration (ppm)	Area(Y)	Yi=bx+a	$(Yi-Y)^2$
1	12	504,4	505,36	0,93
2	14	588,3	590,96	7,08
3	18	773,8	762,16	135,55
4	24	1007	1018,95	142,83
5	32	1365,3	1361,34	15,66
		Total		302,05
		LOD		0,7
		LOQ		33,45

System Suitability Test

The suitability results can be seen in **table 6** using 5 concentrations.

Table 6. Suitability Test Results of the Hydroquinone System with Reversed Phase HPLC

Replication	AUC	Retention Time	TF	N	HETP (cm)
1	781,561	1,588	1,21	4107	0,00365
2	781,870	1,589	1,25	4087	0,00367
3	773,797	1,588	1,22	4081	0,00368
4	791,669	1,588	1,22	4120	0,00364
5	788,999	1,587	1,24	4161	0,00360
X	783,579	1,588	1,228	4111	0,00365
RSD(%)	0,897	0,04452813	1,338084424	0,77615407	0,00772

Determination of Hydroquinone

The results of the hydroquinone content test on 8 whitening toners were replicated 3 times using HPLC with a retention time of 1.5 minutes. The levels obtained can be seen in **table 7**

Table 7. Results of Determination of Hydroquinone Levels

Sample	Replication	AUC	% Hydroquinone Levels (b/v)	Average (%) ± SD
1	-	-	-	-
2	1	15661,40	0,36612	0,36499 ± 0,00108
	2	15569,89	0,36398	
	3	15607,22	0,36486	
3	-	-	-	-
4	1	15154,85	0,35429	0,35248 ± 0,00197
	2	15090,55	0,35278	
	3	14987,85	0,35038	
5	-	-	-	-
6	1	531,78	0,01262	0,01253 ± 0,00022
	2	534,54	0,01268	

ISSN 3031-0911

	3	517,22	0,01228	
7	1	15383,77	0,35963	0,35271 ± 0,00603
	2	14966,48	0,34988	
	3	14912,60	0,34863	
8	1	144,36	0,00357	0,00371 ± 0,00018
	2	159,20	0,00391	
	3	147,82	0,00365	
9	1	268,88	0,00647	0,00516 ± 0,00142
	2	147,82	0,00365	
	3	221,22	0,00536	
10	-	-	-	-
11	-	-	-	-
12	1	192,96	0,00470	0,00272 ± 0,00172
	2	64,71	0,00170	
	3	66,45	0,00174	
13	1	57,139	0,00153	0,00152 ± 0,000027
	2	57,425	0,00153	
	3	55,294	0,00148	
14	-	-	-	-
15	-	-	-	-

In this study, 15 samples of the whitening toner used were coded T1-T15. These samples were obtained from the online marketplace X based on the criteria in this study. This study uses a reversed-phase because the mobile phase is relatively polar, and the stationary phase is nonpolar. It is known that the ε of hydroquinone is 3.104, so it can be analyzed using HPLC, which requires ε > 1,000. The value of A1% 1cm of hydroquinone at a wavelength of 295 nm is known to be 282 (Moffat, 2011). Hydroquinone can be analyzed at trace levels using HPLC with a UV-Vis detector. The hydroquinone colour reaction test using Benedict's reagent will undergo an oxidation-reduction reaction. If it is positive for hydroquinone, a red brick will be formed because hydroquinone is in the phenol group. From the study results, it was found that 8 samples were positive for hydroquinone due to changes. The results of this colour test can be confirmed by carrying out quantitative analysis using HPLC to determine the level of hydroquinone contained in the sample. Based on the results of the research that has been done, the maximum wavelength is 295 nm. This result is to the maximum hydroquinone length requirement of 293 ± 2 nm (Pharmacopeia, 2020). This maximum wavelength will be used to measure the absorbance of the whitening toner sample.

The results obtained from the specificity test have a resolution value of 4.042. These results meet the requirements because they have Rs ≥ 1.5 (Pharmacopeia, 2020). Based on the % recovery value obtained at 3 concentrations, it was 91.54%-102.76%, so the results obtained were by the accuracy requirements at a concentration of 10 ppm with a range of 80-110%. The results of the linear regression equation relate the hydroquinone concentration to the area obtained from the HPLC injection. The requirement for the coefficient of determination is r2 ≥ 0.999 (ICH, 2006). Of the 3 replications chosen, the best r2 value is replication 3 because it has the highest r2 value than the others, namely r2= 0.9994 and the value of r = 0.9996 with the equation Y = 42.799x-8.2248, then you get the results the calibration curve is linear and can be used for analysis of hydroquinone levels. From this research, the RSD result was 0.801%, which shows that the method used has good precision because the RSD or CV value is <2% (Pharmacopeia, 2020).

From this test that has been carried out, results were found for a LOD value of 0.7 ppm, which means that the tool is unable to detect if it is smaller than 0.7 ppm and if it is more significant than 0.7 ppm, the tool can detect with good precision and accuracy values. The LOQ value obtained was 33.45 ppm, which means that the tool can carry out measurements at this concentration with careful analysis results. This test is an integral part of the chromatography used to verify that the resolution and reproducibility of the HPLC system are sufficient for the analysis to be carried out with short retention times, resolution (Rs) ≥ 1.5, tailing factor (TF) ≤ 2 and theoretical plates (N) ≥ 3500 and meets the RSD value ≤ 2% (Pharmacopeia, 2020). Based on the results obtained in Table 6, the T2 sample obtained an average of 0.36499%; sample T4 0.35248%; sample T6 0.01253%; sample T7 0.35271%; sample T8 0.00371%; sample T9 0.00516%; samples T12 0.00272% and samples T13 0.00272%, while samples T1, T3, T5, T10, T11, T14 and T15 did not contain hydroquinone as evidenced by the colour test using Benedict's reagent. Based on BPOM NO 23 of 2019,

ISSN 3031-0911

hydroquinone levels are not allowed for cosmetics, and the research showed that the whitening toner sample did not meet BPOM requirements because it contained more than 0% hydroquinone.

Conclusions

The result of the 15 samples studied, 8 samples were positive hydroquinone, so it can be concluded that these samples do not meet the requirements of BPOM No. 23 of 2019

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Formulation and evaluation test of lip balm from ethanol extract of fruit (*Salacca zalacca (Gaertener)* Voss.)

Syarifah Purbaningrum[1*], Maya Uzia Beandrade[2], Melania Perwitasari[3], and Reza Anindita[4]
[1,2,3,4] Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga Bekasi, Indonesia

*Correspondence author : maya.uzia@stikesmitrakeluarga.ac.id

Abstract

Lip balm is a product to temporarily prevents dryness of the lips and helps to treat chapped lips. The content of salak fruit, namely flavonoids and phenols, has benefits as an antioxidant. The difference in concentration is found in the ethanol extract of salak fruit, namely 1%, 3% and 5%. This type of research is quantitative with a pre-experimental design. The phytochemical screening test was carried out using reagents of Mg and HCl (for flavonoids) and FeCl3 (for phenol). Physical stability tests were carried out by organoleptic, homogeneity, and pH tests and characteristic tests, namely the melting point of 15 days of storage. Data analysis was carried out using a descriptive approach**.** The results of the phytochemical screening were positive for the presence of flavonoids and phenols as antioxidant compounds. The results of the melting point test at 1% F1, 3% F2, and 5% F3 showed a melting point of 51°C. Organoleptic test F1 1%, F2 3%, and F3 5% showed white results, had no aroma, and had a semi-solid form, pH test F1 1%, F2 3%, and F3 5% was 6, where all formulations were stated homogeneous. It can be concluded that lip balm preparations with concentrations of 1% F1, 3% F2, and 5% F3, which were stored for 15 days, were declared stable.

Keywords: Ethanol extract of salak fruit; Lip Balm; Physical Stability Test

Introduction

Currently, cosmetics play an important role in lifestyle. Makeup products used on the lips, especially by women, are used to add attractiveness and femininity, but men can use some products; usually, they are colorless products and are used to moisturize and protect from the sun (Baki *et al.*, 2019). One cause of lip problems is the use of K-10 and Sudan IV dyes. Lip balms are known to trigger allergic reactions or irritation of the lips in sensitive individuals (Prakash *et al.*, 2020). Chapped, dry, or peeling lips are a very common beauty dilemma due to direct contact with the chemicals contained in lip balms or lipsticks (caused by dyes and fragrances).

Problems with the lips can be prevented by using products that contain organic ingredients such as honey and vitamin E, which can help keep the lips hydrated and healthy (Vinodkumar *et al*., 2019). Lip problems can also be prevented by using lip balm products that contain extracts of natural ingredients, one of which is salak fruit. Natural ingredients can be formulated in the form of lip balm.

Salacca zalacca fruit contains active compounds such as flavonoids and polyphenols and has high antioxidant activity (Tilaar *et al*., 2017; Bunghez *et al*., 2016). In a study by Tilaar *et al*. (2017), 5% ethanol extract of salak fruit had an antioxidant potential of 99.5%. The study by Bunghez *et al*. (2016) informed that salak fruit has an antioxidant activity of 82.67%. Susiloningsih *et al.* (2016) reported that carrots have an antioxidant activity of 8.35%. Tambunan *et al.* (2018) reported that tomatoes have an antioxidant activity of 44.72%.

Based on these results, it is known that salak fruit has the highest antioxidant content among the others. Because of the problems, consequences, and previous research, the authors are interested in researching the physical stability of lip balm preparations from the ethanol extract of salak fruit as the active ingredient in the formulation because it has a higher antioxidant activity than tomatoes and carrots. The novelty of this research is the use of an ethanol extract of salak fruit at concentrations of 1%, 3%, and 5%. This study is expected to provide information regarding the physical stability of lip balm preparations with the formulation of ethanol extract of salak fruit, glycerin, cera alba, propyl paraben, butyl hydroxytoluene, and Vaseline album for 15 days.

Methods

This study used experimental design with descriptive analysis. This research was conducted at the Pharmaceutical Technology Laboratory, and the phytochemical screening test for flavonoids and phenols was carried out at the Phytochemical Laboratory of STIKes Mitra Keluarga Bekasi from February to March 2023. The population and

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sample in this study were fruit *Salacca zalacca* (*gaertener*) Voss.), which were obtained from a supplier of extracts from Palapa Muda Perkasa, Depok. The independent variable in this study was independent variableThe independent variables in this study were organoleptic, pH, homogeneity, melting point, and phytochemical screening.

Plant determination was performed at the Bogorinse Herbarium, BRIN Cibinong. The salak fruits were extracted using the maceration method. Salak fruit simplicia powder (3 kg) was macerated by soaking in 5 L 96% ethanol. The powder was soaked for three days at room temperature (25-30°C). The extract was then filtered. The filtrate obtained from the maceration process was then concentrated using a rotary evaporator at 40°C to remove unwanted solvents to obtain a 137 g thick extract of Salak fruit. The flavonoid test was carried out by putting 0.5g of the extract into a test tube, adding 5 mL of 96% ethanol, then heating it in the test tube for 2 minutes, adding concentrated hydrochloric acid (HCl), then adding magnesium (Mg) powder. A positive result in the flavonoid test produces a red color. Quercetin was used as a positive control, and the extract with the addition of 96% ethanol was used as a negative control.

The phenol test was performed by placing the extract (0.5 g) into a test tube and adding 1% iron (III) chloride ($FeCl_3$). A positive result from the phenol test produces a dark green, blue, or black color. Quercetin was used as a positive control, and the extract with the addition of 96% ethanol was used as a negative control. The preparation was carried out by weighing the base vaseline album, and cera alba melted at a temperature of 62–65^{0}C (mixture A), propylparaben, BHT, and glycerin mixed (mixture B) were added to the melted base while stirring continuously, then added the ethanol extract of salak fruit. g while stirring. The preparation mixture was placed in a lip balm container and left at room temperature until it solidified (Handayani *et al.*, 2021). The lip balm ethanol extract of the salak fruit formula is shown in **Table 1.**

Table 1. Lip Balm Formula

Material Composition	Formula 1	Formula 2	Formula 3	Function
Ethanol extract of Salak fruit	1%	3%	5%	Active ingredient
Glicerin	8%	8%	8%	Emollient
Cera Alba	15%	15%	15%	Stiffening agent
Propyl Paraben	0,2%	0,2%	0,2%	Preservative
BHT	0,02%	0,02%	0,02%	Prevent rancidity in fats and oils
Vaseline Album	Ad 5g	Ad 5g	Ad 5g	Base *lip balm*

The Melting Point Test is carried out by melting the lip balm in the oven with an initial temperature of 50^{0}C for 15 minutes and then observing it. If it does not melt, the oven temperature can be increased every 15 min by 1^{0}C and the temperature at which the lip balm melts. Evaluation of Lip Balm Ethanol Extract of Salak Fruit: Organoleptic tests were carried out by observing lip balm preparations using senses. Organoleptic testing includes observation of the color, smell, and dosage form of the lip balm. Organoleptic tests were performed by examining the color, smell, and form of F1, F2, and F3 of the lip balm preparation (Ambari *et al.*, 2020). The final pH of the lip balm was measured using a universal pH indicator. A pH test was performed to avoid the risk of irritation to the skin of the lips when the lip balm was used. This test was carried out by applying lip balm preparations F1 1%, F2 3%, and F3 5% to a universal pH indicator. The pH of lip balm preparations is acidic or alkaline (Ambari *et al.*, 2020). A homogeneity test was performed by observing the presence or absence of coarse grains during the preparation. This test is carried out by taking an adequate amount of lip balm, smearing it on the watch glass, and then touching and rubbing the lip balm mass to show the absence of coarse particles (Amalia, 2021).

Results and Discussions

Determination of plants in this study was carried out at the Herbarium Bogoriense, Botany Field, Research Center for Biology BRIN, Cibinong. The results of the determination confirmed that the sample of the type of salak fruit used was *Salacca zalacca (Gaertener) Voss*, with the Arecaceae tribe. Based on the results of phytochemical screening, it can be seen that the flavonoid test, and phenol test showed positive results.

The melting point test is a characteristic of the physical properties of lip balm preparations F1 (1%), F2 (3%), and F3 (5%) carried out using an oven for 15 days of storage at a temperature range of 50°C to 70^{0}C. The melting point test

ISSN 3031-0911

was carried out to determine the temperature range where all the ingredients in the lip balm preparations at F1 (1%), F2 (3%), and F3 (5%) actually melted. The results of the lip balm melting point test in this study can be seen in table 2.

Table 2. Melting Point Test

Day	F1 (1%)	F2 (3%)	F3 (5%)
1	51°C± 0,00	51°C± 0,00	51°C± 0,00
3	51°C± 0,00	51°C± 0,00	51°C± 0,00
6	51°C± 0,00	51°C± 0,00	51°C± 0,00
9	51°C± 0,00	51°C± 0,00	51°C± 0,00
15	51°C± 0,00	51°C± 0,00	51°C± 0,00

Examination of lip balm organoleptic tests on F1 (1%), F2 (3%), and F3 (5%). Organoleptic testing was carried out for 15 days and observed changes in color, odor and shape of the lip balm preparations. The results of the organoleptic test can be seen in **table 3.**

Table 3. Organoleptic Test

Formula	Hasil Uji Organoleptik	
	Hari ke-1	Hari ke-15
F1 (1%)	Color : White Odor : Odorless Form : Semi-Solid	Color : White Odor : Odorless Form : Semi-Solid
F2 (3%)	Color : White Odor : Odorless Form : Semi-Solid	Color : White Odor : Odorless Form : Semi-Solid
F3 (5%)	Color : White Odor : Odorless Form : Semi-Solid	Color : White Odor : Odorless Form : Semi-Solid

The pH test was carried out to determine the pH value of the lip balm at F1 (1%), F2 (3%), and F3 (5%) whether it is acidic, basic or neutral. pH stability test on F1 (1%), F2 (3%), and F3 (5%) was carried out for 15 days of storage. The results of the pH test in this study can be seen in **table 4.**

Table 4. pH Test

Day	F1 (1%)	F2 (3%)	F3 (5%)
1	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00
3	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00
6	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00
9	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00
15	6,00 ± 0,00	6,00 ± 0,00	6,00 ± 0,00

Research on the formulation and evaluation of lip balms from the ethanol extract of salak fruit (*Salaccazalacca* (Gaertener) Voss.) was carried out in several stages, namely the preparation of samples of salak fruit, extraction of salak fruit, phytochemical screening of the ethanol extract of salak fruit, and formulation and evaluation of lip balm from the ethanol extract of salak fruit. The evaluations included organoleptic, pH, homogeneity, and melting point tests.

Salak fruit extraction was performed using the maceration method with 96% ethanol as the solvent. This study used the maceration method because the compounds taken, namely phenols and flavonoids, which are polyphenolic compounds, are easily damaged when heated. The maceration method was selected to prevent damage to the compound due to heating. The solvent used was 96% ethanol, which was chosen based on safety considerations and the ease of evaporation. Ethanol (96%) can dissolve polar, semi-polar, and non-polar substances optimally to extract flavonoids and phenolic compounds (Ramadhani *et al*., 2020).

The yield is the ratio between the final weight of the ethanol extract of the salak fruit and the weight of the simplicial powder before extraction. In 3000 g of simplicia powder of salak fruit, 137 g of brown viscous extract was obtained, with a distinctive aroma and an extract yield of 7.22%. An extract yield of less than 10% w/w was considered a low yield. According to the Indonesian Ministry of Health (2008), extracts with an optimal yield above 10% w/w. The less-than-optimal extract is probably caused by factors such as the type of solvent, extraction time, ratio of materials and solvents, and particle size, which affect the extraction process (Chairunnisa *et al*., 2019). The higher the yield of the extract, the higher the content of the substance of interest in the sample (Senduk *et al*., 2020).

This phytochemical screening test was performed on viscous extracts to identify the presence of secondary metabolites using color reagents. This examination was performed to determine the contents of the extract preparations (Ramadhani *et al*., 2020). The flavonoid test was carried out using quercetin as a positive control and ethanol extract-

ISSN 3031-0911

salak fruit as a negative control. Testing for flavonoids on ethanol extract-salak fruit as a negative control was carried out by putting 0.5 g of the extract into a test tube, adding 96% ethanol, and then heating it for 5 minutes. Subsequently, ten drops of concentrated HCl were added, after which 0.2 g of Mg powder was added (Pangow *et al*., 2018). The addition of Mg powder and concentrated HCl causes a reduction process in flavonoid compounds to produce Mg-flavonoid complexes that are red or orange (Pratiwi *et al*., 2021). The phenol test was carried out using quercetin as a positive control and ethanol extract-salak fruit as a negative control. The phenol test for ethanol extract-salak fruit as a negative control was carried out by putting 0.5 g of the extract into a test tube and adding $FeCl_3$ (Putri *et al.*, 2019). The addition of FeCl31% produces a polyphenol complex compound with a central atom, Feber, which is blackish-green (Iskandar, 2020).

The purpose of the melting point test is to identify the melting point, which is defined as the temperature range in which all materials melt. Based on the results of testing the melting point of Formula 1 (F1) 1%, Formula 2 (F2) 3%, and Formula 3 (F3) 5%, the results obtained have an average melting point of 51°C. According to Amalia (2021), due to the use of the same amount of additives between formulas 1, 2, and 3 so that the melting point of the lip balm preparations does not differ, the melting point test results obtained for each formula are the same. The preparations made have a good melting point, and each formula meets the standards by the requirements of SNI 16-4769-1998.

An *organoleptic test* is a procedure that utilizes the five human senses to assess texture, color, and smell (Tanone & Prasetya, 2019). In Formula 1, which uses 1% extract, lip balm preparations are obtained in a semisolid form, white in color, and odorless. Formula 2, which uses 3% extract, is obtained as a lip balm preparation in semisolid form, white in color and odorless. In Formula 3, which uses 5% extract, lip balm preparations are obtained in a semisolid form, white in color, and odorless. Based on the results obtained, the lip balm preparations did not change in color, smell, or shape, both before and after storage for 15 days. According to Amalia (2021), these results indicate that the formula used is stable because it does not cause interactions between the active substance and other ingredients.

A pH test was performed to determine the safety of the lip balm preparation. Based on the physiological pH of the skin the recommended pH for cosmetic products ranges from 4.5 to 6.5 according to the physiological pH of the skin (Permatananda, 2021). Based on the results of testing the pH values obtained in Formula 1 (F1) 1%, Formula 2 (F2) 3%, and Formula 3 (F3) 5%, the average pH value obtained was 6. According to Ambari *et al.* (2020), lip balm preparations are said to be good if they have a pH in the pH range of the lips, namely 4.5-6.5. Results from an average pH range of 6 are known to meet the requirements, namely, entering the range of 4.5-6.5. Cosmetic products with a pH range between 4 and 6.5 are considered a normal pH for the skin, including lips (Azmin *et al.*, 2020). These results indicated that the results of the pH test fulfilled the requirements, and the preparations made were safe and did not irritate the lips. Acidic or alkaline pH can irritate the lips, so the pH of the formulation is kept as close as possible to a neutral pH (Waykule *et al*., 2022).

The acquisition of the same pH value was six because the pH meter used was a universal pH indicator paper. This was done because of the limited ability of the pH meter to measure the pH of lip balm preparations, because the lip balm preparations had a semisolid form, making it difficult to measure the pH of lip balm preparations, and the limited availability of pH meter preparations. In a study conducted by Amalia (2021), the pH of lip balm preparations from strawberry extract was measured using a universal pH indicator paper obtained at pH 5 in Formulas 1, 2, and 3. In a study conducted by Shaikh (2022), pH measurements of lip preparations balm from beetroot extract using a pH meter (model HI-2211-01) obtained an average pH of 5.6.

The homogeneity test aims to determine whether the ingredients are mixed when creating a lip balm. This also determines whether the lip balm mixture is homogeneous or contains coarse grains or not. Based on the homogeneity test that was carried out to prove that all preparations of salak extract lip balm do not show any coarse particles when applied to the watch glass, this proves that the salak extract lip balm has a homogeneous composition of components. These results indicate that the homogeneity obtained has met the requirements of homogeneity, namely homogeneous or no coarse grains, according to the requirements of SNI 16-4769-1998.

Conclusions

The ethanol extract of salak fruit (*Salacca zalacca* (*Gaertener*) Voss.) can be formulated into lip balm preparations with concentrations of 1% F1, 3% F2, and 5% F3 by obtaining good physical stability results in all formulas. The results of physical stability showed that in the organoleptic test for 15 days, there was no change in color, smell and shape, the pH value was stable with an average pH value of 6, and all preparations had good homogeneity,

ISSN 3031-0911

namely, there were no coarse grains. Thus, the conclusion of this study was that lip balm preparations with concentrations of 1% F1, 3% F2, and 5% stored for 15 days were declared stable. Suggestions in this study are an antioxidant testing is also needed to determine the activity of antioxidant compounds in lip balm preparations with a concentration of 1% F1, 3% F2, and 5% F3, and future researchers are expected to test the physical stability of lip balm preparations for one to three months.

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Physical Evaluation Test Of Serum Facial Ethanol Extracts Of Purple Passion Fruit Peel (*Passiflora edulis* Sims)

Unggul Fernando Sinambela[1*], Maya Uzia Beandrade[2], Reza Anindita[3,] Intan Kurnia Putri[4]
[1,2,3,4] Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga Bekasi, Indonesia
*Correspondence author : unggulfernando@gmail.com

Abstract

A *facial serum* is a slightly viscous liquid that contains active ingredients to treat skin problems. Purple passion fruit peel extract is proven to contain flavonoids and tannins, which have antibacterial activity that causes acne. Hence, it is necessary to test the formula (F) of facial serum with the composition of ethanol extract of purple passion fruit peel, niacinamide, carboxymethylcellulose sodium, and distilled water. The concentration differences in the percentage of ethanolic extract of purple passion fruit peel were 4%, 5%, and 6%. This type of research is quantitative with a pre-experimental design. A phytochemical screening test was carried out on the presence or absence of flavonoids and tannins. Physical stability tests were carried out by organoleptic, homogeneity, pH, and viscosity tests for 30 days of storage. Data analysis was performed with a descriptive test. The phytochemical screening test on the flavonoid test was positive, and the tannins were positive. The results of the physical stability test only yielded stability until the 5th day, when the organoleptic test did not change color and smell; F1 was pale yellow, F2 was yellow, and F3 was dark yellow and had a distinctive aroma of the extract. pH test results at F1 4% (6.22-6.28), at F2 5% (5.93-6.28), and at F3 6% (5.41-5.82). In the viscosity test, F1 4% (1.833-5.667 cP), F2 5% (3.333-8.000 cP), and F3 6% (4.167-12.000 cP). The homogeneity test for the three formulas showed homogeneous results. In this study, the best formula cannot be achieved because, during stability testing for 5 days of storage, the viscosity decreased with a percentage of 67% F1, 58% F2, and 65% F3. It can be found that the facial serum preparation became liquid and unstable.

Keywords: Facial serum: purple passion fruit peel extract: Physical Stability Test: Passiflora edulis sims

Introduction

Acne vulgaris or acne is an inflammation characterized by blackheads, pustules, and papules, which usually appear on the neck, face, chest, and upper arms or back (Duru & Örsal, 2021). Acne vulgaris can be caused by the bacteria *Propionibacterium acnes*, *Corynebacterium acnes*, *Pityrosporum ovale*, and *Staphylococcus aureus*. These bacteria are bacteria that can cause inflammation, blockages, and the occurrence of acne vulgaris on the skin. Acne vulgaris can have a significant impact, both physically and psychologically, because it can cause a lack of self-confidence, anxiety, and depression for sufferers (Tyasari *et al*., 2022). So far, efforts to overcome the problem of acne vulgaris caused by *P.acnes* are by administering synthetic antibiotics such as doxycycline, tetracycline, and clindamycin. Synthetic antibiotics are given because antibiotics can kill or inhibit *P. acnes* bacteria. However, prolonged use of synthetic antibiotics can cause quite severe side effects, such as damaged organs, irritation, and resistance (Harefa *et al*., 2022).

Referring to the impact and problems caused by acne vulgaris, researchers are trying to prevent acne vulgaris, namely by making product innovations using natural raw materials that have potential as antibacterials. Natural ingredients may be developed as antibacterial among others by using the skin of purple passion fruit (*Passiflora edulis*). The antibacterial potential of purple passion fruit peel is due to secondary metabolites such as flavonoids and tannins (Anabel *et al*., 2020). The study of antibacterial activity test against *Propionibacterium acnes* bacteria conducted by Harefa *et al.* (2022) stated that the use of purple passion fruit peel extract extracted using 70% ethanol solvent with variations extract concentrations of 5%, 10%, and 15% obtained an average inhibition zone in each extract of 14.9; 15.3; 17.2mm. Based on research, Nugraha *et al.* (2019) investigated the antibacterial activity test of 96% ethanol extract of purple passion fruit peel against Staphylococcus aureus bacteria with extract concentrations of 5%, 7.5%, and 10%. The results obtained inhibition zone diameters of 11.43, 12.37, and 14.2 mm. Based on this background, researchers are interested in making a facial serum formulation using the ethanol extract of purple passion fruit peel as the active substance with concentrations of F1 4%, F2 5%, and F3 6% and knowing the physical characteristics of each formula.

Methods

This study used a pre-experimental design with a descriptive analysis method. This research was conducted at the Pharmaceutical Technology Laboratory, and the phytochemical screening test for flavonoids and phenols was carried out at the Phytochemical Laboratory.of STIKes Mitra Keluarga Bekasi in February-March 2023. The population and sample in this study are purple passion fruit peel (*Passiflora edulis* Sism), which was from a supplier of extracts from

ISSN 3031-0911

Palapa Muda Perkasa, Depok. The variable in this study was the independent variable. The independent variables in this study were organoleptic, pH, homogeneity, viscosity, and phytochemical screening.

Plant determination was performed at Bogorinse Herbarium, BRIN Cibinong. Purple passion fruit peel was extracted using the maceration method. 1531 g of purple passion fruit peel 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 was then concentrated with a rotary evaporator at a temperature of 40°C to remove unwanted solvents to obtain 125.3 g of thick purple passion fruit peel extract. The flavonoid test was carried out by putting ethanol extract of purple passion fruit peel, then putting it in a test tube, adding sufficient pieces of Mg band, and adding a few drops of concentrated HCl solution. Positive test results from the flavonoid test will produce red, yellow, or orange. The Tanin test was carried out by putting 5 ml of ethanol extract of purple passion fruit peel into a test tube and then adding a few drops of $FeCl_3$ solution. The result will show a black-green color if the sample is positive tannin.

The preparation was carried out by taking 100mL of aquadest and putting it in a beaker, then heating it using a hot plate until the temperature reaches 90, then putting 2 g of CMC-Na into the beaker glass, adding the aquadest little by little stir using a mixer until a serum base is formed (mixture 1), then dissolve 2 g of niacinamide with 10 mL of aquadest separately (mixture 2), after that slowly add mixture 2 into mixture 1 little by little, finally add 4, 5, and 6 grams of purple passion fruit peel ethanol extract into a beaker glass, stir until evenly distributed then add aquadest ad 100 ml (Ariyanti *et al*., 2020). The formulation of Fase Serum can be seen in table 1.

Table 1. Face Serum Formulation

Material	Face Serum Formula (%)			Function
	F1	F2	F3	
Purple Passion Fruit Pell Extract	4	5	6	Active ingridient
Ni Niacinamide	2	2	2	Active Substance
CMC-NA	2	2	2	Gelling Agent
Aquadest Ad (mL)	100	100	100	Solvent

Facial Serum evaluation tests include organoleptic tests, pH tests, viscosity tests, and homogeneity tests. The organoleptic test was carried out by observing the color and aroma of the face serum, which was stored at room temperature (25±2°C) for 30 days. The pH test is done by dipping the calibrated pH meter into the face serum mixture. After that, read the pH value present in the pH meter indicator. The purpose of the pH test is to see the acidity level of facial serum so it can guarantee the safety of the product when applied to the skin. Homogeneity tests are done by dripping the facial serum onto the surface of a glass, flattening it, and then feeling if there are still rough particles. A homogeneous facial serum is when there are no more rough particles inside the mixture. Viscosity tests are performed by dipping most parts of the spindle connected to the viscometer. The purpose of this test is to look at the viscosity level of the facial serum so it can determine whether the facial serum viscosity goes into a good viscosity range.

Results and Discussions

The results of the research on the formulation of face serum purple passion fruit peel extract consist of determination, phytochemical screening, and physical stability test. The result of physical stability tests includes organoleptic, pH, viscosity, and homogeneity tests. Determination of plants in this study was carried out at the Herbarium Bogoriense, Botany Field, Research Center for Biology BRIN, Cibinong. The results of the determination confirmed that the sample of the type of Passiflora edulis Sims in the family of Passifloraceae. Based on the results of phytochemical screening, it can be seen that the flavonoid test and tannin test showed positive results. There is no best formula because the face serum is only stable for 5 days within the requirements of 30 days of storage. The organoleptic test result can be seen in **Table 2.**

Table 2. Face Serum Organoleptic Results

Formula	Color	Aroma
F1	Pale Yellow	Aromatic Extract
F2	Yellow	Aromatic Extract
F3	Dark Yellow	Aromatic Extract

ISSN 3031-0911

The viscosity test is carried out to see whether the viscosity level of a facial serum preparation has entered the required range, namely 3,000-50,000 cP. The viscosity test on F1 4%, F2 5%, and F3 6% showed instability on the fifth day. The results of the facial serum viscosity test can be seen in **Table 3.**

Table 3. Viscosity tests

Day	Viskosity (Cp)		
	F1	F2	F3
	Average ± SD	Average ± SD	Average ± SD
0	5.667 ± 289	8.000 ± 500	12.000 ± 500
2	3.333 ±289	4.833 ± 764	5.833 ± 289
5	1.833 ± 289	3.333 ± 289	4.167 ± 289

All formulas enter the pH range of the skin. The results of the purple passion fruit peel facial serum pH test are shown in **Table 4.**

Table 4. pH tests

Day	pH		
	F1	F2	F3
	Average ± SD	Average ± SD	Average ± SD
0	5.667 ± 289	8.000 ± 500	12.000 ± 500
2	3.333 ±289	4.833 ± 764	5.833 ± 289
5	1.833 ± 289	3.333 ± 289	4.167 ± 289

The results of homogeneity showed that all formulas are homogeneous. The result of our facial serum homogeneity test can be seen in **Table 5.**

Table 5. Homogeneity tests

	Day-1	**Day-15**
F1	Homogeneous (no rough particles)	Homogeneous (no rough particles)
F2	Homogeneous (no rough particles)	Homogeneous (no rough particles)
F3	Homogeneous (no rough particles)	Homogeneous (no rough particles)

Formulation research and evaluation of purple passion fruit peel (*Passiflora edulis* Sims) ethanol extract facial serum with variations in concentrations of purple passion fruit peel ethanol extract aims to obtain the best formula in terms of physical stability of the formulated facial serum. This research was carried out in several stages, which are purple passion fruit peel preparation, purple passion fruit extraction, phytochemical screening of purple passion fruit peel extract, formulation, and evaluation of facial serum. The evaluations are organoleptic test, pH test, Viscosity test, and homogeneity test. Extraction of purple passion fruit peel was carried out with maceration method with 96% ethanol as a solvent. The maceration method was chosen because of the blending method; the tools used are simple, and this method is an extraction located suitable for the active substance contained in strawberry fruit that is not resistant to high heat (Handoyo, 2020). The 96% ethanol solvent is chosen because it has the same polarity as the active substance contained in purple passion fruit peel.

Phytochemical screening is the initial stage to provide an overview of the compounds contained in the plant. In this study, phytochemical screening was carried out on the active compounds contained in purple passion fruit peel, which are flavonoids and tannin. The flavonoid test was performed by adding concentrated Magnesium (Mg) and HCl bands to the reaction tubes already available. The addition of concentrated HCl and Magnesium (Mg) bands aims to reduce the benzopyran core present in the structure of flavonoids so that flavilium salts form and undergo discoloration (Dewi *et al*., 2021). The positive result of flavonoids is the formation of yellow, red, or orange. In this test, the color of the sample changed from pale yellow to concentrated yellow, indicating that the sample of purple passion fruit skin ethanol extract contained flavonoid compounds. Tannin testing is performed by adding a 10% FeCl solution. In accordance with previous studies, the positive results of the tannin test will be marked with blackish green (Wahid & Safwan, 2020). The working mechanism of the tannin test reaction using a 10% FeCl3 reagent is that when a 10% FeCl3 is added, the sample containing tannin forms a complex Fe3+-tannin with a coordination bond, resulting in a blackish-green change as a sign of the phenol group contained in the sample (Yanti & Vera, 2019).

The results of the obtained viscosity measurements are known that in each formulation, it decreases with storage for 5 days at room temperature. The decrease in viscosity as storage time progresses can also be due to mixing factors using mixers with too large rpm since CMC-Na is a gelling agent with pseudoplastic flow properties. This pseudoplastic flow property has a property that, as the shear rate increases, decreases the viscosity of the gel (Ramadhani *et al*., 2022). In addition, additional factors, such as suspending agents, have a considerable influence on viscosity. Suspending agents such as PGA are used because they can increase the stability of the preparation by increasing the viscosity of a

ISSN 3031-0911

preparation. Research conducted by Wijaya and Lina (2021) proved that using PGA as a suspending agent results in good quality properties and viscosity values after 4 weeks of storage despite a decrease but not very significant and still falls under the requirements.

Another factor that can cause a decrease in viscosity is the storage factor. In this study, the preparation of facial serum was not kept in an airtight container and thus affected the viscosity of the facial serum. Facial serum stored in airtight containers will reduce the inflow of air from the outside, which can lead to increased gel moisture in the facial serum (Rinaldi *et al.*, 2021). Organoleptic test results of facial serum, F1 produced pale yellow facial serum with aromas of extract. F2 produced yellow with aromas of extract. Furthermore, F3 produced dark yellow facial serum with aromas of extract. The difference in color in F3 6% is darker in yellow compared to F1 4% and F2 4%. The difference in pale yellow to dark yellow color due to the increasing number of extracts used will result in a darker color serum (Iskandar *et al*., 2021). This is in line with the research done by Raharjeng *et al.* (2021), where the study used a variation in extract concentration of 5%, 10%, and 15%, producing a more concentrated color if the extract concentration was more excellent.

pH results of facial serum showed that pH values remained in the range of 4.5–6.5 after 5 days of storage. Based on the results of the tests, the preparation of the facial serum at the time of the stability test at room temperature showed an increase and decrease in pH during 5 days of storage. The increase and decrease in facial serum pH is attributed to environmental factors, such as poor storage and unstable room temperature, that can change the intake condition to slightly acidic but still fall within the skin pH requirements (Ariyanti *et al*., 2020). Another factor that can cause a decrease in the pH of each formulation is the release of hydrogen ions or ion contamination in a 5-day stored serum (Raharjeng *et al*., 2021).

Homogeneity results Facial serum homogeneity testing is carried out to determine whether or not the active ingredients and excipients are evenly mixed so that each part of the preparation contains the same amount of active substances and excipients. Based on the results of a 5-day homogeneity test at room temperature indicating that all three formulations did not show any rough grains when applied to the object glass, this indicates that the facial serum preparation of purple marquis fruit skin extract had homogeneous results. The results have met the homogeneity requirements, namely that the gel preparation does not have rough grains or clumps in the preparation following SNI requirement No. 1. 06–2588 (Putri *et al*., 2019).

Conclusions

The facial serum preparation stability test at F1 4%, F2 5%, and F3 6% was conducted for 30 days, but on the 5th day of the test, there was a decrease in the 5th day in F1 4% by 67%, F2 5% by 58% and F3 6% by 65%, where on the 5th day the texture became liquid. Thus the preparation formulation test results in physical instability on the 5th day so that this preparation has no potential to be developed into a product of perfarmacy innovation. The suggestions from this study, the formula F1 4%, F2 5%, and F3 6% had unstable physical stability, therefore it is recommended to reformulate facial serum preparations with the addition of other additional ingredients such as suspending agents so that they can produce good physical stability.

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Relationship of Body Mass Index-For-Age to Body Fat Percentage Using Relative Fat Mass in Teenagers in Surakarta

Nais Volum Firdaus[1]*, Tonang Dwi Ardyanto[2], Isna Qadrijati[3]

[1] Department of Nutrition science, Sebelas Maret University, Surakarta, Indonesia
[2] Department of Medical science, Sebelas Maret University, Surakarta, Indonesia
[3]Department of of Medical science, Sebelas Maret University, Surakarta, Indonesia
*Correspondence author : nais_volum@student.uns.ac.id

Abstract

Adolescence is a period of rapid growth and physical, psychosocial, and cognitive changes, which can influence nutritional needs. Adolescent groups are one of the leading health concerns because of their unhealthy lifestyles compared to other age groups. One of the problems facing young people is related to weight, such as underweight, overweight, and obesity. This study aims to determine the relationship between the body mass index for age (BMI-for-age) and the percentage of body fat using relative fat mass (RFM) in adolescents in Surakarta. The research method used is quantitative research with a cross-sectional design. The total number of respondents was 117, consisting of 40 males and 77 females. BMI-for-age measurements include age, height, and weight in the formula. Body fat percentages are measured using the Relative Fat Mass (RFM), and body height and waist circumference measurements are included in the form. Spearman's rank test results showed a significant relationship between BMI-for-age and body fat percentage ($p<0.05$), with a positive correlation. The conclusion is that there is a relationship between body mass index according to age and percent body fat using relative fat mass in adolescents in Surakarta.

Keywords: BMI-for-age, RFM, Teenagers

Introduction

Adolescents are the most susceptible to nutritional problems, as adolescents experience rapid physical, psychosocial, and cognitive changes and growth compared to the previous age group (Hardinsyah & Supariasa, 2017). Teenagers between the ages of 15 and 19 are more susceptible to obesity (Jebeile *et al.*, 2022) and other nutritional problems due to excessive eating. Obesity can occur when energy consumption exceeds energy use over a long period (Lin & Li, 2021), as shown by the increase in Body Mass Index-for-age (BMI-for-age) of teenagers. The prevalence of obesity between the ages of 16-18 in Surakarta City is the highest in the city or district, with the highest prevalence of obesity rates in Central Java, with 9.98% overweight and 11.47% obese (Riskesdas, 2018).

Obesity that lasts until adulthood can increase the risk of cardiovascular disease (Powell-Wiley *et al.*, 2021). Genetic factors, environmental factors, high fat consumption, and high levels of spontaneous activity in adolescents are the main factors in obesity (Puspitasari *et al.*, 2019). Overweight or obesity prevention interventions have always focused on behavioral modification, such as increasing daily physical activity or improving the quality of a diet by limiting calorie intake (Kansra *et al.*, 2021).

One of the prevention strategies for obesity is the screening of people with measurements of BMI-for-age and body fat percentage (Yao, 2013). Relative Fat Mass (RFM) is a method to calculate the percentage of body fat. Scientists at the U.S. Cedars-Sinai Medical Center in California invented this measurement method. This measurement method puts a person's height and waist circumference into the formula, approximately equivalent to their body fat percentage. RFM can estimate body fat percent and diagnose overweight or obesity in teenagers aged 15 to 19 (Woolcott & Bergman, 2018). Research has shown that using RFM is more practical in measuring fat mass in obese individuals (Woolcott & Seuring, 2022). This study aimed to determine the relationship between the body mass index-for-age (BMI-for-age) and the percentage of body fat using relative fat mass (RFM).

Methods

This research method is descriptive with a cross-sectional study design. This study was conducted in 4 schools in Surakarta City, namely State Senior High School 4 Surakarta, State Senior High School 5 Surakarta, Al-Abidin Bilingual Boarding School Surakarta, and State Vocational High School 9 Surakarta, which was conducted in July 2023, with inclusion criteria for research subjects in grades X-XI, age 15-18 years, attending school and living in the

ISSN 3031-0911

city of Surakarta. Exclusion criteria for adolescents who use appetite suppressants. Determination of the minimum sample size using the calculation formula of Slovin (1960) is:

n = N / (1 + N x e2)
= 41093 / (1 + 41093 x 0.12)
= 41093 / 411.93
= 99.75 =100 respondents

An estimate of a 15% loss to follow-up is:

n = 100 + (100 x 15%)
= 115 respondents

N: Population size / total population in Surakarta aged 15-19 years of 41093 people
n: Number of samples
e: margin of error (error tolerance) 10% or 0.1(Badan *et al.*, 2021).
The Number of respondents for each school using the formula according to Sugiyono (2007) is n = (x / N) N1
n = Number of desired samples per stratum
N = Total population of teenagers in the School
X = Total population in each stratum
N1 = Sample

Based on the formula, the number of respondents from each school is State Senior High School 4 Surakarta = 1134/4876 x 115 = 26.7 = 27 respondents, State Senior High School 5 Surakarta = 1054/4876 x 115 = 24.8 = 25 respondents, Al-Abidin Bilingual Boarding School Surakarta = 1054/4876 x 115 = 26.1 = 27 respondents, State Vocational High School 9 Surakarta = 1581/4876 x 115 = 37.2 = 38 respondents. The total sample size is 117 adolescents.

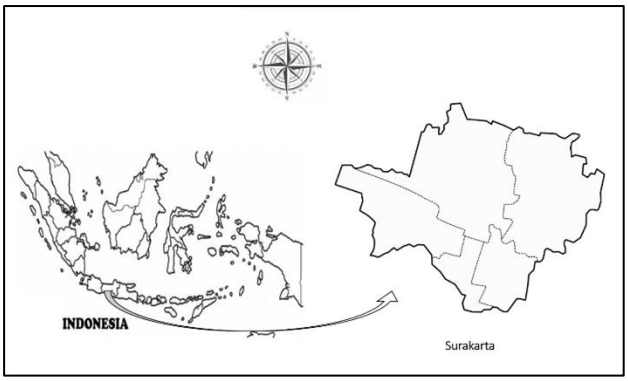

Figure 1. Location of research in Surakarta City at four schools, State Senior High School 4 Surakarta (7°33'22.4"S 110°48'07.7"E), State Senior High School 5 Surakarta (7°33'00.8"S 110°49'50.3"E), Al-Abidin Bilingual Boarding School Surakarta (7°32'16.8"S 110°48'22.7"E), and State Vocational High School 9 Surakarta (7°32'05.0"S 110°48'15.5"E)

This study has received ethical approval from the Health Research Ethics Committee (KEPK) of the Faculty of Medicine, Sebelas Maret University, with the number 95/UN27.06.11/KEP/EC/2023. The data were primary, including body weight using a weight scale, height using a microtome, and waist circumference using a midline to determine BMI/U and RFM values. Statistical tests to determine the relationship between variables used the Person Product Moment test if the data is normally distributed. If the data is not normally distributed, use the *Spearman rank* test.

Results and Discussions

The research results in **Table 1** show that women dominate the characteristics of the gender research subjects compared to men, which is due to the higher number of female students than men. Based on BMI-for-age, most subjects were in the normal category, with 76 (65%) people, while the lowest was fewer than 10 (8.5%). In the percentage category of body fat using RFM, most samples were found at the height of 46 (39.3) people.

Table 1. Characteristics and distribution of samples based on variables studied

Category	n	%
Gender type		

ISSN 3031-0911

Male	40	34.2
Female	77	65.8
BMI-FOR-AGE		
Poor nutrition	10	8.5
Normal nutrition	76	65.0
Overnutrition	20	17.1
Obesity	11	9.4
Body Fat Percentage (RFM)		
Fitness	34	29.1
Average	37	31.6
Obese	46	39.3

Table 2 shows the frequency distribution of body mass index according to age (IMT/U) and per cent body fat using RFM. The respondents' IMT/U had a mean value of 3.27, a median of 3.00, a standard deviation of 0.75, a minimum value of 2.00, and a maximum value of 5.00. The respondents' per cent body fat with RFM had a mean value of 1,39, a median of 1,00, a standard deviation of 0.49, a minimum value of 1.00, and a maximum value of 2.00.

Tabel 2. Frequency Distribution of Body Mass Index Respondents by Age and Percent Body Fat Using Relative Fat Mass

	Mean	Median	Std. Deviation	Minimum	Maximum
IMT/U	3.27	3.00	0.75	2.00	5.00
RFM	1.39	1.00	0.49	1.00	2.00

Table 3 shows the correlation test results between variables using the Spearman rank test. Then, the results obtained for the relationship between BMI-for-age and body fat percentage show, among other things, that there is a significant relationship with the result (p=0,000, r=0.628**) where the value of p<0.05 indicates that the higher a person's BMI-for-age value, the higher the value of the body fat per cent; otherwise, the smaller the BMI-for-age value, the lower the per cent of body fat. This research succeeded in observing the relationship between both.

The results of this study are in line with previous studies, which stated that there was a relationship between body fat percentage and BMI (Akindele *et al.*, 2016). The study conducted by Naulia *et al.* (2023) showed a significant relationship between BMI and body fat percentage (p<0.05) with a positive direction. Another study with the same variable found a correlation between BMI and body fat percentage (p = 0,000) with a sample size of 115 people meeting the productive age criteria (15–64 years), a study conducted in Semarang (Susantini, 2021). Similarly, a study conducted on medical students at Diponegoro University resulted in a positive correlation between BMI and individual body fat percentage, with an average fat content of 72.5% in the sample (Archilona *et al.*, 2016).

To the author, this is the first study to address the relationship between BMI and RFM. The study results show that there is a relationship between BMI/U and RFM. The results of this study are in line with previous studies, which stated that there was a relationship between BMI and the per cent body fat of adult women estimated by BIA (Misra *et al.*, 2019). Other supporting studies show that RFM has high accuracy in estimating body fat percentage (Paek *et al.*, 2019). RFM predicts the proportion of body fat more accurately (Woolcott & Bergman, 2018), so it can easily estimate adiposity in individuals (Corrêa *et al.*, 2021). RFM is a simple-to-calculate, sex-specific obesity indicator more closely approximates whole-body fat percentage than BMI (Suthahar *et al.*, 2023). The reference explains that separately, RFM is more accurate in predicting per cent fat than BMI in obese individuals (Paek *et al.*, 2019).

Obesity is the accumulation of excess fat that can be detrimental to health. Obesity results from an imbalance between energy consumed and energy spent (Jehan *et al.*, 2020). Increased consumption of high-energy foods such as fat and sugar can lead to an increase in body fat percentage (WHO, 2021). The gradual and periodic measurement of anthropometry is one of the methods of preventing obesity. Anthropometric measurement methods include the calculation of age-based body mass index (BMI-for-age), upper arm circumference (LILA), neck circle, waist-to-arm circle comparison (RLPP), and skin fold thickness (SKF), bioelectric impedance analysis (BIA) and double-energy x-ray absorption (DXA) (Supariasa *et al.*, 2016).

The advantage of RFM in measuring body fat percentage is that it is a simple, inexpensive, and non-invasive technique (Corrêa *et al.*, 2021). Thus, its use is more popular. It is known that BMI is correlated with body fat mass

ISSN 3031-0911

(Jeong *et al.*, 2023). One study determined that the body mass index (BMI) correlates strongly with the body fat mass determined by the bioelectrical impedance analysis (BIA) method. Still, the BIA method is superior to the BMI method in determining body mass composition (Wiranata & Inayah, 2020).

A study stated that a person who followed nutrition counselling experienced a decrease in the percentage of overweight women's body fat and obesity among Pilates gym participants (Ranitadewi *et al.*, 2018). Therefore, it is essential to note the importance of keeping the body mass index according to age in adolescents within the normal range. BMI-for-age is closely linked to total body fat levels, so it can trigger the onset of obesity, which may trigger various fatal diseases. Teenagers who are obese or overweight can be prevented by performing routine measurements of BMI-for-age and body fat percentage.

Conclusions

The study was conducted on adolescents in Surakarta City at State Senior High School 4 Surakarta, State Senior High School 5 Surakarta, Al-Abidin Bilingual Boarding School Surakarta, and State Vocational High School 9 Surakarta with a total sample of 117 people where the majority of the sample was female with a percentage of 65.8%. Based on the results of the Rank Spearman test conducted, the data showed a significant solid relationship with a positive direction between body mass index according to age (IMT/U) and per cent body fat using Relative Fat Mass (RFM) (p=0.000). The results showed that the higher a person's body mass index, the higher the body fat percentage. Therefore, excessive fat accumulation can lead to obesity and hurt health. The results of this study are expected to be a scientific reference in school nutrition programs to determine the nutritional status and percentage of body fat in students using IMT/U and RFM, which are conducted periodically.

Acknowledgments

Thank you to all the subjects who participated in this study: State Senior High School 4 Surakarta, State Senior High School 5 Surakarta, Al-Abidin Bilingual Boarding School Surakarta, and State Vocational High School 9 Surakarta, and all those who have been motivated and supported so that this research can be completed.

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Quantitative Test Of Quercetin Content Of Rome Beauty Apple Flour Extract Using The High-Performance Liquid Chromatography Method

Dwi Wulandari Aaas Hamka,[1] Dhani Redhono Harioputro,[2] Ratih Puspita Febrinasari[3]

[1] Department of Nutrition Science, Universitas Sebelas Maret, Surakarta City, Central Indonesia
[2]Department of Internal Medicine, Universitas Sebelas Maret, Surakarta City, Central Indonesia
[3]Department of Pharmacology, Universitas Sebelas Maret, Surakarta City, Central Indonesia

*Correspondence author : dwiwulandari60@gmail.com

Abstract

Introduction: Apple is a fruit containing antioxidant phytochemicals, one of which is quercetin, a flavonoid compound. Apples are one of the natural ingredients that have a beneficial effect on health. Rome beauty apples (*Malus sylvestris Mill.*) are natural antioxidants that are safer for consumption compared to synthetic antioxidants, which have the potential to cause harm. Oxidative stress occurs due to an imbalance in the amount of antioxidants in the body, causing cell damage. High-Performance Liquid Chromatography (HPLC) was conducted to determine the antioxidant content, especially quercetin, in Rome Beauty apple extract.
Objective: to find out how to quantitatively test the quercetin flavonoids from Rome Beauty apple extract (Malus sylvestris Mill.) using HPLC to determine the levels of quercetin flavonoids in Rome Beauty apple extract (*Malus sylvestris Mill.*).
Method: The method of sample preparation was carried out using a maceration extraction technique with 70% ethanol solvent. Determination of the quercetin content in Rome Beauty Apple Extract was carried out using the High-Performance Liquid Chromatography (HPLC) method and a UV spectrophotometer detector.
Results: The results of the quantitative tests that have been carried out conclude that Rome Beauty apple extract (*Malus sylvestris Mill.*) has a quercetin content of 557.999 ppm and a quercetin content of 0.557%.
Conclusion: Rome Beauty apples (*Malus sylvestris Mill.*) positively contain the flavonoid compound quercetin

Keywords: *Rome Beauty Apple, HPLC, Quercetin*

Introduction

Fruit is a natural ingredient source containing various antioxidant compounds (Jideani *et al.*, 2021). Natural antioxidants are generally safer to consume than synthetic ones, which have potentially detrimental effects such as pro-oxidative properties (Liu, 2022). Apple is a fruit with abundant antioxidants (Asma *et al.*, 2023). Apples have beneficial effects against oxidative stress and related diseases (Oyenihi *et al.*, 2022).

Oxidative stress is a condition where there is an imbalance between free radicals and the amount of antioxidants in the body, causing cell damage (Oyenihi *et al.*, 2022). Oxidative stress can cause cancer, diabetes mellitus, neurodegenerative disease, cardiovascular disease, rheumatoid arthritis, kidney disease, and eye disease (Pisoschi *et al.*, 2021). Malang is the largest producer of apples in Indonesia, which have the potential to be a source of natural antioxidants. *Apples* are plants that originate in West Asia. This plant lives in areas with subtropical climates with cold temperatures and air conditions. Batu City is an expansion city of Malang Regency that produces many varieties of apples, one of which is the Rome Beauty apple, which has the potential to have properties as a natural antioxidant (Dohitra *et al.*, 2015). *Rome Beauty apples* are a natural ingredient that has beneficial effects on health. Rome Beauty apples contain catechin, epicatechin, phloridzin, allergic acid, chlorogenic acid, and quercetin, which act as antioxidants. One of the flavonoid compounds in quercetin, which has the potential as an antioxidant, has a working mechanism by reducing free radicals (Rusita *et al.*, 2019; Zaddana *et al.*, 2020). Quercetin is a flavonoid reported to exhibit various biological activities and is used for medical applications (Tronins *et al.*, 2023).

The potential pharmacological activity of flavonoids, especially quercetin compounds, is the basis for using High-Performance Liquid Chromatography (HPLC) to determine the quercetin content of Rome Beauty apple extract, as in previous research (Mizzi *et al.*, 2020). The purpose of this study was to find out how to quantitatively test the flavonoid quercetin from Rome Beauty apple extract (*Malus sylvestris Mill.*) using HPLC to determine the levels of the flavonoid quercetin found in Rome Beauty apple extract (*Malus sylvestris Mill.*).

ISSN 3031-0911

Methods

Design and Material

This research is a descriptive observational study. This study focuses on the quercetin content of Rome Beauty apples (*Malus sylvestris Mill*.). This research was carried out in different laboratories; namely, the manufacture of Rome Beauty apple Simplicia was carried out at the UPT of the Materia Medika laboratory in Batu City Malang, and the manufacture of apple extract and analysis of the quercetin content was carried out in the Food Engineering laboratory of Soegijapranata Catholic University Semarang.

Simplicity-making Process

The study began with preparing the dry extract (simplicial), namely Rome Beauty apples washed thoroughly in running water and then sliced thinly. After that, it was dried in an oven at 50oC for three days. Then, dry Simplicia is obtained, crushed using a blender, and sieved to get uniform Simplicia particles.

Rome Beauty Apple Extract Process

The extract was prepared by maceration using 70% methanol. Simplisia powder weighed as much as 50 grams, dissolved in 250 mL of 70% methanol in a glass vessel, and stirred until homogeneous. The maceration process was carried out for five days. On the fifth day, filtering and remaceration were carried out by adding 250 mL of 70% methanol for two days with the same treatment. On the second day of repatriation, screening is carried out. The results of the filtering (filtrate) of maceration and maceration are mixed and evaporated using a vacuum rotary evaporator at a temperature of 500C until a thick extract is obtained. Pour it into a porcelain cup, heat it with an electric stove and with the help of a fan while continuing to stir, and keep the temperature controlled with a thermometer. This process evaporates ethanol to obtain a thick extract with a concentration of 100%. This maceration method is very advantageous because it is cheap and easy to do and does not damage compounds that cannot withstand heating. It is adjusted to the physical and chemical properties of the class of compounds in Rome Beauty apples (*Malus sylvestris Mill.*), namely the form of flavonoid compounds that are not heat resistant and easily oxidized at high temperatures (Setiani *et al*., 2017).

Quercetin Standard Solution Process

The quercetin standard solution dissolves the standard with methanol and sonicating for 15 minutes, then filters with a 0.22 µm filter membrane. The results of making a standard quercetin solution are soluble and ready for analysis in a High-Performance Liquid Chromatography (HPLC) system.

Standard and Sample Preparation

The first standard preparation performed was 10.0 mg of quercetin standard. Then, the standard was dissolved in a 10 mL measuring flask with methanol solvent up to the mark (Quercetin content became 1 mg/mL or 1000 µg/mL). Then 1000 µg/mL of mother liquor was taken, and as much as 1 mL was dissolved in a 10 mL measuring flask with methanol solvent up to the mark (Quercetin level became 100 µg/mL). A standard curve was made from 100 µg/mL mother liquor and the solution with a 0.22 µL membrane filter. For sample preparation, weigh 25 mg of the sample, dissolve it in methanol up to 10 mL, and filter the sample solution using a 0.22 µm membrane filter. Next, inject 20 µL of sample filtration into the HPLC system. Repeat replication at least five times (Husnia & Budiarti, 2021)

Results and Discussions

The sample characteristics consist of maternal age, maternal education level, and family income, which can be seen in the following table:

Table 1. Characteristic sample

Subject Characteristic	Frequency	Percentage
	n	%
Mother's Age		
No Risk (20-35 th)	**21**	**70**
Risk (>35 th)	**9**	**30**
Gestational Age		
First Trimester (4-13 weeks)	**7**	**23**
Second Trimester (14-26 weeks)	**21**	**70**
Third Trimester (27-40 weeks)	**2**	**7**
Mother's Education level		

ISSN 3031-0911

Subject Characteristic	Frequency	Percentage
	n	%
Elementary School	**3**	**10**
Middle and High School	**24**	**80**
College	**3**	**10**
Family Income		
Below the regionl minimum wage (<Rp2.000.000)	**24**	**80**
Above the regionl minimum wage (>Rp2.000.000)	**6**	**20**

Based on **Table 1**, information was obtained that the majority of pregnant women were not at risk, as many as 21 people (70%), while nine people (30%) were pregnant women who were at risk (>35 years). Pregnant women with the majority of gestational age in the second trimester (14-26 weeks) were 21 people (70%), while in the first trimester, there were seven people (23%), and in the third trimester, there were two people (7%). Most pregnant women had a secondary education level (SMP-SMA), as many as 24 people (80%). In comparison, the maternal education levels were primary and higher (university) as many as three people (10%). The majority of the mother's family had an income below the regional minimum wage (<Rp. 2,000,000), as many as 24 people (80%), while the family income was above the provincial minimum wage (>Rp. 2,000,000) as many as six people (20%).

Table 2. Analyzed Univariate Independent and Dependent Variables

Independen and Dependen Variable	Frequency	Percentage
	n	%
Iron Intake		
Not enough	**24**	**70**
Enough	**6**	**30**
Vitamin D intake		
Not Enough	**24**	**70**
Enough	**6**	**30**
Nutritional Status		
Malnutrition	**24**	**70**
Good Nutrition	**6**	**30**
Anemia Occurrence		
Anemic	**25**	**75**
Non Anemic	**5**	**25**

Based on **Table 2.** Most pregnant women's food intake is deficient in iron, as many as 24 people (70%), while six people (30%) have sufficient iron intake in pregnant women. Most pregnant women's food intake of vitamin D is deficient, as many as 24 people (70%), while pregnant women with sufficient vitamin D intake are six people (30%). Most pregnant women experienced poor nutritional status, 24 people (70%), while six pregnant women experienced good dietary grade (30%). Most pregnant women also experience anemia, as many as 25 people (75%), while five people (25%) do not experience anemia

Table 3. Relationship between Iron Intake, Vitamin D, and Nutritional Status on the Incidence of Anemia Using Bivariate Analysis

Variable	Anemia Occurrence				OR	CI 95%		P^a
	Anemic		Not Anemic			Lower	Upper	
	n	%	n	%				
Iron Intake					15,00	1,652	136,172	0,016*
Not enough	19	45	5	25				
Enough	4	20	2	10				
Vitamin D Intake					0,427	0,033	4,228	0,417
Not enough	19	45	6	30				
Enough	4	20	1	5				
Nutritional Status					0,787	0,165	10,743	0,787
Malnutrition	19	45	5	25				
Good Nutrition	4	20	2	10				

P[a]: *Chi-Square Test* *) *P<0.05 Significant* *n: number of people*

ISSN 3031-0911

Table 3 shows that iron intake is significantly related to the incidence of anaemia (OR=15, 95% CI, p=0.016), namely that 19 pregnant women (45%) who experienced insufficient iron intake resulted in anaemia. The OR value is 15, meaning inadequate iron intake can increase the risk of anaemia by 15 times compared to sufficient iron intake. Vitamin D intake was not significantly related to the incidence of anaemia (OR=0.427, 95% CI, p=0.417). Nineteen pregnant women (45%) who experienced insufficient vitamin D intake resulted in anaemia. The OR value is 0.427, meaning inadequate vitamin D intake can increase the risk of anaemia by 0.427 times compared to sufficient vitamin D intake. Nutritional status was not significantly related to the incidence of anaemia (OR=0.787, 95% CI, p=0.787); namely, 19 pregnant women (45%) who experienced poor nutritional status resulted in anaemia. The OR value is 0.287, meaning poor nutritional quality can increase the risk of anaemia by 0.287 times compared to good nutritional status.

Table 4. Relationship between Mothers age, Family Income, Mother's Education Level, and Gestational Age on the Incidence of Anemia Using Bivariate Analysis

Variable	Anemia Occurrence				OR	CI 95%		*P*[a]
	Anemic		Not Anemic			Lower	Upper	
	n	%	n	%				
Mother's Age								
No Risk (20-35 th)	6	30	3	15	0,287	0,048	2,457	0,279
Risk (>35 th)	7	35	4	20				
Family Income								
Below the regionl minimum wage (<Rp2.000.000)	9	45	5	25	0,139	0,616	32,069	0,128
Above the regionl minimum wage (>Rp2.000.000)	4	20	2	10				
Mother's Education level								
Elementary School	2	10	1	5	1,009	0,240	4,233	0,419
Middle and High School	9	45	5	25				
College	2	10	1	5				
Gestational Age								
First Trimester (4-13 weeks)	7	35	4	20	2,151	0,346	13,392	0,895
Second Trimester (14-26 weeks)	5	25	3	15				
Third Trimester (27-40 weeks)	1	5	0	0				

**) p 0.05 Significant n: number of people*

Based on **Table 4**, information was obtained that maternal age was not significantly related to the incidence of anaemia (OR=0.287, 95% CI, p=0.279), namely that six mothers (30%) were at risk of developing anaemia. Seven pregnant mothers (35%) are not at risk of anaemia. The OR value is 0.287, which means that the age of a mother at risk can increase the risk of anaemia by 0.287 times compared to that of a mother who is not at risk. Family income was not significantly related to the incidence of anaemia (OR=0.139, 95% CI, p=0.128). That is, nine pregnant women (45%) whose income was below the minimum wage (<Rp. 2,000,000) experienced anaemia, and as many as four pregnant women (20%) who earned above the minimum wage (>Rp. 2,000,000) experienced anaemia. The OR value = 0.139 means that mothers whose income is below the minimum wage can increase the risk of anaemia 0.139 times compared to mothers who earn above the minimum wage. The level of education was not related to the incidence of anaemia (OR=1.009, 95% CI, p=0.419). Namely, nine pregnant women (45%) had secondary education (middle and high school), and two pregnant women (10%) had essential (primary) and tertiary (college) education levels. An OR value of 1.009 means that pregnant women with a basic education level can increase the risk of anaemia by 1.009 compared to mothers with a higher education level. Gestational age was not significantly related to the incidence of anaemia (OR 2.151, 95% CI, p=0.895), namely seven pregnant women (35%) in the first trimester (4-13 weeks) and five pregnant women (25%) in the second trimester. (14-26 weeks), as many as one pregnant woman (5%) in the third trimester (27-40 weeks).

Table 5. Multiple Analyzed Independent Variable and Confounding

Variable	B	Wald	Sig	OR	CI95%	
Nutrition status	-1.544	0.382	0.537	0.214	Lower	Upper
Mother's age	-1.374	0.587	0.444	0.253	0.002	28.616
Gestational age	38.254	0.000	0.998	4.107	0.008	8.509
Level of education	-0.281	0.020	0.889	0.755	0.000	
Family income	36.991	0.000	0.998	1.162	0.015	38.579
Iron intake	-58.885	0.000	0.997	0.000	0.000	
vitamin D intake	16.935	0.000	0.999	22.64	0.000	

Pa: Multiple linear regression test *) p<0.05 significant

ISSN 3031-0911

Based on **Table 5,** it can be concluded that of all the independent variables (nutritional status, maternal age, education level, family income, iron intake, and vitamin D intake it is thought not to influence anaemia in pregnant women. The largest OR value was 22.64, meaning that vitamin D intake has a 22.64 times chance of causing anaemia in pregnant women.

Based on **Table 1,** information was obtained that the majority of mothers were not at risk (20-35 years) as many as 11 people (55%). Meanwhile, nine mothers are not at risk (45%). Maternal age < 20 years or above and > 35 years can cause anaemia. This is because iron consumption for 20 years is divided by the fetus in the womb and its biological growth, which, of course, still requires a lot of iron consumption (George *et al*., 2021). After the age of 35, they enter an early degenerative stage when body function is not optimal, and they have various health problems. Pregnancies under 20 years and over 35 years are pregnancies with a risk of anaemia (Bellakhal *et al*., 2019; Sunuwar *et al.*, 2019).

According to Parischa *et al*. (2023), mothers of at-risk ages can reduce the incidence of anaemia by 0.68 times compared to mothers of non-risk ages. The results of this study show that there is conformity with the theory put forward by (Guo *et al*., 2022) that the ideal maternal age in pregnancy is the 20-35 year age group and at this age, mothers have healthy reproduction and are less at risk of pregnancy complications. The age group < 20 years is at risk of anaemia because reproductive development is not yet optimal, and according to Kumar and Lahiri (2023), pregnancy in the 35-year age group is associated with deterioration and decreased endurance as well as various diseases that often occur at this age. Pregnancy at >35 years of age is a high-risk pregnancy because, at this age, chronic health problems often occur, one of which is the risk of anaemia. Bleeding that occurs during childbirth, if not handled properly, will cause anaemia. Apart from that, age is not the only factor that causes anaemia. There are other factors, namely socio-economic factors (Kang *et al*., 2023).

Based on **Table 1**, information was obtained that the majority of mothers had a secondary education level (SMP-SMA), as many as 14 people (70%). Meanwhile, there were three mothers (15%) who had primary (primary) and higher (university) education. According to research by Edison (2019), the relationship between education level and the incidence of anaemia in pregnant women shows that the prevalence of anaemia in mothers who have a low level of education reaches 90.3% compared to mothers who have a high level of education, which is only 9.7%. The Chi-Square test results obtained a value of ρ= 0.001. This is due to the mother's lack of knowledge on how to process good food so that it does not damage the nutritional content in it and also due to low education, the mother does not work, which reduces household income, and the mother cannot buy nutritious food so that due to this limitation anaemia occurs (Panchal *et al* ., 2022). Based on interviews during the preliminary study, mothers only received education from health workers at the Community Health Center only once a month. So, the information obtained is lacking because, during the education class for pregnant women, only one theme is given, such as consuming blood supplement tablets regularly.

Based on **Table 1**, information was obtained that the majority of family income was below the minimum wage (<Rp. 2,000,000), as many as 14 people (70%). Meanwhile, six people (30%) had family income above the minimum wage (>Rp. 2,000,000). The prevalence of anaemia is greater in pregnant women with incomes lower than the Regional Minimum Wage (UMR). This affects the purchasing power of food families consume because >57% of family income is spent on purchasing food (Gibore et al., 2021; Pasricha et al., 2023). According to Septiasari (2019) research, it was found that 25 out of 39 pregnant women (61.0%) of mothers with income < minimum wage experienced anaemia, while among pregnant women with income ≥ minimum wage, there were 16 out of 47 people (39.0 %) have anaemia. The results of the chi-square statistical test show that the p-value = 0.005 (p ≤0.05), RP 3.460 (95% CI = 1.421 – 8.425), so it can be concluded that mothers with an income <UMR increase the incidence of anaemia by 34 times compared to mothers with an income >UMR causes mothers not to get adequate nutrition, thereby risking anaemia. The number of families will influence the food distribution within the family. The lack of family income causes a reduction in the location and purchase of daily food, thereby reducing the quantity and quality of the mother's food per day, which has an impact on reducing nutritional status. A common nutritional disorder in pregnant women is anaemia. The food sources needed to prevent anaemia generally come from protein sources, which are more expensive and difficult for those with low incomes. This deficiency increases the risk of anaemia in pregnant women and accelerates the risk of morbidity in mothers (Mekonen & Alemu, 2021; Maugliani & Baldi, 2023). Based on preliminary study interviews, as many as 65% of pregnant women said they consumed two pieces of vegetable protein (tempeh, tofu, processed nuts) 3-4 times per week.

Based on **Table 1,** information was obtained that the majority of pregnant women with poor nutritional status were 14 people (70%). Meanwhile, there were six pregnant women with good nutritional status (30%). Steven et al. (2022) stated the relationship between nutritional status and the incidence of anaemia. *Nutritional status* is also defined

ISSN 3031-0911

as health status resulting from a balance between nutrient needs and input and is a basic need for pregnant women. Nutritional status and the incidence of anaemia were also shown by Dewi and Mardiana (2021), who found that the risk of anaemia in pregnant women was 2.9 times higher for pregnant women with poor nutritional status than those with good nutritional status. This comparative figure has a large role in influencing the health of pregnant women. The estimated determinant of R2 is 0.047, meaning that nutritional status contributes 4.7% in influencing the incidence of anaemia. Even though the contribution value is small, as long as the regression coefficient β1 is not statistically zero, scientifically, it can be proven that there is an influence between nutritional status and the incidence of anaemia (Guo *et al*., 2022)

Based on **Table 1**, information was obtained that the majority of mothers' iron intake was less than 14 people (70%). Meanwhile, six people (30%) had sufficient iron intake, according to research conducted in Jatinangor District regarding the consumption patterns of pregnant women, which shows that the intake of food sources of iron (Fe) in pregnant women is still inadequate, namely 53 people (93%) out of 57 pregnant women (Putri *et al*., 2020). This happens because the source of iron consumed does not come from heme, such as meat and animal food sources, so it is not easy to absorb and does not support the presence of iron in the body. The same thing was done in research in TuaTunu Pangkal Pinang Village regarding the relationship between nutritional intake and the incidence of anaemia in preconception women, which showed that the intake of food sources of iron (Fe) in preconception women was still insufficient, namely 98.4% (Devriany & Wardani, 2019). This is because the consumption of less balanced foods can interfere with the absorption of iron in the body, and the majority of respondents consume more vegetable protein than animal protein. In addition, the lack of consumption of side dishes, which are a source of iron which is useful during pregnancy for the formation of new haemoglobin, compensates for the small amount of iron which is constantly excreted by the body (especially through urine, faeces and sweat), and replaces iron losses during pregnancy—lactation for milk secretion (Devriany & Wardani, 2019).

Mothers who are malnourished are at risk of having a difficult or long labour, giving birth to a baby prematurely (not yet full term), bleeding in the mother after giving birth and usually during labour, the mother also lacks the strength to push during the labour process so giving birth using a high surgical procedure for pregnant women lack nutrition (Xu *et al*., 2022). There are 41% of pregnant women suffer from malnutrition. The emergence of nutritional problems in pregnant women, such as the incidence of CED, cannot be separated from the social, economic, and biosocial conditions of pregnant women and their families, such as education, income level, food consumption, age, parity and so on (Andersen *et al*., 2022). Malnutrition can cause the mother to suffer from anaemia; the blood supply that delivers oxygen and food to the fetus will be hampered, so that the fetus will experience impaired growth and development. Therefore, monitoring the nutrition of pregnant women is very important (Nuru *et al*., 2021).

Based on **Table 1**, it was found that the majority of mothers' vitamin D intake was less than 14 people (70%). Meanwhile, six people (30%) had sufficient vitamin D intake. Vitamin D is a vitamin that is needed for various metabolic processes in the body. It is fat-soluble and is produced by human skin with energy obtained from food intake. Vitamin D deficiency in pregnant women has an impact on the fetus and newborn. The prevalence of vitamin D deficiency is 63% in Indonesia and Malaysia. According to Smith M. Ellen and Tang Pricha Vin (2019), vitamin D can increase erythropoiesis by increasing the proliferation of erythroid progenitors and reducing proinflammatory cytokines. Additionally, by decreasing proinflammatory cytokines that stimulate hepcidin and through direct transcriptional regulation of the HAMP gene, vitamin D can suppress hepcidin expression. Reducing proinflammatory cytokines and hepcidin may increase iron bioavailability for erythropoiesis and haemoglobin synthesis by restoring iron recycling, preventing iron absorption in macrophages, and eliminating impaired iron absorption, thereby protecting against anaemia.

Conclusions

Iron intake is significantly related to the incidence of anaemia ($p=0.016<0.05$) and can increase the risk of anaemia 15 times. Meanwhile, vitamin D intake is not significantly related to the incidence of anaemia ($p=0.417>0.05$). Nutritional status is not significantly related to the incidence of anaemia ($p=0.787 >0.05$). Factors that have a significant influence on anaemia are family income and gestational age. There is a need for further research regarding interviews using recall to determine the food ingredients consumed by respondents and education using questionnaires regarding anaemia in pregnant women.

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The Effect of Body Image and Pocket Money on Food Choice Attitudes in Obseous Adolescents In Surakarta

Afifah Istiqomah[1]*, Eti Poncorini Pamungkasari[2], Sri Mulyani[3]

[1]Department of Nutrition Science, Sebelas Maret University,Surakarta, Indonesia.
[2] Department of Medicine, Sebelas Maret University,Surakarta, Indonesia.
[3] Vocational School, Sebelas Maret University, Surakarta, Indonesia.

*Correspondence author : afhisti@student.uns.ac.id

Abstract

Obesity is a condition of nutritional status due to the accumulation of fat in the body, which causes social and emotional problems. Said to be obese if the body weight is more than 20% of the ideal body weight. In obese adolescents, physical appearance becomes important. The assessment called body image is a feeling of satisfaction or dissatisfaction with the state of the body and its appearance. Furthermore, in giving pocket, money is a tool in the attitude of choosing food. The greater or higher the pocket money received will support a person in the activity of consuming food or drinks. In adolescents, there can be an increased risk of obesity due to the consumption of foods with low nutrition, high fat, and high carbohydrates. The objective of this study is to know whether there is an influence of body image and pocket money on food selection attitudes in obese adolescents. Methods: Cross-sectional research design. The study population was adolescents of SMAN 6, SMAN 8, and SMA Muhammadiyah 1 Surakarta, with a sample of 48 respondents. The sampling technique used purposive sampling and quota sampling. Data analysis using multiple linear regression test. Results: The multiple linear regression analysis test obtained a p-value of 0.150>0.05. Furthermore, body image and pocket money together affect the attitude of food selection by 8.1%. The remaining 91.9% is influenced by factors not examined in this study. Conclusion: There is no influence between body image and pocket money on the attitude of food selection in obese adolescents in Surakarta City.

***Keywords*:** Adolescent, Body Image, Food selection attitude, Obesity, Pocket money

Introduction

Obesity is a condition of nutritional status due to the accumulation of fat in the body, which causes social and emotional problems. It is said to be obese if the body weight is more than 20% of the ideal body weight (Ferdianti, 2021). The prevalence of adolescent obesity at the age of 16-18 years in Indonesia has increased from 2013 to 2018 by 1.6% and 4%. The prevalence in Central Java was 3.7%, while in Surakarta City, it was 11.47% (Riskesdas, 2018). According to data from the Surakarta City Health Office (2015), the highest prevalence of obesity in adolescents is in Banjarsari District, which is 68.57%, and the second is 27.85% in Jebres District.

The process of rapid growth and development occurs in adolescence, so energy and nutrients are also needed by the body. Fulfilling nutrition in adolescents is so that optimal and balanced nutritional intake can be achieved to support growth and development. However, this goal is often not achieved in connection with the attitude of food choices in adolescents who often diet to achieve a positive body image that is not by the guidelines and nutritional needs in the body (Amraini *et al*., 2020).

Attitudes towards wrong or inappropriate eating choices are influenced by several things, namely that many adolescents are very concerned about their body shape, especially adolescent girls. In general, adolescents are in the stage of paying great attention to their body shape according to the desired body image. Body image is a behaviour that leads to an evaluation of the individual's assessment of his physical appearance, as well as individual experiences in the form of perceptions or thoughts about the shape and weight of his body (Intantiyana *et al*., 2018). Another factor that causes incorrect or inappropriate food choices is the provision of pocket money from parents. According to Septiani *et al.* (2019), the increase in variations in adolescents' purchasing power toward diet is based on parents' income, which has an impact on the high amount of pocket money given by parents.

Methods

ISSN 3031-0911

This study was an analytic cross-sectional study that measured risk factors and was conducted at the same time (concurrent). The study was conducted in June 2023 at SMAN 6, SMAN 8, and SMA Muhammadiyah 1 Surakarta. The sample size was calculated using the minimum sample formula Sugiyono (2017), with as many as 48 respondents. Sampling techniques using Non-probability Sampling in the type of Purposive Sampling and Quota Sampling. Data collection consists of data on age, gender, pocket money, body image and food selection attitudes.

In this study, multiple linear regression test data analysis. Researchers first conducted a normality test on the two variables, namely Body Image (X1) and Pocket Money (X2), on the attitude of food selection (Y) using the SPSS (Statistical Product and Service Solutions) application. Inclusion criteria for this study were respondents of the target population actively enrolled in SMAN 6, SMA Muhammadiyah 1, and SMAN 8 Surakarta City, Willing and agreeing to be a respondent, and Anthropometric screening results showed that the student had BMI >27 (Obesity). Exclusion factors were respondents not present when the study was conducted and Suffering from diseases that must carry out certain diets (diabetes, kidney, heart, hypertension).

Data related to body image using the Body Shape Questionnaire (BSQ)-16. The BSQ-16 questionnaire consists of 16 questions about body shape and appearance. This questionnaire uses a Likert scale to measure responses or answers from respondents. The score is obtained from the sum of all questions, then categorized into positive body image if the score is <38 and negative body image if the score is ≥38 (Merita *et al*., 2020).

Data related to food selection attitudes using a questionnaire developed by Rosdianti (2016), consisting of 10 questions. Research respondents were asked to provide statements or reactions according to the choices in the questionnaire. Attitude was measured by Agree (S) and Disagree (TS). Processing of questionnaires to identify negative food selection using scores < mean and positive scores ≥ mean. This study obtained ethical journal approval from the Research Ethics Committee of the Faculty of Medicine, Sebelas Maret University, Surakarta, in the form of a statement of Ethical *Approval* with number No.110/UN27.06.11/KEP/EC/2023 dated May 22, 2023.

Results and Discussions

Respondent Characteristics

The sample used in the study amounted to 48 respondents from SMAN 6, SMAN 8, and SMA Muhammadiyah 1 Surakarta. The following is the distribution of respondent characteristics

Table 1. Distribution of Respondent Characteristics

Respondent Characteristics	n	%
Gender		
Male	21	43,8
Female	27	56,2
Age		
15	9	18,8
16	25	52,1
17	12	25
18	2	4,2

Table 1 shows that in SMA N 6, SMA N 8, and SMA Muhammadiyah 1 Surakarta, 27 respondents (56.2%) were female. And the male gender is 21 respondents (43.8). According to age characteristics, the proportion of respondents at the age of 16 years was more, namely 25 respondents (44%) and 18-years-old respondents were less, namely 2 respondents (4.2%). In research, Qoirinasari *et al*. (2018) said that in women, the influence of hormones also results in the risk of obesity. According to Wahyono (2019), women are more at risk of obesity than men. The high rate of obesity in women is due to differences in levels of physical activity and energy intake in men and women. Based on research by Weni (2015) states that adolescence (10-18 years) is a period of nutritional vulnerability due to various reasons, namely, adolescents require higher nutrients due to increased physical growth, changes in lifestyle and eating habits, and special nutritional needs. Adolescents are one of the target groups at risk of overnutrition or obesity. Adolescents have a risk of obesity that may continue into adulthood. This risk of obesity can occur in early adolescence, as evidenced in this study, and relatively young adolescents are more at risk of obesity (Nugroho, 2020).

ISSN 3031-0911

Distribution of respondents based on pocket money, Body image, and meal selection attitude

In **table 2**, it is known that in SMAN 6, SMAN 8, and SMA Muhammadiyah 1 Surakarta, the characteristics of respondents' pocket money obtained data mostly have a high category, namely >Rp. 18,300 which amounted to 37 respondents (77.1%). And for 39 respondents (81.2%) had negative body image and 9 respondents (18.8%) had positive body image. Attitude for 20 respondents (41.7%) have negative food selection attitudes, and 28 respondents (58.3%) have positive food selection attitudes.

Table 2. Distribution of respondents

Variable	n	%
Pocket money/day		
Low (<IDR 11,700)	0	
Medium (IDR 11,800 - 18,299)	11	22,9
High (>IDR 18,300)	37	77,1
Body image		
Negative (< 38)	39	81,2
Positive (≥ 38)	9	18,8
Attitude		
Negative (score < mean)	20	41,7
Positive (score ≥ mean)	28	58,3

According to the characteristics of the respondents, most of the pocket money obtained data has a high category, namely >Rp. 18,300 (77.1%). Based on research by Widyoningsih *et al.* (2016), giving high pocket money to children can cause children to be more able to consume a variety of foods, such as unhealthy snacks, so the amount of pocket money has a significant effect on behaviour in choosing snacks.

Body image can be linked to psychosocial images of self-assessment, body size and body shape. Disruption of body image causes dissatisfaction with his low self-confidence (Abdul Muhith, 2015). In the results of the study, most respondents had a negative body image (81.2%) and a positive (18.8%). This is due to several factors that can affect negative body image, namely gender and age. In the characteristics of the gender and age of respondents, most of them were female, 27 respondents (56.2%) and the age of all respondents was 16-18 years (100%). In adolescence, a new element will arise, namely awareness of personality and physical life, so that adolescents, especially women, begin to pay attention to their physical appearance. In general, there is dissatisfaction with body shape, so adolescent girls tend to make efforts to get the desired weight (Nugroho, 2020). Therefore, many respondents avoid wearing clothes that make them aware of their body shape (35%).

Attitude is the most important component in influencing food choices. The attitude in choosing good food will be able to affect a person's health condition. This is influenced by the ability to accept, practice and respond to healthy food. Some types include food selection, namely the type, amount and time of consuming these foods (Laenggeng & Lumalang, 2015).

In the results of the study, there were negative (41.7%) and positive (58.3%) food selection categories. This can be due to the availability of canteens and food vendors outside the school that provide varied and colourful food so that it can potentially consume unhealthy food. Most respondents preferred fatty foods (meat, chicken skin, cheese, butter, ice cream) (91.7%) and fast foods (instant noodles, instant porridge) (83.3%). This can be due to one of the factors that can directly influence food choices, namely the individual and the surrounding environment, which will show how a person's lifestyle is (Ratih, 2020).

The effect of body image and pocket money on food choice attitudes in obese adolescents

Based on **Table 3.** the results of the analysis obtained for the food selection attitude variable, the Z K-S value is 1.266 with asymp sig. 0.081>0.05, it can be concluded that the data on the attitude variable of food selection is normally distributed. The body image variable has a Z K-S value of 0.766 with an asym sig of 0.600 (>0.05), it can be concluded that the data distribution is normally distributed. And the pocket money variable has a Z K-S value of 1.520 with an asym sig of 0.020 (<0.05), meaning that the data distribution is not normally distributed

ISSN 3031-0911

Table 3. Normality Test

	Normal parameters			
	Mean	**Std. Deviation**	**Kolmogrov-Smirnov Z**	**AsympSig. (2-tailed)**
N	48	48		
Attitude	50,21	14,065	1,266	0,081
Body image	58,73	18,839	0,766	0,600
Pocket money	19,47	6,782	1,520	0,020

The results of this study indicate that each increase of 1 score on body image will reduce attitudes in food selection by 0.193 (p=0.079) but not statistically significant. Each increase of 1 score on pocket money will reduce attitudes in food selection by 0.195 (p=0.517) but not statistically significant. Body image and pocket money together affect the attitude of food selection by 8.1%, the remaining 91.9% is influenced by factors not examined in this study.

Table 3. Result of multiple linear regression analysis of the effect of body image and pocket money on food selection attitudes

Variabel	**ß (Koefisien regresi)**	**P**	**CI 95%**	
			Lower limit	**Upper limit**
Constant	65,341	0,00	48,544	82,139
Body image	-0,193	0,079	-0,409	0,023
Pocket money	-0,195	0,517	-0,795	0,406
N observation	48			
Adjusted R2	0,081			
P model	0,150[a]			

Based on the results of statistical tests, it was found that there was no effect of body image and pocket money on food selection attitudes. This is in line with previous research by Ritan *et al.* (2018), which shows that there is no effect of body image on diet with a p-value of 0.137 >0.05. This is based on several factors, namely interpersonal or peer relationships, that provide an assessment of body shape that can build perceptions of themselves. So many adolescents need more confidence and satisfaction with their body shape, which is not ideal. Some respondents feel very often (23%) and always (27%) when their slim peers make them aware of their body shape and assume that the ideal body is thin and tall (54.2%). In line with the provision of pocket money Jung *et al* (2010) stated that receiving more pocket money gives adolescents more free-spending power, and they can choose preferred foods and tend to prefer unhealthy foods such as snacks with various flavours and higher energy intake.

According to research by Wulandari *et al.* (2016), the level of parental income is related to the ability of parents to meet their needs, choose the type and amount of food, and influence the family lifestyle, which will also have an impact on children. High income can also lead to the selection of foodstuffs that are more delicious, ready to eat, fast, and contain more fat, oil, and other ingredients that can cause obesity. In line with the research of (Intantiyana *et al*., 2018), there are differences in food selection in adolescents who have a positive body image and adolescents who have a negative body image. Adolescents with a positive body image tend not to limit their diet, so in the selection of food, they pay less attention to the content of the nutritional value of the foods they consume. The choice of food tends to lead to fast food, favouring certain foods and also following the development of new food products that are currently popular among the community. Obese adolescents want to limit their diet, so the selection of food is limited, and they follow the wrong diet, such as slimming drugs. In addition, psychological disorders can also occur in adolescents with obesity. A body that is too fat often makes the teenager often ridiculed by their peers. So, it has a less good impact on psychological development, and another impact that is often overlooked is that obese adolescents can interfere with the psyche, namely often feeling less confident and experiencing learning disorders in school children. Especially if the child is in adolescence and is obese, it will usually become passive and depressed (stressed) because it is often not involved in activities carried out by their peers (Lidiawati *et al.*, 2020).

ISSN 3031-0911

However, in body image and pocket money, some factors can have no effect due to family environmental factors in providing attention related to diet. Based on research results, Septiani *et al.* (2019) showed a significant influence on the attitude of choosing staple foods, choosing vegetables, and choosing vegetables and fruits with each p-value <0.05. Food ingredients that the family has provided can provide examples of good food choices. The study of Berge *et al.* (2010) also revealed that the role of the family has a major impact on food selection and diet in adolescents, so the attitude toward food selection in respondents depends entirely on the family environment. Other factors include heredity (parents), living habits and the type of food consumed. The risk of adult obesity is greater in obese or non-obese children if at least one parent is overweight. There are other factors, such as excessive food intake in the selection of meals, influenced by the family environment, especially those with hereditary factors (parents), living habits and types of food consumed. The risk of adult obesity is greater in obese or non-obese children if at least one parent is overweight (Lidiawati *et al*., 2020).

Conclusions

There is no effect between body image and pocket money on food selection attitudes among obese adolescents in Surakarta city. Based on the results, discussion and conclusions obtained, suggestions can be made for parents to student pocket money at school and urge children not to buy fast food and snacks excessively. It is hoped that the attention of parents who are obese is limited further and supervise children's eating habits that are excessive, high in calories but low in fibre, so that children can consume these foods not excessively. Moreover, for schools, it is necessary to hold health programs for obese people through school health efforts (UKS) or counselling guidance (BK), such as healthy adolescent diets, balanced nutrition counselling, and providing education about healthy food.

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The Effect of Nutrition Education Through *Flashcard* Media on Knowledge of Iron Sources Food Consumption in Adolescent Women at SMP N 33 Bekasi City

Miswah Alfaresti Sutrisna[1]*, Arindah Nur Sartika[2].

[1,2]Department of Nutrition Science,Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Bekasi, Indonesia.

*Correspondence author : miswahresti@gmail.com

Abstract

In young women the nutritional problems that are still experienced are nutritional anemia caused by a lack of consumption of food sources of iron. Adolescent girls who consumed food sources of iron were still relatively low in the animal food group (meat, chicken, liver, fish, eggs) 39% and from the vegetable side dishes (tofu and tempeh) 31.2%. One of programs to improve nutritional anemia that can be done is through nutrition education. In the process of nutrition education it is necessary to have visual aids that can help the learning process to be effective and attract students' attention regarding the consumption of iron intake in young women at school. *Flashcards* can be applied to junior high school students, because students can understand from the contents of the picture cards which contain information related to knowledge of consumption of food sources of iron. This study aims to analyze the effect of nutrition education through flashcard media on knowledge of consumption of food sources of iron in young women at SMPN 33 Bekasi City. The research design is a quasi-experimental design in the form of a one group pretest-posttest design. The research subjects consisted of 34 female students as the intervention group. The media used is flashcard media for food sources of iron from the carbohydrate, animal, vegetable, legumes, vegetables, fruits, and dairy product groups referring to the 2017 Indonesian Food Composition Table. The results of statistical analysis using the Wilcoxon Rank Test showed that providing nutrition education using flashcard media on knowledge of consumption of food sources of iron in female adolescents was p-value = 0.00005 (<0.05). This means that there is an effect before and after providing nutrition education using flashcard media on knowledge of consumption of food sources of iron in young women at SMPN 33 Bekasi City

***Keywords*:** *Nutrition Education; Flashcard; Consumption of Food Sources of Iron.*

Introduction

The nutritional problem experienced in adolescents is nutritional anemia. Adolescent girls need to pay attention to their health because they experience anemia caused by factors such as long periods of menstruation resulting in a lack of nutritional intake in the formation of hemoglobin. Based on the 2018 Basic Health Research, the prevalence of anemia in young women is 48.9%. The leading causes of adolescent girls experiencing anemia are : low intake of food sources of iron, increased iron requirements during pregnancy and breastfeeding, significant blood loss during menstruation.

One effort to improve the situation of nutritional anemia in adolescents is by fulfilling iron intake, one of which is consuming food sources of *heme* iron found in animal food sources and *non-heme* iron found in plant foods. However, in Indonesia, there still needs to be more knowledge about preventing anemia and consuming iron intake by nutritional adequacy rates in adolescent girls. One program to improve nutritional anemia is through nutritional education. Efforts to provide nutrition education in schools have several advantages, namely that the knowledge and attitudes received can become the basis for consumption patterns for young women at school. Providing nutritional education to young women at school is a strategic step in improving nutritional anemia because the impact is directly related to preventing stunting in Indonesia.

Nutrition education provides information related to nutrition so that school teenagers can change their food consumption behavior and increase their knowledge because it can form positive attitudes and behavior. In the nutrition education process, there is a need for teaching aids that can help the learning process be effective and attract students' attention regarding the consumption of iron intake in young women at school. *Flashcards* can applied to all age groups, including junior high school students, because students already read well and can already understand the contents of the picture cards, which contain information related to knowledge of consuming food sources of iron. According to Wahyuni's (2020) research, there was an increase in the average knowledge score after being given education using flashcard media in the pretest from 69.00 to 80.40 in the implementation of SIKLUS II learning activities.

ISSN 3031-0911

Based on this background, researchers are interested in examining the influence of nutritional education through *flashcard* media on knowledge of consuming food sources of iron among young women at SMPN 33 Bekasi City.

Methods

This quantitative research uses a quasi-experimental design as a one-group pretest-posttest design and has received approval from the Muhammadiyah University Research Ethics Commission, Prof. Dr. Hamka, with No.03/23.03/02357. This research was conducted in March at SMPN 33 Bekasi City.

The sampling technique in this study used a consecutive sampling technique. The inclusion criteria in this study were a) All female students in class VII-IX of junior high school, b) Female students aged 12–15 years, and c) Students willing to be respondents during the study. In the intervention group, 34 female students met the inclusion criteria.

The data taken included data on the characteristics of female student respondents, data on the demographic characteristics of the female respondent's family, as well as data on knowledge of source food consumption. Knowledge data was obtained by filling out an independently prepared questionnaire totaling 18 questions in *Multiple Choice* form, which validated and accepted a *Cronbach's Alpha* value of 0.912. The questions asked were related to the consumption of food sources of iron. The questionnaire refers to flashcard media food sources of iron. In the intervention group, education was given using flashcard media on food sources of iron from the carbohydrate, animal, vegetable, legumes, vegetables, fruits, and dairy product groups referring to the 2017 Indonesian Food Composition Table.

The statistical test was used to see whether or not there was an effect of nutrition education through *flashcard* media on knowledge of the consumption of food sources of iron. The statistical test method used is the parametric statistical test of the Dependent Paired T-test if it is not normally distributed using the *Wilcoxon Test.* Testing the normality of this data uses the *Shapiro-Wilk* normality test.

Results and Discussions

In the intervention group (SMPN 33 Bekasi City), the total number of children sampled in this study was 34 students who were in class VII, where the highest results were found in students aged 13 years (70.6%) and students with nutritional status of most were nutritional status normal (79 .4%). In addition to age and nutritional status, there is a student school pocket money of 0-50.000, whereas, in the intervention group, the highest results were found in respondents with a student school pocket money of 0-10.000 (41.2%) **(Table 1).**

Table 1. Characteristics of Female Student Respondents

Variable		Treatment	
		Number (n)	Percentage (%)
Gender	Female	34	100.0
Student Class	VII. G	17	50.0
	VII. H	17	50.0
Age	12 years	10	29.4
	13 years	24	70.6
Nutritional Status	Malnutrition	1	2.9
	Normal	27	79.4
	Overweight	4	11.8
	Obesity	2	5.9
Student School Pocket Money (day)	0 – 10.000	14	41.2
	11.000 – 20.000	13	38.2
	21.000 – 30.000	5	14.7
	31.000 – 40.000	0	0
	41.000 – 50.000	2	5.9
Total		**34**	**100.0**

In the demographic characteristics of the family of the respondent's parents' occupation, it was found that the results were more significant for the work of businessman fathers (44.1%) and housewife (79.4%). Regarding the educational characteristics of fathers and mothers, the average respondent has parents who are educated with an educated status of senior high school **(Table 2).**

ISSN 3031-0911

Table 2. Demographic Characteristics of Respondent's Family

Variable		Treatment	
		Number (n)	Percentage (%)
Father's Occupation	PNS/TNI/Polri	7	20.6
	Private Employes	11	32.4
	Businessman	15	44.1
	Doesn't work	1	2.9
Father's Last Education	Elementary School	2	5.9
	Junior High School	4	11.8
	Senior High School	22	64.7
	College	6	17.6
Mother's Occupation	PNS/TNI/Polri	2	5.9
	Private Employees	3	8.8
	Business woman	2	5.9
	Housewife	27	79.4
Mother's Last Education	Elementary School	3	8.8
	Junior High School	5	14.7
	Senior High School	19	55.9
	College	7	20.6
Total		**34**	**100.0**

Table 3. Distribution of Knowledge Scores on Food Consumption of Iron Sources

Variable	n	Min-Max	Median	*Interquartile Range*	$\Delta \bar{x}$
Pre-test					
Knowledge	34	50 - 100	66.67	78 – 61	50
Post-test					
Knowledge	34	78 - 100	94.44	94 - 94	22

Based on **(Table 3)** above, in the sample, it can be seen from the variable knowledge of consumption of food sources of iron that the median score before providing education (*pre-test*) has a value of 66.67 while the median score after providing education (*post-test*) has a value of 94.44.

Table 4. Data Normality Test Results

Variable	n	*P-Value (Shapiro Wilk)*	Information
Knowledge			
Before Providing Education	34	0,027	Abnormal Distribution (P-Value <0.05)
After Providing Education	34	0,000	Abnormal Distribution (P-Value <0.05)

In **(Table 4)** the results of the normality test with shapiro wilk (N<50) show that the p-value for knowledge before providing education and after providing education is 0.027 and 0.000, respectively. So, the knowledge variables before and after schooling are not normally distributed. So, the analysis test used is the *wilcoxon rank test.*

Table 5. Distribution of Knowledge Level of Respondents Before and After Participating in Education

Variable	Median	*Interquartile Range*	*P-Value*
Knowledge			
Before Providing Education	67	78 – 61	**0,00005**
After Providing Education	94	94 - 94	

N = 34 ; Wilcoxon rank test; significant if the p-value is <0.05

ISSN 3031-0911

Figure 2. Providing Education to the Intervention Group

In **(Table 5),** the statistical test results showed that the p-value was 0.00005, so it can be concluded that there was an increase in respondents' knowledge before and after providing education regarding knowledge of consuming food sources of iron using flashcard media.

DISCUSSION

Description of Sample Characteristics

Women have higher memory than men because men's memory centers are smaller than women's (Ariska *et al*., 2023). School-age adolescents are between 12-15 years old. During this period, children naturally experience a period of growth and development both psychologically and cognitively (Mauliya, 2019). According to Rizky *et al* (2019), nutritional status is influenced by a person's food consumption and physical activity. The choice of food ingredients and determining the amount of food consumed is influenced by nutritional knowledge. Father's and mother's education is related to consumption of food sources of iron, as well as pocket money, family income, parental education, and the availability of other food ingredients which have a significant positive relationship with consumption of food sources of iron such as animal, vegetable, vegetable, and fruit among students (Oktavia *et al.*, 2019).

Based on the parents' employment data results, it was found that most of the father's work was as a businessman, and the mother's work was as a housewife. According to research by Yonatha *et al* (2019), the better a person's job, the higher the income will be used to access family food, including providing a source of food at home.

Distribution of Respondents' Level of Knowledge Before and After Participating in Education

There was an increase in knowledge scores after carrying out nutrition education through flashcard media regarding the consumption of food sources of iron in adolescent girls in line with research by Rusdi *et al* (2021) which stated that there was an increase in knowledge in adolescent girls regarding changes in balanced nutritional behavior to prevent anemia after intervention in the form of nutrition education using instagram media. Research conducted by Febriyanto and Yanto (2019) also shows that using flashcard media affects students' knowledge. Education is a process of ongoing interaction between humans and the environment, producing changes in knowledge, attitudes, and skills.

According to research by Dwi *et al* (2021), which provided nutrition education to junior high school teenagers through *flashcard* media, showed that there was an increase in knowledge scores in the first pre-test and post-test by 48.76 points and between the pre-test and post-test In the second test, there was an increase in the knowledge score of 72.27 points. Through education, someone will learn from what they didn't know to become knowledgeable (Mordayanti *et al.*, 2022).

ISSN 3031-0911

Effect of *Flashcard* Media on Food Consumption Knowledge of Substance Sources during *Pre-test* and *Post-test*

There is an influence of nutritional education through flashcard media regarding the consumption of food sources of iron on young women's knowledge. This is in line with research conducted by Mardiana (2018), which stated that nutritional education about iron consumption to prevent anemia using flashcard media influenced knowledge of food choices among female students at SMP Negeri 45 Surabaya and SMP Unggulan Bina Insani Surabaya. One factor that can affect the knowledge level is the information source. So, the presence of media in education can make students more active (Utami and Alvionita, 2019).

When providing education using flashcard media, researchers explained the cards' contents in a unique way, namely playing, which included nutritional education about consuming food sources of iron. Pictures on the cards are easily recognized by female students, thereby triggering female students' interest in observing during the learning process. This is reinforced by research by Hasanah *et al* (2019), which states that the presence of pictures with a few words or sentences in media that are easily recognized by students can easily influence them in receiving information so that each picture card can influence changes in knowledge—accompanied by a shift in attitude.

Conclusions

Based on the research and discussion results, providing education using flashcard media is going well and has improved. Female students experience changes in terms of students' understanding and values. During the process of delivering education, students are always active. Students pay attention to explanations and respond to what is explained.

There is an effect of education through *flashcard* media on knowledge of the consumption of food sources of iron in class VII students of SMPN 33 Bekasi City. It is hoped that the students will be able to absorb the knowledge they have received through education using flashcard media to apply it to their daily lives and remind each other to be fond of consuming food sources of iron. Future researchers are expected to be able to pay attention to educational success factors such as timing by making a rundown of events and a closed room that is comfortable for conducting education well.

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Literature Review: Effectiveness 0f Online Nutrition Education on Mother Nutrition Knowledge of Stunting Children

Alfi Rahmatillah Nur Fithriah[1*], Sumardyono[2], Anik Lestari[3]

[1]Department of Nutrition Science, Sebelas Maret University,Surakarta, Indonesia.
[2]Vocational School, Sebelas Maret University, Surakarta, Indonesia.
[3]Department of Medicine, Sebelas Maret University,Surakarta, Indonesia.

*Correspondence author : alfi.rahmatillah@students.uns.ac.id

Abstract

One of the unresolved nutritional problems is stunting. Short stature, known as stunting in childhood, is the result of chronic malnutrition or failure to thrive in the past. This study aims to analyze general articles related to the effectiveness of online nutrition education on mother nutrition knowledge of stunting children. Researchers used a literature review study based on data collection from the period between 2019 and 2023 with the keywords stunting/short toddlers, nutrition education, effectiveness of online education, knowledge of stunting mothers' nutrition and stunting mothers' knowledge of nutrition. Data related to topics originating from within and outside the country have been studied and selected from Google Scholar, PubMed, Proquest, and ScienceDirect. The results of the article search process show that 28 articles match the keywords Indonesian and English. After the data was reviewed and re-selected, the remaining 14 articles met the inclusion criteria and were relevant to the research objectives. The conclusion from the results of the literature review of this journal shows that there is an effectiveness of online nutrition education on the nutritional knowledge of stunting mothers.

Keyword: Mother Nutrition Knowledge, Online Education, Stunting

Introduction

Short stature, known as stunting in childhood, is the result of chronic malnutrition or failure to grow and develop in the past. Children who are stunted can be seen from their body size, whether they are in accordance with the growth rate at their age or not. Stunting is also associated with impaired neurocognitive development and the risk of developing non-communicable diseases in the future (Kemenkes, RI 2016).

According to the 2013 Basic Health Research, the prevalence of stunting at the national level was 37.2%; this decreased in 2018 to 30.8% (Kementerian Kesehatan RI 2018). Meanwhile, according to SSGBI (Study of Nutritional Status of Indonesian Toddlers), in 2019, the stunting rate also decreased to 27.67% (Izwardy, 2020). In the SSGI (Indonesian Nutrition Status Study Survey), this figure decreased again in 2021 to 21.4% (Kementerian Kesehatan Republik Indonesia 2021). Based on this prevalence, it is still below the figure expected by the WHO (World Health Organization), namely <20% (Kementerian Desa, 2017). This figure is also still far from the target of reducing stunting in Indonesia by 14% in 2024 (Kementerian Sekretariat Negara RI 2020).

Many factors influence the occurrence of stunting, one of which is the level of consumption and the baby's mother's lack of knowledge regarding nutritional requirements. So, a mother plays a major role in maintaining the nutritional status of her toddler (Amaliah *et al*., 2012). This statement is in line with research conducted in Kupang Regency, stating that there is a real correlation between the incidence of stunting in toddlers and the level of education of parents, number of family members, and knowledge about maternal diet, especially regarding child nutrition, as well as protein and carbohydrate intake. (Zogara and Pantaleon, 2020).

In order to prevent and overcome stunting caused by low levels of maternal nutritional knowledge, online nutrition education is carried out. This is confirmed by research that online learning and direct learning have the same effect on improvement (Nisrina *et al*., 2017). In the other research from Setiani and Sriwiyati (2022), online health education is effective in increasing stunting prevention behaviour. According to the research from Kadek *et al*. (2022), there was a significant increase in knowledge before and after education and group discussions using WhatsApp media. We know that WhatsApp media is part of online media. Then, online education about nutrition in stunting cases is also important and can be the choice for prevention. So, this research aims to analyze articles related to the effectiveness of online nutrition education on the nutritional knowledge of stunting mothers of toddlers.

Methods

ISSN 3031-0911

This study conducted a literature study using the systematic PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) method. The article search process was carried out by operating Google Chrome and databases from Google Scholar, PubMed, Proquest, and ScienceDirect. The sources used come from articles at home and abroad in Indonesian and English. Researchers searched the Google Scholar database by applying the keywords " *Stunting*/ Balita Pendek, Edukasi Gizi, Efektivitas Edukasi Online, Pengetahuan Gizi Ibu Balita S*tunting* dan Pengetahuan Ibu Balita *Stunting* Tentang Gizi ". To search the PubMed, Proquest, and ScienceDirect databases, use the keywords "Stunting, Online Education, Mother's Knowledge, and Knowledge of Toddler Nutrition".

This research uses data with criteria, still in the 2019-2023 range, the data used is still relevant to stunting, nutrition education, the effectiveness of online education, nutritional knowledge of stunting mothers and knowledge of stunting mothers about nutrition, the data taken comes from research conducted at home or abroad and data or articles use Indonesian and English.

Results and Discussions

Data or articles that researchers have searched will then be selected and adjusted according to predetermined criteria. The number of articles that have been found is 28 articles, within the scope of the criteria. Fourteen articles met the criteria: 12 articles in Indonesian and 2 articles in English. Fourteen articles were excluded because they needed to meet the criteria. Then, each article is reviewed based on the completeness of the article as a whole (full text). Based on the review results, 14 articles that met the inclusion criteria were selected and included for analysis.

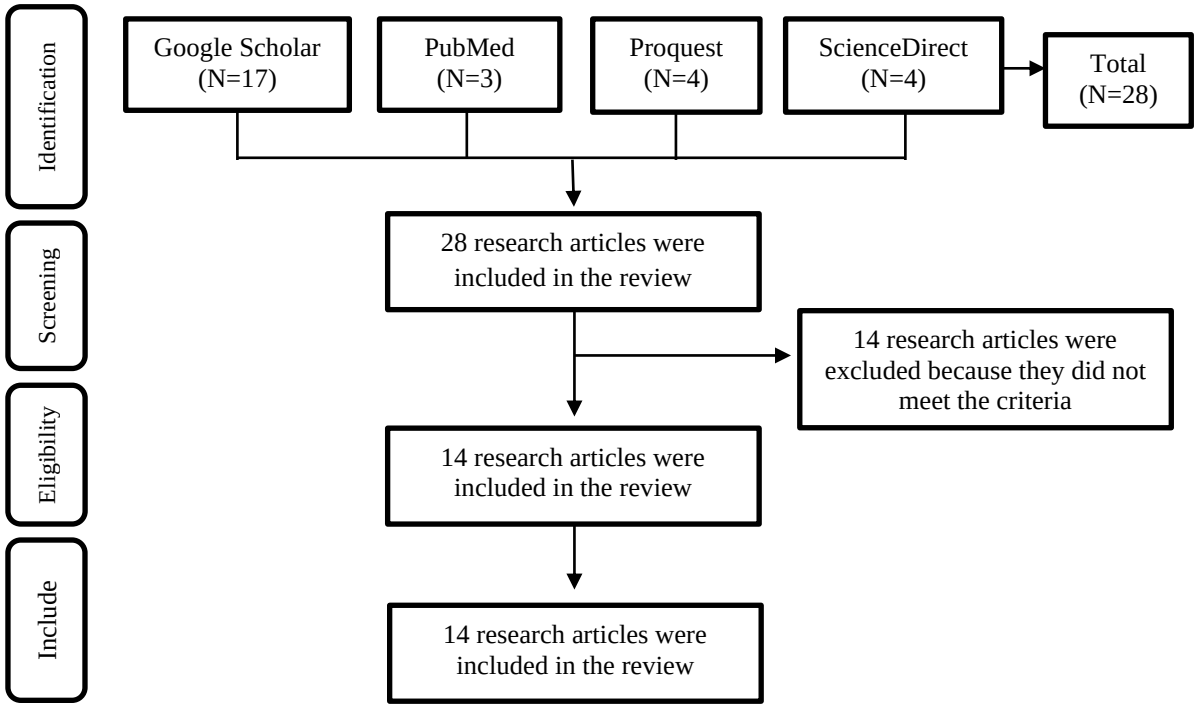

Figure 1. PRISMA flowchart literature search

The following are the results of article analysis using the literature review method and using the observed journals, namely:

Table 1. The result of article analysis

No	Author (Year)	Title	Method	Research Subject	Research Result
1.	Hikmiyah et al. (2019)	Pengaruh Pendampingan Gizi Online Terhadap Pengetahuan Dan Konsumsi Energi Ibu Hamil KEK Di Puskesmas Bululawang	Pre-experimental studies with randomized whitout control pre and posttest design	The total sample was 90 pregnant women who were then randomly divided into 3 groups and received different treatments (group 1 with e-booklets, group 2 with video media,	There were significant differences in knowledge and energy consumption before and after providing online assistance using e-booklet, video, or e-booklet and video media, and the highest average increase in

ISSN 3031-0911

				and group 3 with e-booklets and videos).	knowledge and energy consumption was in the e-booklet and video media group.
2.	Mahmudah, Aba, and Nggawu (2020)	Efektivitas Pemberian Edukasi secara Online melalui Media Video dan Leaflet terhadap Tingkat Pengetahuan Pencegahan Covid-19 di Kota Baubau	Quasi-Experiment with The Non-Randomized Without Control Group Pretest And Posttest Design approach	The sample was 120 people. The sample was then divided into 3 groups based on the type of educational media provided, namely video media, leaflets and both (video and leaflets).	Education carried out online is effective in increasing public knowledge in Baubau City regarding preventing Covid-19 by using video media as well as leaflets.
3.	Siregar and Koerniawati (2021)	Edukasi Gizi Seimbang Menggunakan Aplikasi Whatsapp pada Siswa Madrasah Aliyah di Kabupaten Bogor	One Group Pretest and Posttest Design	Students in grades 10-12 of Madrasah Aliyah Daarul Fataa, Bojonggede District, Bogor Regency	Increasing the knowledge of MA Daarul Fataa students about balanced nutrition through online education using Whatsapp.
4.	Tane and Sembiring (2021)	Edukasi Online Pemberian MPASI terhadap Praktik Pemberian Makan dan status Gizi Anak Usia 6-24 Bulan	Quasi-experimental pre-post test with a control group	The number of research respondents was 23 for the intervention group and 18 for the control group.	Online education on the practice of giving MP-ASI can improve feeding practices for mothers so that they can be applied periodically to educate mothers about how to give complementary foods. However, there were no significant differences between the intervention and control groups in feeding practices and children's nutritional status.
5.	Ditya Yankusuma Setiani and Sriwiyati (2022)	Efektifitas Pendidikan Kesehatan *Stunting* dengan Metode Daring Terhadap Perilaku Pencegahan Stunting	Quasi Experimental with pre-test and post-test	31 Mothers in Klaten Regency, Central Java	Online health education is effective for increasing stunting prevention behavior
6.	Hendrawati et al. (2023)	Penggunaan Edukasi Online untuk Pencegahan Stunting pada Masyarakat Umum di Era Pandemi Covid-19	One group design with pre-test and post-test	196 Respondents from Aceh, Sumatra, West Java, Central Java, East Java and Kalimantan.	Online education has been proven to be effective in increasing public knowledge and attitudes in preventing stunting.
7.	Kadek et al. (2022)	Efektivitas Edukasi Stunting dengan Whatsapp terhadap Pengetahuan Orang Tua Balita Stunting	Pre Experiment design, using a one group design with pre-test and post-test	40 Respondent Parents of Stunted Toddlers in Payangan District	There was a significant increase in knowledge before and after education and group discussions using WhatsApp media
8.	Naulia, Hendrawati, and Saudi (2021)	Pengaruh Edukasi Gizi Terhadap Pengetahuan dan Sikap Ibu dalam Pemenuhan Nutrisi Balita Stunting	Quasi experiment non-equivalent control post-test	60 Respondents Mother Toddler Stunting	Nutrition education can increase knowledge and attitudes in fulfilling nutrition so that nutrition can be an alternative intervention to improve health behavior in preventing stunting
9.	Putra et al. (2022)	Efektifitas Aplikasi Edukasi Gizi Remaja Berbasis Android Untuk Pencegahan Stunting	Quasi-experiment with pre-test and post-test	30 teenagers spread across 2 different schools but with almost the same characteristics.	Android-based education or educational applications are an effective medium for increasing teenagers'

ISSN 3031-0911

					knowledge and attitudes towards stunting
10.	Andoyo et al. (2022)	Pemanfaatan Teknologi Virtual Meeting Dalam Upaya Peningkatan Efektifitas Kegiatan Penyuluhan Kesehatan: Edukasi Pencegahan Stunting Dengan Pangan Tinggi Protein	Quantitative using the Participatory Action Research (PAR) method.	19 Posyandu Cadres	There is an increase in knowledge after being given education
11.	Anjani et al. (2022)	Efektivitas Metode Edukasi Berbasis Mobile Edu App Sebagai Upaya Intervensi Penurunan Stunting Dengan Pendekatan Asuh, Asih, Asah	Experimental analysis with a cross sectional method using a pre-post test design	20 people who are representatives of cadres in Tanjung Mas sub-district who are considered representative as representatives of each RW	The use of Android applications in educational methods as an effort to prevent stunting using a nurturing, asih and asah approach provides effectiveness in efforts to increase the knowledge of posyandu cadres.
12.	Simanjuntak *et al*., (2022)	Pengaruh inovasi edukasi gizi masyarakat berbasis social media marketing terhadap pengetahuan, sikap, dan perilaku dalam upaya pencegahan stunting	Quasi experiment with one group pre-test and post-test design.	The intervention was carried out on nutrition education social media viewers with a total of 150 people who were determined voluntarily	The results of the research indicate that knowledge, attitudes and behavior in efforts to prevent stunting have increased so that it shows the success of the interventions carried out in the research
13.	Ernawati et al. (2021)	The Effectiveness of Web-Based Audiovisual Media Applications in Monitoring Children's Growth to Prevent Stunting	Pre-test and post-test quasi-experimental design with a control group	The sample used as the intervention group was 50 people, and the control group was 50 people	Public health education through web-based audiovisual media is effective in increasing parents' knowledge about monitoring the growth and development of toddlers.
14.	Dimitri et al. (2023)	Accelerating Digital Health Literacy for The treatment of Growth Disorders: The Impact of A Massive Open Online Course	Quantitative	219 Users The Mooc	MOOCs can improve digital health literacy in the management of growth disorders

One of the unresolved nutritional problems is stunting. Short stature, known as stunting in childhood, is the result of chronic malnutrition or failure to thrive in the past (Kemenkes RI, 2016). One of the interventions that can be given to increase the nutritional knowledge of stunting toddler mothers is conducting education. Nutrition education can increase knowledge and attitudes toward fulfilling nutrition so that nutrition can be an alternative intervention to improve health behaviour in preventing stunting (Naulia *et al*., 2021). Nutrition education can be done face-to-face or online. In this research, the authors used online education for the media. So, in the previous articles, they found that online education can increase mother nutrition knowledge in stunting children. This assumption can be linked to the following discussion.

According to research by Setiani dan Sriwiyati (2022), online health education is effective in increasing stunting prevention behaviour. After getting the knowledge, providing online education about appropriate MPASI is also important to improve children's feeding practices (Tane and Sembiring 2021). Apart from that, education carried out online is effective in increasing public knowledge in Baubau City regarding preventing COVID-19 by using video media as well as leaflets (Mahmudah *et al.*, 2020). The same method but a different subject also can be effective.

Online education can be done in various ways, for example, through Zoom meetings, the WhatsApp application, or other Android-based applications. This is explained in research by Kadek *et al.* (2022) that stunting education using WhatsApp media is effective on the level of knowledge of parents who have stunted toddlers in Payangan sub-district in

ISSN 3031-0911

2022 and is also effective in providing information, then increasing knowledge among Madrasah Aliyah students (Siregar & Koerniawati, 2021). Meanwhile, the use of Android applications in educational methods as an effort to prevent stunting using a nurturing, asih and asah approach provides effectiveness in efforts to increase the knowledge of posyandu cadres (Anjani *et al*., 2022). This is also similar to research by Andoyo *et al*. (2022) that there was an increase in knowledge after being provided with education via the WhatsApp, Zoom and Trello applications.

Online education has been proven to be effective in increasing public knowledge and attitudes in preventing stunting. Providing education through posters directly or through media that can be accessed online/virtually can increase public knowledge and attitudes towards stunting prevention (Hendrawati *et al*., 2023). Android-based educational or education applications are also an effective medium in increasing teenagers' knowledge and attitudes towards stunting in this research (Putra *et al*., 2022). Online mentoring using videos and e-booklets simultaneously, targeting pregnant women, has more influence on increasing the average knowledge and energy consumption of respondents compared to using e-booklets alone or videos alone (Hikmiyah *et al*., 2019). So, online education or face-to-face education can be used to increase the knowledge of mothers in stunting children. At the end of this discussion, online education or face-to-face education can be chosen depending on the situation and condition.

Conclusions

Based on several articles that have been reviewed and analyzed regarding online nutrition education, the nutritional knowledge of mothers of children under five who are pregnant can be applied as an alternative for providing education, with the aim of conveying information well. Online nutrition education on the nutritional knowledge of mothers of stunting toddlers is known to be effective, considering that in several studies, it has been proven that there is an increase in knowledge after being given online nutrition education.

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The relationship between intake of iron, vitamin D and nutritional status on the incidence of anemia in pregnant women at Kebak Kramat 1 community health center, Karanganyar

Baarizah Febriana Badri[1*], Dono Indarto[2], Tonang Dwi Ardyanto[3]

[1,2,3] Department of Nutritios Science, Universitas Sebelas Maret, Surakarta City, Central Indonesia
*Correspondence author : baarizahfebriana@student.uns.ac.id

Abstract

Anaemia is one of the World Health Organization's five global health challenges for 2025. The causes of anaemia during pregnancy are linked to iron intake and nutritional status, which may result in severe consequences such as miscarriage, preterm birth, pre/postpartum haemorrhage, and low birth weight. This study aimed to investigate the association between iron intake, vitamin D, nutritional status, and the prevalence of anaemia during pregnancy. This was an analytical cross-sectional study involving a population of 132 pregnancies. The sample included 30 pregnancies. The data was analyzed through the Chi-square, logistic, and multiple linear regression tests. Iron deficiency demonstrated a 15-fold increase in the risk of anaemia (p-value 0.016) without correlation. Vitamin D intake is unrelated to anaemia, while nutritional status is unrelated to anaemia (p-value 0.787). Insufficient iron intake may increase the risk of anaemia, whereas a good vitamin D and adequate nutritional status may lower this risk. Therefore, further research is needed on the particularities of anaemia knowledge education during pregnancy.

Keywords : *Iron intake, Vitamin D, Nutritional State, Anemia, Pregnancy*

Introduction

Anaemia in pregnant women in Indonesia is still relatively high. According to Riskesdas data (2018), anaemia in pregnant women has increased by 48.9%. Meanwhile, in Karanganyar Regency in 2021, 1,163 cases of moderate anaemia (8-11 mg/dl) were found in pregnant women in Karanganyar Regency covering 21 regions (Karanganyar Public Health Service, 2021). Factors influencing the causes of anaemia in pregnant women. Risk factors for anaemia in pregnant women are related to iron intake, and the mother's nutritional status also influences cases of anaemia (El-Kholy *et al*., 2023; Yang *et al*., 2023). This research by Tadesse *et al.* (2021) said that Fe intake is eight times less likely to cause anaemia due to the less varied diet of pregnant women, especially foods that are sources of iron.

The source of iron that pregnant women often consume is vegetable side dishes because they are affordable. Apart from that, you also often consume sweet tea twice a day, which can inhibit iron absorption. Drinking tea has a 2.785 times greater risk of developing anaemia compared to pregnant women who never drink tea (Santhakumar *et al*., 2023; Wiafe *et al*., 2023)

An indicator of success in preventing iron anaemia is consuming food sources of iron such as animal protein (processed meat, processed chicken, processed fish) (Sukmawati *et al.*, 2019). Apart from that, the government has taken several steps to reduce cases of iron deficiency anaemia, namely by fortifying minerals and vitamins (such as Fe, zinc, folic acid, thiamine, and riboflavin) already on the market (Nugraheni *et al*., 2019). This study aims to determine the effect of differences in iron intake and nutritional status on anaemic pregnant women in the working area of the Kebak Kramat 1 Health Center, Karanganyar Regency.

Methods

This cross-sectional study was conducted in the Kebak Kramat 1 Community Health Center, Karanganyar Regency, for one month in July 2023. Based on the data of the health report from the Karanganyar public health office in 2022, there were 132 pregnant women with anaemia in the Kebak Kramat 1 Community Health Center. Research subjects in research are calculated using the formula from the application http://www.www.openepi.com/SampleSize/SSPropor.htm (Probandari *et al.*, 2020) from which we got at least 30 research subjects. The inclusion criteria included pregnant women in the first and second trimesters of pregnancy, aged 20-35 years old, and haemoglobin level <9-10 g/dl. Pregnant women who had high blood pressure (>120/80 mmHg), parity history >3, postpartum bleeding, and pregnancy history <2 years were excluded from this study.

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Data on basic characteristics and food frequency were collected by direct interviews using open and SQ-FFQ (semi-quantitative food frequency questionnaire) questionnaires, respectively. Data on the nutritional status were collected by measuring MUAC (mid-upper arm circumference). Numerical data were presented as mean and standard deviation and statistically analyzed using a linear regression test. Categorical data were presented as frequency and percentage and statistically analyzed using the chi-square test. Multiple logistic regression test was used to analyze independent and confounding variables against the dependent variable with a significant value <0.05.

Results and Discussions

The sample characteristics consist of maternal age, maternal education level, and family income, which can be seen in the following table:

Table 1. Characteristic sample

Subject Characteristic	Frequency n	Percentage %
Mother's Age		
No Risk (20-35 th)	**21**	**70**
Risk (>35 th)	**9**	**30**
Gestational Age		
First Trimester (4-13 weeks)	**7**	**23**
Second Trimester (14-26 weeks)	**21**	**70**
Third Trimester (27-40 weeks)	**2**	**7**
Mother's Education level		
Elementary School	**3**	**10**
Middle and High School	**24**	**80**
College	**3**	**10**
Family Income		
Below the regionl minimum wage (<Rp2.000.000)	**24**	**80**
Above the regionl minimum wage (>Rp2.000.000)	**6**	**20**

Based on **Table 1**, information was obtained that the majority of pregnant women were not at risk, as many as 21 people (70%), while nine people (30%) were pregnant women who were at risk (>35 years). Pregnant women with the majority of gestational age in the second trimester (14-26 weeks) were 21 people (70%), while in the first trimester, there were seven people (23%), and in the third trimester, there were two people (7%). Most pregnant women had a secondary education level (SMP-SMA), as many as 24 people (80%). In comparison, the maternal education levels were primary and higher (university) as many as three people (10%). The majority of the mother's family had an income below the regional minimum wage (<Rp. 2,000,000), as many as 24 people (80%), while the family income was above the provincial minimum wage (>Rp. 2,000,000) as many as six people (20%).

Table 2. Analyzed Univariate Independent and Dependent Variables

Independen and Dependen Variable	Frequency n	Percentage %
Iron Intake		
Not enough	**24**	**70**
Enough	**6**	**30**
Vitamin D intake		
Not Enough	**24**	**70**
Enough	**6**	**30**
Nutritional Status		
Malnutrition	**24**	**70**
Good Nutrition	**6**	**30**
Anemia Occurrence		
Anemic	**25**	**75**
Non Anemic	**5**	**25**

Based on **Table 2.** Most pregnant women's food intake is deficient in iron, as many as 24 people (70%), while six people (30%) have sufficient iron intake in pregnant women. Most pregnant women's food intake of vitamin D is

ISSN 3031-0911

deficient, as many as 24 people (70%), while pregnant women with sufficient vitamin D intake are six people (30%). Most pregnant women experienced poor nutritional status, 24 people (70%), while six pregnant women experienced good dietary grade (30%). Most pregnant women also experience anemia, as many as 25 people (75%), while five people (25%) do not experience anemia

Table 3. Relationship between Iron Intake, Vitamin D, and Nutritional Status on the Incidence of Anemia Using Bivariate Analysis

Variable	Anemia Occurrence				OR	CI 95%		*P*[a]
	Anemic		Not Anemic			Lower	Upper	
	n	%	n	%				
Iron Intake					15,00	1,652	136,172	0,016*
Not enough	19	45	5	25				
Enough	4	20	2	10				
Vitamin D Intake					0,427	0,033	4,228	0,417
Not enough	19	45	6	30				
Enough	4	20	1	5				
Nutritional Status					0,787	0,165	10,743	0,787
Malnutrition	19	45	5	25				
Good Nutrition	4	20	2	10				

P[a]: *Chi-Square Test* *) *P<0.05 Significant n: number of people*

Table 3 shows that iron intake is significantly related to the incidence of anaemia (OR=15, 95% CI, p=0.016), namely that 19 pregnant women (45%) who experienced insufficient iron intake resulted in anaemia. The OR value is 15, meaning inadequate iron intake can increase the risk of anaemia by 15 times compared to sufficient iron intake. Vitamin D intake was not significantly related to the incidence of anaemia (OR=0.427, 95% CI, p=0.417). Nineteen pregnant women (45%) who experienced insufficient vitamin D intake resulted in anaemia. The OR value is 0.427, meaning inadequate vitamin D intake can increase the risk of anaemia by 0.427 times compared to sufficient vitamin D intake. Nutritional status was not significantly related to the incidence of anaemia (OR=0.787, 95% CI, p=0.787); namely, 19 pregnant women (45%) who experienced poor nutritional status resulted in anaemia. The OR value is 0.287, meaning poor nutritional quality can increase the risk of anaemia by 0.287 times compared to good nutritional status.

Table 4. Relationship between Mothers age, Family Income, Mother's Education Level, and Gestational Age on the Incidence of Anemia Using Bivariate Analysis

Variable	Anemia Occurrence				OR	CI 95%		*P*[a]
	Anemic		Not Anemic			Lower	Upper	
	n	%	n	%				
Mother's Age								
No Risk (20-35 th)	6	30	3	15	0,287	0,048	2,457	0,279
Risk (>35 th)	7	35	4	20				
Family Income								
Below the regionl minimum wage (<Rp2.000.000)	9	45	5	25	0,139	0,616	32,069	0,128
Above the regionl minimum wage (>Rp2.000.000)	4	20	2	10				
Mother's Education level								
Elementary School	2	10	1	5	1,009	0,240	4,233	0,419
Middle and High School	9	45	5	25				
College	2	10	1	5				
Gestational Age								
First Trimester (4-13 weeks)	7	35	4	20	2,151	0,346	13,392	0,895
Second Trimester (14-26 weeks)	5	25	3	15				
Third Trimester (27-40 weeks)	1	5	0	0				

**) p 0.05 Significant n: number of people*

Based on **Table 4**, information was obtained that maternal age was not significantly related to the incidence of anaemia (OR=0.287, 95% CI, p=0.279), namely that six mothers (30%) were at risk of developing anaemia. Seven pregnant mothers (35%) are not at risk of anaemia. The OR value is 0.287, which means that the age of a mother at risk can increase the risk of anaemia by 0.287 times compared to that of a mother who is not at risk. Family income was not significantly related to the incidence of anaemia (OR=0.139, 95% CI, p=0.128). That is, nine pregnant women (45%) whose income was below the minimum wage (<Rp. 2,000,000) experienced anaemia, and as many as four pregnant

ISSN 3031-0911

women (20%) who earned above the minimum wage (>Rp. 2,000,000) experienced anaemia. The OR value = 0.139 means that mothers whose income is below the minimum wage can increase the risk of anaemia 0.139 times compared to mothers who earn above the minimum wage. The level of education was not related to the incidence of anaemia (OR=1.009, 95% CI, p=0.419). Namely, nine pregnant women (45%) had secondary education (middle and high school), and two pregnant women (10%) had essential (primary) and tertiary (college) education levels. An OR value of 1.009 means that pregnant women with a basic education level can increase the risk of anaemia by 1.009 compared to mothers with a higher education level. Gestational age was not significantly related to the incidence of anaemia (OR 2.151, 95% CI, p=0.895), namely seven pregnant women (35%) in the first trimester (4-13 weeks) and five pregnant women (25%) in the second trimester. (14-26 weeks), as many as one pregnant woman (5%) in the third trimester (27-40 weeks).

Table 5. Multiple Analyzed Independent Variable and Confounding

Variable	B	Wald	Sig	OR	CI95%	
Nutrition status	-1.544	0.382	0.537	0.214	Lower	Upper
Mother's age	-1.374	0.587	0.444	0.253	0.002	28.616
Gestational age	38.254	0.000	0.998	4.107	0.008	8.509
Level of education	-0.281	0.020	0.889	0.755	0.000	
Family income	36.991	0.000	0.998	1.162	0.015	38.579
Iron intake	-58.885	0.000	0.997	0.000	0.000	
vitamin D intake	16.935	0.000	0.999	22.64	0.000	

Pa: Multiple linear regression test *) p<0.05 significant

Based on **Table 5,** it can be concluded that of all the independent variables (nutritional status, maternal age, education level, family income, iron intake, and vitamin D intake it is thought not to influence anaemia in pregnant women. The largest OR value was 22.64, meaning that vitamin D intake has a 22.64 times chance of causing anaemia in pregnant women.

Based on **Table 1,** information was obtained that the majority of mothers were not at risk (20-35 years) as many as 11 people (55%). Meanwhile, nine mothers are not at risk (45%). Maternal age < 20 years or above and > 35 years can cause anaemia. This is because iron consumption for 20 years is divided by the fetus in the womb and its biological growth, which, of course, still requires a lot of iron consumption (George *et al*., 2021). After the age of 35, they enter an early degenerative stage when body function is not optimal, and they have various health problems. Pregnancies under 20 years and over 35 years are pregnancies with a risk of anaemia (Bellakhal *et al*., 2019; Sunuwar *et al*., 2019)
According to Parischa *et al*. (2023), mothers of at-risk ages can reduce the incidence of anaemia by 0.68 times compared to mothers of non-risk ages. The results of this study show that there is conformity with the theory put forward by (Guo *et al*., 2022) that the ideal maternal age in pregnancy is the 20-35 year age group and at this age, mothers have healthy reproduction and are less at risk of pregnancy complications. The age group < 20 years is at risk of anaemia because reproductive development is not yet optimal, and according to Kumar and Lahiri (2023), pregnancy in the 35-year age group is associated with deterioration and decreased endurance as well as various diseases that often occur at this age. Pregnancy at >35 years of age is a high-risk pregnancy because, at this age, chronic health problems often occur, one of which is the risk of anaemia. Bleeding that occurs during childbirth, if not handled properly, will cause anaemia. Apart from that, age is not the only factor that causes anaemia. There are other factors, namely socio-economic factors (Kang *et al*., 2023).

Based on **Table 1**, information was obtained that the majority of mothers had a secondary education level (SMP-SMA), as many as 14 people (70%). Meanwhile, there were three mothers (15%) who had primary (primary) and higher (university) education. According to research by Edison (2019), the relationship between education level and the incidence of anaemia in pregnant women shows that the prevalence of anaemia in mothers who have a low level of education reaches 90.3% compared to mothers who have a high level of education, which is only 9.7%. The Chi-Square test results obtained a value of ρ= 0.001. This is due to the mother's lack of knowledge on how to process good food so that it does not damage the nutritional content in it and also due to low education, the mother does not work, which reduces household income, and the mother cannot buy nutritious food so that due to this limitation anaemia occurs (Panchal *et al* ., 2022). Based on interviews during the preliminary study, mothers only received education from health workers at the Community Health Center only once a month. So, the information obtained is lacking because, during the education class for pregnant women, only one theme is given, such as consuming blood supplement tablets regularly.

ISSN 3031-0911

Based on **Table 1**, information was obtained that the majority of family income was below the minimum wage (<Rp. 2,000,000), as many as 14 people (70%). Meanwhile, six people (30%) had family income above the minimum wage (>Rp. 2,000,000). The prevalence of anaemia is greater in pregnant women with incomes lower than the Regional Minimum Wage (UMR). This affects the purchasing power of food families consume because >57% of family income is spent on purchasing food (Gibore et al., 2021; Pasricha et al., 2023). According to Septiasari (2019) research, it was found that 25 out of 39 pregnant women (61.0%) of mothers with income < minimum wage experienced anaemia, while among pregnant women with income ≥ minimum wage, there were 16 out of 47 people (39.0 %) have anaemia. The results of the chi-square statistical test show that the p-value = 0.005 (p ≤0.05), RP 3.460 (95% CI = 1.421 – 8.425), so it can be concluded that mothers with an income <UMR increase the incidence of anaemia by 34 times compared to mothers with an income >UMR causes mothers not to get adequate nutrition, thereby risking anaemia. The number of families will influence the food distribution within the family. The lack of family income causes a reduction in the location and purchase of daily food, thereby reducing the quantity and quality of the mother's food per day, which has an impact on reducing nutritional status. A common nutritional disorder in pregnant women is anaemia. The food sources needed to prevent anaemia generally come from protein sources, which are more expensive and difficult for those with low incomes. This deficiency increases the risk of anaemia in pregnant women and accelerates the risk of morbidity in mothers (Mekonen & Alemu, 2021; Maugliani & Baldi, 2023). Based on preliminary study interviews, as many as 65% of pregnant women said they consumed two pieces of vegetable protein (tempeh, tofu, processed nuts) 3-4 times per week.

Based on **Table 1,** information was obtained that the majority of pregnant women with poor nutritional status were 14 people (70%). Meanwhile, there were six pregnant women with good nutritional status (30%). Steven et al. (2022) stated the relationship between nutritional status and the incidence of anaemia. *Nutritional status* is also defined as health status resulting from a balance between nutrient needs and input and is a basic need for pregnant women. Nutritional status and the incidence of anaemia were also shown by Dewi and Mardiana (2021), who found that the risk of anaemia in pregnant women was 2.9 times higher for pregnant women with poor nutritional status than those with good nutritional status. This comparative figure has a large role in influencing the health of pregnant women. The estimated determinant of R2 is 0.047, meaning that nutritional status contributes 4.7% in influencing the incidence of anaemia. Even though the contribution value is small, as long as the regression coefficient β1 is not statistically zero, scientifically, it can be proven that there is an influence between nutritional status and the incidence of anaemia (Guo *et al*., 2022)

Based on **Table 1**, information was obtained that the majority of mothers' iron intake was less than 14 people (70%). Meanwhile, six people (30%) had sufficient iron intake, according to research conducted in Jatinangor District regarding the consumption patterns of pregnant women, which shows that the intake of food sources of iron (Fe) in pregnant women is still inadequate, namely 53 people (93%) out of 57 pregnant women (Putri *et al*., 2020). This happens because the source of iron consumed does not come from heme, such as meat and animal food sources, so it is not easy to absorb and does not support the presence of iron in the body. The same thing was done in research in TuaTunu Pangkal Pinang Village regarding the relationship between nutritional intake and the incidence of anaemia in preconception women, which showed that the intake of food sources of iron (Fe) in preconception women was still insufficient, namely 98.4% (Devriany & Wardani, 2019). This is because the consumption of less balanced foods can interfere with the absorption of iron in the body, and the majority of respondents consume more vegetable protein than animal protein. In addition, the lack of consumption of side dishes, which are a source of iron which is useful during pregnancy for the formation of new haemoglobin, compensates for the small amount of iron which is constantly excreted by the body (especially through urine, faeces and sweat), and replaces iron losses during pregnancy—lactation for milk secretion (Devriany & Wardani, 2019).

Mothers who are malnourished are at risk of having a difficult or long labour, giving birth to a baby prematurely (not yet full term), bleeding in the mother after giving birth and usually during labour, the mother also lacks the strength to push during the labour process so giving birth using a high surgical procedure for pregnant women lack nutrition (Xu *et al*., 2022). There are 41% of pregnant women suffer from malnutrition. The emergence of nutritional problems in pregnant women, such as the incidence of CED, cannot be separated from the social, economic, and biosocial conditions of pregnant women and their families, such as education, income level, food consumption, age, parity and so on (Andersen *et al*., 2022). Malnutrition can cause the mother to suffer from anaemia; the blood supply that delivers oxygen and food to the fetus will be hampered, so that the fetus will experience impaired growth and development. Therefore, monitoring the nutrition of pregnant women is very important (Nuru *et al*., 2021).

ISSN 3031-0911

Based on **Table 1**, it was found that the majority of mothers' vitamin D intake was less than 14 people (70%). Meanwhile, six people (30%) had sufficient vitamin D intake. Vitamin D is a vitamin that is needed for various metabolic processes in the body. It is fat-soluble and is produced by human skin with energy obtained from food intake. Vitamin D deficiency in pregnant women has an impact on the fetus and newborn. The prevalence of vitamin D deficiency is 63% in Indonesia and Malaysia. According to Smith M. Ellen and Tang Pricha Vin (2019), vitamin D can increase erythropoiesis by increasing the proliferation of erythroid progenitors and reducing proinflammatory cytokines. Additionally, by decreasing proinflammatory cytokines that stimulate hepcidin and through direct transcriptional regulation of the HAMP gene, vitamin D can suppress hepcidin expression. Reducing proinflammatory cytokines and hepcidin may increase iron bioavailability for erythropoiesis and haemoglobin synthesis by restoring iron recycling, preventing iron absorption in macrophages, and eliminating impaired iron absorption, thereby protecting against anaemia.

Conclusions

Iron intake is significantly related to the incidence of anaemia ($p=0.016<0.05$) and can increase the risk of anaemia 15 times. Meanwhile, vitamin D intake is not significantly related to the incidence of anaemia ($p=0.417>0.05$). Nutritional status is not significantly related to the incidence of anaemia ($p=0.787>0.05$). Factors that have a significant influence on anaemia are family income and gestational age. There is a need for further research regarding interviews using recall to determine the food ingredients consumed by respondents and education using questionnaires regarding anaemia in pregnant women.

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Analysis of the effectiveness of herbal drink consumption on emesis gravidarum in pregnant women in the senapelan, Pekanbaru city

Desy Winda[1]*, Endah Purwani Sari[2], Fatma Richa Rahmana[3], Mona Dewi Utari[4]

[1,2,3,4] Department of Midwifery, Sekolah Tinggi Ilmu Kesehatan Pekanbaru, Indonesia
*Correspondence author : desywinda12@gmail.com

Abstract

Emesis gravidarum is a common condition during the first trimester. However, in some instances, this condition can escalate to emesis gravidarum, elevating the risk of pregnancy complications. Pregnant women have long recognized the use of herbal drinks containing ginger and honey as an alternative approach to mitigate the symptoms of nausea and vomiting. The objective of this research is to evaluate the efficiency of herbal drink consumption in mitigating emesis gravidarum symptoms, with a specific focus on pregnant women living in the Senapelan Subdistrict of Pekanbaru City. The research involved 14 respondents who were observed periodically, employing a Group Pre-Post Test by Quasi-Experimental design. Statistical analysis revealed a positive impact of herbal drink consumption. Before the intervention, 57.1% of respondents experienced moderate Emesis, 35.7% experienced severe Emesis, and 7.1% experienced mild Emesis. Following the consumption of herbal beverages, these percentages shifted to 71.4% experiencing mild Emesis and 28.6% experiencing moderate Emesis. The significant efficacy of herbal drink consumption is confirmed in alleviating emesis gravidarum symptoms among pregnant women in the Senapelan Subdistrict of Pekanbaru City by the bivariate analysis results with a p-value of 0.000. Consequently, it is recommended that healthcare professionals provide non-pharmacological education to pregnant women regarding the benefits of consuming herbal drinks to mitigate nausea and vomiting during pregnancy.

Keywords: Herbal Drink, Ginger, Honey, Pregnant Women, Emesis Gravidarum

Introduction

Emesis gravidarum (EG) is a commonly encountered early pregnancy condition characterized by severe nausea and vomiting, typically occurring during the first trimester. While it often improves by the second or third trimester, persistent EG is rare (Pudiastuti, 2012). EG can adversely affect maternal and fetal health, including a reduced appetite, leading to electrolyte imbalances such as potassium, calcium, and sodium, ultimately affecting maternal metabolism. The fetus may also suffer from nutritional deficiencies and inadequate fluid intake, potentially resulting in low birth weight (LBW) and developmental disruptions (Rose & Neil, 2016).

The global prevalence of Emesis Gravidarum is approximately 15% among pregnant women, as reported by the World Health Organization in 2015. However, the incidence rates of EG vary significantly worldwide. For example, Indonesia has a relatively high incidence, with rates ranging from 1% to 3%. In Sweden, the rate is lower at 0.9%, while California reports a rate of 0.5%. In contrast, Turkey reports a higher prevalence of 1.9%, and the United States has an incidence range of 0.5% to 2%. Taking a closer look at Indonesia, among 2,203 observed pregnancies, 543 pregnant women experienced EG, indicating that approximately 10% of pregnant women in the country are affected by this condition. In a specific case, the Riau region reported a prevalence of 10% to 15% among 182,815 pregnant women in 2015, as documented by the Indonesian Ministry of Health (Depkes, 2015).

Furthermore, at the Senapelan Primary Health Center, there were 116 cases of Emesis Gravidarum in 2022, a number similar to the 112 cases reported in 2020. It is important to note that these cases encompass various levels of severity, including levels 1, 2, and 3 of Emesis. Overall, Emesis Gravidarum is notably more prevalent among women experiencing their first pregnancies. Midwives play a crucial role in providing care to EG patients, including gathering relevant client information, identifying client issues, developing care plans, implementing midwifery care, and evaluating client progress. They should also educate patients about pregnancy and childbirth as physiological processes, reassuring them that nausea and vomiting are expected in early pregnancy and tend to subside after the first four months. Midwives may recommend dietary modifications, such as consuming smaller, more frequent meals, avoiding oily or pungent foods, and serving hot or cold meals (Lisnawati, 2013).

According to Ayuputri (2017), non-pharmacological therapy with ginger-infused herbal drinks can reduce nausea and vomiting in EG patients. The research demonstrates that an herbal drink containing ginger and honey effectively alleviates these symptoms. The herbal drink is prepared by infusing ginger with hot water, sweetened with honey, and consumed in the morning. These herbal drinks are administered three times a week on Mondays, Wednesdays, and Saturdays, with follow-up interviews to assess the frequency of nausea and vomiting.

A preliminary survey at the Private Clinic in Senapelan, Pekanbaru City, revealed that out of 10 pregnant women, 6 experienced excessive nausea and vomiting and were unaware of the potential benefits of ginger-infused herbal drinks. Based on this research, the researcher was motivated to investigate the effectiveness of herbal drink consumption in managing Emesis Gravidarum in pregnant women in Senapelan, Pekanbaru City.

Methods

This study utilizes quantitative methodology, specifically adopting a Quasi-Experimental design featuring a Group Pre-Post Test. It was conducted at the Private Clinic in Senapelan, Pekanbaru City, during June 2023. Initial preparations encompassed a preliminary survey conducted in February 2023 involving ten pregnant women, six of whom were grappling with severe nausea and vomiting. According to Ayuputri (2017), non-pharmacological therapy involving herbal drinks and a modest amount of honey can effectively alleviate nausea and vomiting in individuals suffering from emesis gravidarum. The herbal drink consists of 2 pieces of ginger root boiled in 1 litre of water for 10 minutes until it yields one cup of infusion. To sweeten it, add one tablespoon of honey, and it should be consumed while still warm, given three times a week.

Ginger, a versatile plant with a rich history of medicinal applications, holds a crucial place as a spice in culinary practices, beverages, and traditional healing methods. Ginger, scientifically known as ZingiberZingiber, belongs to the Zingiberaceae family and has gained global recognition due to its wide-ranging versatility. Its composition includes essential oils (such as bisabolene, cineol, phellandrene, citral, borneol, citronella, geranial, linalool, limonene, Zingiber, zingiberene, camphene), oleoresins (gingerol, shogaol), phenolic compounds (gingerol, zingerone), proteolytic enzymes (Zingiber), vitamins B6 and C, as well as essential minerals like calcium, magnesium, phosphorus, and potassium. Additionally, it contains linoleic acid and gingerols, which are alcohol compounds found in oleoresins, making up around 1-3% of ginger's composition (Permenkes, 2016).

Alyamaniyah and Mahmudah (2014) propose that to alleviate emesis gravidarum in pregnant women, administering ginger-infused herbal drinks should involve a regimen of twice-daily consumption for four consecutive days, with a maximum daily intake of 250 ml. According to Nasution et al. (2016), the measurement scale for evaluating the administration of ginger-infused herbal drinks to pregnant women can be classified as follows: categorized as mild if maternal nausea and vomiting occur 1-3 times daily following the consumption of ginger-infused herbal drinks, classified as moderate if maternal nausea and vomiting happen 4-7 times daily after ginger-infused herbal drink consumption, designated as severe if maternal nausea and vomiting manifest 8-10 times daily following the intake of these herbal drinks.

Results and Discussions

The researcher conducted research results through observations on 14 pregnant women to assess the effectiveness of herbal drink consumption in managing Emesis gravidarum at a Private Clinic in Senapelan, Pekanbaru City.

Table. 1. Frequency Distribution Before and After Herbal Drink Consumption

No	Emesis	Before		After	
		Frequency	%	Frequency	%
1.	Mild	1	7,1	10	71,4
2.	Moderate	8	57,1	4	28,6
3.	Severe	5	35,7	0	0,0
	Total	**14**	**100**	**14**	**100**

It is known that before the herbal drink was administered, eight individuals (57.1%) experienced moderate Emesis, five individuals (35.7%) experienced severe Emesis, and one individual (7.1%) experienced mild Emesis. After administering an herbal drink, there was a decrease in emesis severity, with ten individuals (71.4%) experiencing mild Emesis and four individuals (28.6%) experiencing moderate Emesis.

Table 2. Data Normality Testing

Variable	p.value
Before	0,271
After	0,022

Table 2 shows that before an herbal drink was administered, the p-value was 0.271. After administering an herbal drink, the p-value was 0.022, as determined using the Shapiro-Wilk test. The Shapiro-Wilk test was chosen because the sample size was less than 50 respondents. These results can be interpreted as the data distribution is non-normal, as one p-value is less than 0.05. Given that the data used is not normally distributed, the appropriate test is the non-parametric Wilcoxon test or the Signed Rank Test.

Table 3. The Effectiveness of Herbal Drink Consumption on Emesis Gravidarum in Pregnant Women

Method	Treatment	Deviation Standart (Min-Max)	P value
Consumption of Herbal Drink	Before	1,939 (3 – 9)	0,000
	After	1,188 (0 – 4)	
Deviation		3 (21,4%)	

Table 3 presents data showing that before herbal drink consumption, the standard deviation was 1.939. This indicates that, on average, pregnant women experienced emesis gravidarum about two times a day, with a minimum occurrence of 3 and a maximum of 9. Following the consumption of ginger infusion, the standard deviation decreased to 1.188, signifying those pregnant women experienced Emesis gravidarum an average of 1.1 times, with a minimum of 0 and a maximum of 4. The difference between the occurrences before and after herbal drink consumption was 3. The effectiveness assessment, utilizing the Wilcoxon or Signed Rank Test, resulted in a p-value of 0.000, which is less than α (alpha). This signifies a notable effectiveness in reducing Emesis gravidarum among pregnant women through the consumption of the drink. This is in line with Chandranita (2011), which states that Emesis gravidarum is a common complaint in early pregnancy due to hormonal changes, namely an increase in the hormones estrogen, progesterone, and the release of human chorionic gonadotropin in plasma. Non-pharmacological and pharmacological interventions can manage nausea and vomiting during pregnancy. Non-pharmacological measures often recommended by healthcare professionals include suggesting pregnant women consume ginger in ginger tea, relaxation techniques, and aromatherapy (Runiari, 2015).

Ginger (*Zingiber officinale*) has a long history as an antiemetic remedy. Its effects are believed to be related to increased peristaltic movement in the digestive tract due to its anti-cholinergic and anti-serotonin properties. Ginger directly affects the

digestive tract and does not have any impact on the central nervous system. In traditional medicine, ginger is sometimes discouraged for use during pregnancy due to concerns that it might trigger menstruation or bleeding. In traditional medicine, ginger is often considered a contraindication for use by pregnant women because it is believed to induce menstruation or bleeding. However, no clinical evidence supports the theory that ginger acts as an abortifacient (Pratimin, 2016).

According to Rofi'ah (2017), ginger is a powerful aromatic stimulant that can regulate vomiting by enhancing intestinal peristalsis. Several research findings indicate potential advantages of ginger, including cancer prevention, relief from pregnancy-related nausea and vomiting, mitigation of nausea and vomiting in chemotherapy patients, and postoperative nausea and vomiting. Pregnant women can consume ginger in any form they prefer. By Alyamaniyah (2014), it is asserted that ginger comprises a minimum of 19 components that offer various benefits to the body. Among these components, gingerol is the most notable compound, known for its proven antiemetic (anti-vomiting) properties, primarily achieved through serotonin blockade, a chemical messenger. This compound triggers contractions in the stomach, so when it is obstructed, the muscles in the digestive tract relax, resulting in a significant reduction in nausea. Following the administration of ginger infusion, the occurrence of Emesis gravidarum decreased, attributable to the influence of ginger's constituents, particularly gingerols and shogaols, which effectively block serotonin—a chemical substance responsible for inducing nausea.

The findings of this research align with those from a study conducted by Aini *et al.* (2010) regarding the impact of ginger herbal drinks on nausea and vomiting in pregnant women suffering from Emesis gravidarum within the Wirobrajan Health Center's jurisdiction in Yogyakarta. The paired t-test analysis resulted in a p-value of 0.000 (<0.05), indicating the influence of ginger infusion on pregnant women experiencing Emesis gravidarum in that area. Furthermore, a study by Hasanah *et al.* (2014) supports these findings, as it yielded a p-value of 0.000 (<α=0.05), signifying a notable difference in Emesis gravidarum frequency among pregnant women before and after receiving ginger infusion at the Tebalo Manyar Village Health Center in Gresik in 2014. These research outcomes are consistent with Rusman (2017), which proposes that ginger infusion effectively reduces nausea and vomiting in pregnant women during the first trimester. Prior to the intervention, most pregnant women experienced Emesis gravidarum up to 13 times daily. However, on average, after the ginger infusion intervention, the frequency of nausea and vomiting decreased by 3.18 times per day.

The researcher believes that providing ginger infusion can offer a beneficial solution for mitigating vomiting and can be utilized by pregnant women to alleviate morning sickness. Following the consumption of ginger infusion, there was a notable shift in the occurrence of nausea and vomiting among pregnant women, underscoring the considerable effectiveness of ginger infusion in alleviating these symptoms during the initial trimester. As indicated by the findings, women who incorporated ginger infusion into their regimen experienced a warming sensation in their stomachs, accompanied by a reduction in Emesis gravidarum following its consumption. Ginger contains essential oils, zingiberene, zingiberol, bisabolenes, curcumin, gingerol, and landrene, as well as vitamin A and bitter resin. Ginger is believed to provide comfort in the stomach to alleviate nausea and vomiting. Ginger also has a direct effect on the digestive tract and absorbs toxins and acids because the substances in ginger can block serotonin, a neurotransmitter in the central nervous system, and enterochromaffin cells. Thus, it inhibits the induction of HCG in the stomach. Finding ginger is easy because this plant is widely used as a spice, flavouring in various foods and drinks, and traditional medicine ingredients. Another advantage of consuming ginger infusion to manage Emesis gravidarum is that it is inexpensive and readily available, making it easily accessible in the market. Ginger infusion is safe from harmful ingredients because it can be made at home, so pregnant women do not need to worry about endangering their pregnancy and their fetus. Based on the research results obtained from 14 respondents, the average frequency of nausea and vomiting before drinking the herbal drink was six times. After consuming ginger and honey herbal drinks, the average frequency of nausea and vomiting among respondents was three times. The difference between before and after consumption was three times. The percentage of the effectiveness of consuming herbal drinks was 50%. Based on the results, women who consumed the herbal drink felt warmth in their stomachs, and Emesis gravidarum decreased afterwards.

Conclusions

This research concluded that before the administration of ginger and honey herbal drink, it was found that eight individuals (57.1%) experienced moderate Emesis, five individuals (35.7%) experienced severe Emesis, and one individual (7.1%) experienced mild Emesis. After the administration of ginger infusion, it was found that ten individuals (71.4%) had mild Emesis, and four individuals (28.6%) had moderate Emesis. Herbal drink that contained ginger infusion and honey is effective with a p-value of 0.000 < 0.05 against Emesis Gravidarum at a Private Clinic in Senapelan, Pekanbaru City.

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Assessment of the needs and role of nutritionists in schools in indonesia? How important? : A literature review

Imtiyaz Irbatunnisa[1*] , Delita Septia Rosdiana[2] , Anisa Nur Wulan[3] , Nanda Fitria Salsabila[4] , Tsaltsa Najwa Fitria[5]

[1,2,3,4,5]Department of Nutrition Science, Sports and Health Education, Universitas Pendidikan Indonesia, Bandung, Indonesia

*Correspondence author : imtiyazirbtn17@upi.edu

Abstract

School age is a crucial period in the children's growth and development. Adequate food intake is needed to support children's ability to learn so that children's growth and development can be achieved optimally. Health problems in the group of school-age children are of particular concern to parents and parts of the school. Parents or teachers pay less attention to the food consumed by students in the school, which can lead to nutritional health problems such as deficiency or excess nutrition in school children. School public health can be intended as a vehicle for implementing health programs, such as programs for nutrition, reproductive health, environmental health, and treatment. The implementation of school health efforts is closely related to the experts in charge of implementing children's food at school. This study aims to determine how vital nutritionists' role in the school environment is. The method used is a literature review by analyzing the literature that intersects with the objectives of this research. One example of a food service program is school catering or healthy canteens assisted by nutritionists in compiling food menus so that children can continue to consume healthy snacks and meet their daily energy needs. Based on these results, the presence of a nutritionist at school can play an essential role in organizing meals and monitoring the nutritional status of children regularly.

Keywords: assessment, nutritionists, policy and needs, school feeding program

Introduction

School-age children are the country's most influential human resource assets. School-age children are also the nation's investment as successors who determine the nation's future quality (Kaluku & Junaeni, 2022). Nutrition is one of the factors that has a vital role in shaping quality human resources. Nutritional adequacy is very influential for human health and work productivity. Physical, intellectual, mental, and social growth occurs quickly during school age. School-age children will need more nutrition to support their development and activities. At this age, more attention is required so that children can meet the needs of nutritional intake that has good quality and quantity (Uce, 2018). School-age children are an age group that is prone to nutrition and disease, so it is necessary to monitor children's nutritional intake, especially at school age (Permatasari *et al*., 2023). School-age is one of the crucial periods in the growth and development of children, with nutritional problems in school-age children that can result in low quality of education, high absenteeism, and high dropout rates (Sari, 2022).

During their development, school-age children are categorized as having a relatively dense level of physical activity because they are usually more active in extracurricular activities, sports training, and private lessons (Juniar, 2020). Problems related to lack or excess nutrition in children can affect children's health during their development. Children can experience decreased concentration in learning if they have a poor nutritional status (Utami, 2019). The parents must pay attention to the health and adequacy of food for children, especially if children have activities outside of school hours, such as lessons or other training.

Under Law No. 36 of 2009 Article 79 on health, the Act has mandated nutrition improvement efforts to improve the quality of individual and community nutrition, among others, through improving food consumption patterns, improving nutrition, physical activity, and health awareness behaviours behaviours, and increasing access and quality of nutrition services following advances in science and technology so that the lunch program with appropriate nutrition services can improve the ability of healthy living of students in a healthy living environment so that students can learn, grow and develop harmoniously and to the greatest extent so that they are expected to become quality human resources (BPOM, 2013).

The School Feeding Programme is a school feeding program launched in many countries, especially those with a high prevalence of malnutrition and hunger. The program has been implemented in more than 70 low-income countries with support and supervision provided by the World Food Programme to improve children's performance as students by providing school meals. According to the World Food Programme's official website, the school feeding program has

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existed for six decades. It has successfully made a difference in more than 50 countries around the world, with the initial goal not only focusing on improving the health of school children but also achieving agricultural goals (World Food Programme, 2022).

A report published by the African Union noted that food delivery programs have successfully reduced the prevalence of hunger and nutritional diseases such as malnutrition and micronutrient deficiencies. Developing Countries such as South Africa have maximized the implementation of the school meals program (SFP) by providing healthy meals with additional fortification of some foods to address high-prevalence nutritional problems such as iron deficiency, folate, vitamin A, and various other macronutrients in children (World Food Programme, 2021).

This research is justified by the need to know how the presence of nutritionists affects school feeding programs in Indonesia compared to other countries that have been working around this program for years or decades. The findings of this study will describe why nutritionists have an essential role in school feeding programs.

Methods

Publications were identified by searching various electronic databases on the last ten years of publications (2013-2023). Search terms incorporating school feeding programs, nutritionists, school-aged, policy and needs, developed country, low- or middle-income country, and Indonesia were combined to retrieve relevant literature. In addition, manual searches were conducted, and articles were retrieved from those listed in critical papers. In this paper, we used search engines like Google Scholar and Publish or Perish to complete information for this research and inspect for further accuracy towards the articles. According to the operational definition used, the literature review used four journals.

Results and Discussions

The study results were analyzed by comparing School Feeding Programs in several countries that have included nutritionists in their implementation to success rates and benchmarks. The data is presented in the following articles:

Table 1. Comparison of the Implementation of School Feeding Programmes and the role of nutritionists in different countries

No.	Article/ Journal	Title	Country	Nutritionists' Role	Level of Success	Benchmark
1.	Kitaoka (2018)	The National School Meal Programme in Brazil: A Literature Review	Brazil	They are involved in menu planning and guidance, monitoring food handling, training cooks and managers, and nutritional education.	Nutritional management, quality control, education, nationalized systems, nutritional standards, and nutritionist arrangements are relatively advanced.	The school meal program in Brazil has broad coverage, particularly in secondary schools and education units for youth and adults. Furthermore, it includes elements such as nutritional management, quality control, nutritional education, nationalized systems, nutritional standards, and nutritionist arrangements.
2.	Rivera & MPA-HM (2017)	The Implementation of The School-Based Feeding Programme (SBFP) In The Schools Division Of Tarlac Province	Tarlac, Philippines		Outstanding in its coverage while very satisfactory in its personnel performance, adequate in its financial allocation and facilities.	The primary objectives of the SBFP were mainly accomplished, particularly in restoring a minimum of 70% of severely malnourished individuals to good nutritional health within 100 to 120 feeding days. Furthermore, the program effectively reached its goal of ensuring that 85% to 100% of recipients regularly attended classes, and it also made notable advancements in enhancing the well-being, nutritional status, and behavior of children.
3.	Aliyar (2015)	A review of Nutritional Guidelines and	Kenya	Improving health and academic performance in both	The program functions by the Government,	Enhance enrollment, attendance, and persistence in schools while raising the nation's literacy

ISSN 3031-0911

		Menu Compositions for School Feeding Programmes in 12 Countries		public and private schools	providing funds directly to the School Management Committees (SMCs) for the procurement of food items.	achievement.
4.			Italy		Strong focus on organic food and sustainability, along with the endorsement of Italian agricultural methods and the rich culinary tradition.	The Italian Government invests in ingredients and the school meal service. Local authorities purchase foodstuffs for meal preparation.
5.			USA		It is enhancing the physical and emotional welfare of students in schools.	At the national level, the USDA administers the National School Breakfast Program and the National School Lunch Programme, while at the community level, state education agencies manage these initiatives.
6.	Ishida (2015)	Role of School Meal Service in Nutrition	Japan	The school nutritionist is responsible for food service management and facilitating food and nutrition education.	The influence of school meal service on eating habits is not as prevalent	In many cases, the direct impact on the physical condition cannot be substantially ascertained except in terms of limited food and company supplies with nutritional deficiencies. Japan is currently facing a scenario where schoolchildren show little change in their physique, and the impact of school meal services is difficult to ascertain.

Some countries have programs that have yet to be measured in terms of their achievements. The direct impact of school feeding in Japan on students' physical condition cannot be concretely ascertained, with little physical change. The school feeding program in England needs to be more equitable in its implementation as some students consume the provided food at school, and some bring food from home due to the unclear food standards set at school.

A comparison was made of the four journals in **Table 1** covering six countries: Brazil, Philippines, Kenya, Italy, Japan, and the USA. Based on this comparison, Brazil has set up nutritionists' roles such as menu planning and guidance, monitoring how food is handled, training cooks and managers, and food and nutritional education. Meanwhile, the Philippines still needs to explain nutritionists' role further, but the program has reduced the prevalence of severe hunger by up to 70%. In Kenya, nutritionists were expected to improve health and academic performance in school. In Italy, no nutritionist role is stated either, but the nutritionist strongly focuses on organic food and sustainability while keeping the Italian culture. In Japan, the school nutritionist manages food service and facilitates food and nutrition education. Lastly, no nutritionists' roles are explained in the United States of America, but they have already succeeded in enhancing students' physical and emotional welfare in schools.

School Feeding Programmes

School Feeding Programmes (SFPs) or School Meals Programmes are policies that provide healthy meals to children in schools. This policy is an intervention to reduce hunger and nutritional problems such as micronutrient deficiencies and obesity. It can improve cognitive and academic abilities so that it is considered to enhance the quality of human resources (Aliyar *et al*., 2015). School Feeding Programmes have been implemented in various countries ranging from high-income to medium to low-income, albeit with limited coverage (Wang & Fawzi, 2020).

Research conducted in two elementary schools by Palupi *et al.* (2020) shows that both schools do not use nutritionists in organizing school lunches, and the results obtained based on these data show that the fulfilment of energy and nutrients lower than 80% due to lunch intake does not meet the nutritional needs of students. In addition, the menu has been considered quite varied, but one school still needs to provide fruit in implementing the menu. This result

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is also supported by research conducted by Cohen (2013) in one of the schools that organized lunch, which showed that students only consumed less than the nutritional recommendations of 85%.

In addition, kitchen facilities and school infrastructure with school feeding programs are also influential in providing meals because they can directly impact the quality of food served. Schools with school feeding programs that operate within the school grounds with sturdy buildings, good sanitation and hygiene, and direct distribution can minimize the occurrence of food spoilage or a decrease in the quality of the food provided (Ronitawati, 2016). This situation has an impact on the occurrence of problems that hinder the implementation of this program, such as low food acceptance, resulting in high rates of food waste from menus that students do not eat, so food service management is needed that can support the level of food acceptance by students (Setyaningtyas *et al.*, 2022).

The role of the school nutritionist

Referring to Ronitawati's (2016) study comparing two other primary schools, it was found that schools that had directly involved qualified nutritionists in the provision of food by considering age, gender, and the budget provided by the foundation had better outputs. When compared to other schools that do not directly involve nutritionists, schools with nutritionists have better organization from input (human resources, raw materials, funds, and methods), process (menu planning, processing, serving distribution, reporting), to output (menu quality and acceptability of student consumption) so that the implementation becomes better. It has a direct effect on the nutritional fulfilment of students at school.

Brazil, one of the countries with the world's most extended school feeding program, states that nutritionists are one of the most critical components of a successful school feeding program. This statement is demonstrated by a document containing the duties and obligations of a nutritionist to succeed in the PNAE or National School Food Programme in the Southern Region of Brazil. The document contains 13 mandatory and nine complementary activities that include carrying out a diagnosis of the students' nutritional status; planning, preparation, follow-up, and evaluation of the school meal menu; human resources training; sanitary hygienic quality control; coordination and implementation of food and nutrition education (EAN) actions (Correa *et al*., 2017).

Best Practice School Feeding Programmes in countries where nutritionists are involved

School feeding programs are crucial in tackling food insecurity, enhancing nutritional education, and improving overall health results (Cupertino *et al*., 2022). In its implementation some countries have successfully implemented policies related to school feeding programs and have achieved several benchmarks of success, including reducing the prevalence of nutritional problems, especially in school children.

Based on the results obtained after comparing the countries in **Table 1**, it is found that school nutritionists in each country have roles that are not much different from other countries, namely being responsible for menu planning and evaluation, monitoring the running of food delivery activities, planning according to budget, and adjusting according to the culture of each country. However, all these countries have their achievements despite the similarities in the duties and obligations of a school nutritionist. The Philippines has managed to reduce the prevalence of severe malnutrition by 70% after 120 days of SFP, while in Kenya, SFP has reduced student absenteeism in schools and even raised the nation's overall literacy achievement. SFP in Japan led to physical changes in students, although not significant. The role of nutritionists in Brazil led to a balance in the coverage of nutritional elements so that the output was relatively advanced. This statement aligns with research conducted by Verguet *et al.* (2020), which states that the School Feeding Programme can enhance learning and support higher educational attainment.

The research of Setyaningtyas *et al.* (2022) stated that many school children experienced dissatisfaction with the menu served at school based on several indicators such as taste, texture, aroma, cleanliness, and colour. The result showed that vegetables became one of the variables with the worst assessment due to their unattractive appearance when served, so they became the most significant percentage of discarded food ingredients. The absence of nutritionists who are directly involved in the organization of school meals can be one of the causes of student dissatisfaction with the food served.

According to the observation results, food performances are strongly influenced by how the food is prepared, and this is where the role of a nutritionist is needed. According to research conducted by Correa *et al.* (2017), nutritionists are in charge of promoting healthy eating habits to students and supervising the organization of food in schools, including preparing menus and monitoring food purchases.

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Regulation, the urgency of the role of school nutritionists in school feeding programs, and the need for nutritionists in the education sector

Under Law No. 36 of 2009 Article 79 on health, the Act has mandated nutrition improvement efforts to improve the quality of nutrition of individuals and communities, among others, through improving food consumption patterns improving nutrition, physical activity, and health awareness behaviour; and improving access and quality of nutrition services following advances in science and technology. Thus, the lunch program with appropriate nutrition services can enhance the ability of students to live a healthy life in a healthy environment so that students can learn, grow, and develop harmoniously and at the highest level so that they are expected to become quality human resources (BPOM, 2013).

One of the competencies that a nutritionist must possess is supervision to design menus following consumers' needs and nutritional status, control food production that meets nutritional adequacy, estimate the cost of acceptability, and develop food standards for students and employees (MOH, 2015). Although regulations on improving nutrition by increasing access have been passed, Indonesia has no mandatory school feeding program (Setyaningtyas *et al*., 2022).

Conclusions

Nutritionists play an essential role in implementing School Feeding Programmes to improve the nutritional status of children in schools, according to countries that have implemented such programs. The need for school nutritionists is intensely urgent in preparing students as future generations with superior health status and human resource values. Career development and the need for nutritionists in Indonesia will expand based on the urgency analysis and the need for legislation that strengthens the presence of the role of nutritionists in schools.

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The Effect of Adding Mocaf Flour *(Modified cassava flour)* and Kepok Banana Peels *(Musa paradisiaca forma typica)* on Vla Pie as Alternative Snack for Hypertension Patients

Laurentine Stefanny Arfenda[1*], Tri Marta Fadhilah[2]

[1,2] Department of Nutrition science, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Indonesia
*Correspondence author : laurentine1811@gmail.com

Abstract

Hypertension is a condition when blood pressure is above 140/90 mmHg. One of the causes of hypertension is a lack of calcium and potassium intake. Mocaf flour and kepok banana peels contain calcium and potassium. This study aims to analyze differences in organoleptic characteristics, public acceptability, calcium levels, potassium levels and water content in vla pie with adding mocaf flour and kepok banana peels. The research design used was experimental with a completely randomized design method, the use of wheat flour and mocaf flour, namely F1(50%:50%), F2(35%:65%), F3(20%:80%) and the addition of 50 grams of kepok banana peels in each treatment. The results of the Friedman statistical test showed no differences in aroma, texture and colour, but there was a significant difference in the taste of the Vla pie. The highest hedonic test results were found in F1. The highest calcium content is found in F3. The highest potassium content is found in F1. The water content is not following the pie quality standards in SNI. The conclusion is that vla pie with mocaf flour and kepok banana peels is acceptable to the public.

Keywords: Calcium, Kepok Banana Peels, Mocaf Flour, Potassium, Vla Pie

Introduction

Hypertension is when the systolic blood pressure is above 140 mmHg and the diastolic blood pressure is above 90 mmHg after a re-examination (Unger *et al.*, 2020). The prevalence of hypertension in Indonesia in 2018 has increased since 2013, from 25.8% to 34.1% (Riset Kesehatan Dasar, 2013; Riset Kesehatan Dasar, 2018). It is far from the global target of overcoming non-communicable diseases, namely, to reduce the incidence of hypertension by 25% in 2025 (World Health Organization, 2021). Uncontrolled high blood pressure can cause complications such as heart disease, stroke, kidney disease, retinal damage, nerve disorders, cerebral disorders and peripheral vascular disease (Kemenkes, 2018). Factors that can influence the onset of hypertension include factors that cannot be changed, namely age and gender, as well as factors that can be changed, namely by preventing a deficiency in calcium and potassium intake (Krummel, 2008; US Department of Health and Human Service, 2006).

The use of drugs for the treatment of hypertension is a significant concern in drug selection because of the safety factor of long-term drug use (Wiryowidagdo & Sitanggang, 2002). Therefore, one way to reduce high blood pressure apart from using drugs is to innovate food products that can help lower blood pressure safely. When a person's calcium and potassium intake is low, it can cause blood pressure to rise and vice versa (Damayati, 2020; Putri, 2014). Therefore, it is necessary to innovate food products with attractive shapes that are liked by various age groups, such as vla pie with the addition of mocaf flour and kepok banana peels. This research aims to analyze the effect of adding mocaf flour and kepok banana peels to vla pie as an alternative snack for hypertension patients regarding differences in organoleptic characteristics, public acceptability, calcium content, potassium content and water content in vla pie.

Methods

Three formula vla pie were developed with the following ratios wheat flour and mocaf flour namely F1(50%:50%), F2(35%:65%), F3(20%:80%) and the addition of 50 grams of kepok banana peels in each treatment. The analysis used in this study included chemical analyses such as calcium, potassium and water content. The statistical test used to process organoleptic and hedonic data is the Friedman test, while to process data on calcium, potassium and water content used Kruskal Wallis test. The calcium and potassium analyses used atomic absorption spectrophotometry (AAS) and for water content used gravimetric method. In this research, organoleptic and hedonic formula tests were also carried out on 35 panelists with inclusion criteria namely willing to fill out a questionnaire sheet and exclusion criteria being the presence of health problems in respondents that affect the five senses (taste, smell, sight), having allergies to eggs, gluten and lactose. This research was carried out at Mitra Keluarga College of Health Sciences from March - June 2023. The research design used was experimental with a completely randomized design.

ISSN 3031-0911

Results and Discussions

Figure 1 shows the determination of the appropriateness of intensity (AIT) value based on the panelist's assessment of the intensity of aroma, texture, color and taste. The scale is from 1 to 4. From the results of AIT, formulas 1, 2 and 3 both smelled a bit like mocaf flour and banana peels, with a crunchy texture, and golden yellow color and tasted a bit like mocaf flour and banana peels. Figure 1 also shows that the highest aroma assessment results in vla pie were found in the F1 treatment, namely 2.93; The highest texture assessment results in vla pie were found in the F2 treatment, namely 2.81; The highest color assessment results in vla pie were found in the F2 treatment, namely 3.61; The highest taste assessment results in vla pie were found in the F1 treatment, namely 2.94.

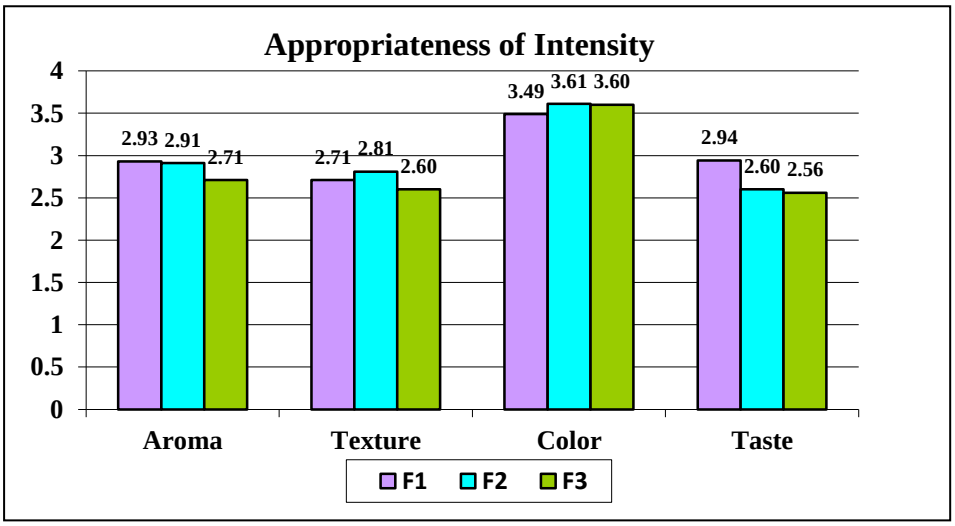

Figure 1. AIT Test

Table 1 shows the results of the vla pie sensory difference test analysis with the Friedman test. The purpose of the Friedman analysis is to determine whether there are significant differences between the three samples. If the results of the data test have a p-value less than 0.05 ($p < 0.05$), then it can be said that there is a difference in the data. From Table 1, it can be seen that there is no difference in aroma, texture and color, but there is a significant difference in the taste of vla pie.

Table 1. Results of the Vla Pie Sensory Difference Test Analysis with the Friedman test

No.	Indicators	Formula	Significant	Information
1	Aroma	F1 F2 F3	0.163 > 0.05	No difference
2	Texture	F1 F2 F3	0.083 > 0.05	No difference
3	Color	F1 F2 F3	0.913 > 0.05	No difference
4	Taste	F1 F2 F3	0.004 < 0.05	There is a difference

Source: Primary Data, 2023

Table 2 shows the results of the hedonic test where the highest hedonic score is found in F1, namely 80.71% with a like interpretation. Meanwhile, F3 has the lowest hedonic score of 75.07% but is still included in the like interpretation and based on the results of the Friedman test on the pie vla hedonic test with the addition of mocaf flour and kepok banana peels, it shows that the p-value is > 0.05 so it can be concluded that there is no difference in the level of liking of the three pie vla formulas.

Table 2. Hedonic Test

No.	Formula	Hedonic Score	Interpretation	Significant	Information
1	F1	80.71%	Like		
2	F2	77.50%	Like	0.129 > 0.05	No difference
3	F3	75.07%	Like		

ISSN 3031-0911

Source: Primary Data, 2023

Table 3 shows the chemical tests which include tests of calcium, potassium and water content of the vla pie. Formula 3 has a higher calcium content due to the greater addition of mocaf flour, which is 80% and based on the results of the Kruskal Wallis test on the calcium content of vla pie with the addition of mocaf flour and kepok banana peel, it shows that the p-value is > 0.05 so it can be concluded that there is no significant difference. Formula 1 has a higher potassium content through the addition of less mocaf flour, which is 50% and based on the results of the Kruskal Wallis test on the potassium content of vla pie with the addition of mocaf flour and kepok banana peel, it shows that the p-value is > 0.05 so it can be concluded that there is no significant difference. The vla pie produced in this study has a relatively high water content which increases the risk of damage and based on the results of the Kruskal Wallis test on the water content of vla pie with the addition of mocaf flour and kepok banana peels, it shows that the p-value is > 0.05 so it can be concluded that there is no significant difference.

Table 3. Vla Pie Chemical Analyze

No.	Chemical Characteristic	Formula 1	Formula 2	Formula 3	Sig.	Information
1	Calcium	45.41 mg/100 g	44.48 mg/100 g	46.06 mg/100 g	0.368 > 0.05	No difference
2	Potassium	182.88 mg/100 g	182.56 mg/100 g	182.05 mg/100 g	0.368 > 0.05	No difference
3	Water content	40.36%	40.73%	40.08%	0.368 > 0.05	No difference

Source: Vicma Lab Laboratory Test Results, 2023

From the results of the AIT test, the highest average aroma value was found in Formula 1 at 2.93. However, Formulas 1, 2 and 3 have the same category, namely they smell a bit like mocaf flour and banana peels with sufficient organoleptic quality criteria. This is because the higher the addition of mocaf flour, the aroma produced by the vla pie product will have a distinctive aroma from mocaf flour. This is in line with research by Ihromil et al. (2018) where the more mocaf flour is added, the more the aroma of the cookies produced will be flavorful with mocaf flour. In research conducted by Alfirochah & Bahar (2014), it was also stated that the greater the number of mocaf flour substitutes used, the more the aroma of the pancakes would be mocaf flour. In this research, apart from the aroma of mocaf flour, there was another aroma produced by adding the same amount of kepok banana peel to each formula to produce a banana peel aroma in the vla pie product.

From the results of the AIT test, the highest average texture value was found in Formula 2 of 2.81. However, in reality, Formula 3 has a higher crispness due to the higher addition of mocaf flour in Formula 3. This is because the starch content in mocaf flour is higher than in wheat flour so the higher the addition of mocaf flour can be increasingly crunchy (Juanda & Cahyono, 2004). This is in line with research by Ihromil et al. (2018) which states that a higher level of mocaf flour addition can make the texture of the dry cake crispier. The results of this research are also in line with research by Widasari & Handayani (2014), where the more mocaf flour added, the crispier the flake product will be.

From the results of the AIT test, the highest average color value was found in Formula 2 of 3.61. However, in reality, Formula 1 has a more golden yellow color than Formula 2 which has a paler white color. This is because the color of mocaf flour is whiter than wheat flour with the whiteness of mocaf flour being 88-91% and wheat flour being 86.5%, so the higher the addition of mocaf flour, the more pale yellow color of the vla pie (Salim, 2011). This is in line with research by Mustaqim *et al.* (2017), where the high use of mocaf flour affected the appearance of the color of the Bhoi cake to become pale so the panelists did not like it. The results of this study are also in line with research conducted by Normasari (2010) where the more addition of mocaf flour, the paler the resulting color will be due to the influence of white mocaf flour.

From the results of the AIT test, the highest average taste value was found in Formula 1 of 2.94. However, Formulas 1, 2 and 3 have the same category, namely they taste a bit like mocaf flour and banana peels with the criteria being that they are of sufficient organoleptic quality. This is because the higher the addition of mocaf flour, the more distinctive the taste produced by the vla pie product is from the mocaf flour. This is in line with research by Badriani et al. (2020), where the more mocaf flour is added to the dough, the kasippi taste will also be more pronounced with mocaf flour so that it can reduce the panelist's level of preference. Apart from the taste of mocaf flour, there are other flavors produced by adding the same amount of kepok banana peel to each formula, but the more dominant taste in the pie vla product comes from mocaf flour.

In the results of the sensory difference analysis using the Friedman test, there were no significant differences in the aroma, texture and color of the three pie vla formulas because the p-value > 0.05. This can be interpreted that the addition of mocaf flour and kepok banana peels does not affect the aroma, texture and color of the vla pie because the ingredients used in each formula are not much different so the aroma, texture and color are quite similar. Regarding aroma, according to Widiantara *et al.* (2018), the aroma of food products can be influenced by the ingredients used and their processing. Regarding texture, according to Affandi & Ferdiansyah (2017), the crunchy texture of a dry cake product is greatly influenced by the characteristics of the raw materials used.

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Regarding color in the research of Mikasari *et al.* (2015), the raw materials used can affect the color of food and drink. However, there was a significant difference in the taste of the three vla pie formulas because the p-value < 0.05, so the Wilcoxon test was continued to determine the difference in taste in the three formulas. The results of the Wilcoxon test on F1 and F2 with F1 and F3 obtained a p-value < 0.05, indicating that there was a real difference in the formula. According to Muchtar & Hastian (2017), the taste of a food product can come from the basic ingredients for making the product itself, the product processing process, and the ingredients added to the product during the processing process.

The results of the hedonic test on vla pie with the addition of mocaf flour and kepok banana peels which got the highest average was in Formula 1 which had a percentage of 80.71 with the liking criteria. F1 uses the lowest addition of mocaf flour, resulting smelled a bit like mocaf flour and banana peels, with a crunchy texture, and golden yellow color and tasted a bit like mocaf flour and banana peels which is well accepted by the panelists. This is because less mocaf flour is used in F1 compared to F2 and F3 with the same ratio of using kepok banana peels in each formula, resulting in an aroma that smelled a bit like mocaf flour and banana peels. This is in line with the research of *Ihromil et al.* (2018), which shows that the level of preference of panelists tends to decrease along with the higher substitution of mocaf flour. However, the texture that the panelists preferred was found in Formula 1 compared to F2 and F3 because the higher the addition of mocaf flour, the texture of the vla pie, apart from being crunchy, would also be more easily crushed or not sturdy. This is not in line with research by Widasari & Handayani (2014), where the higher the addition of mocaf flour, the crunchier the flake texture is, so the panelists like it more. Meanwhile, the color that the panelists liked was found in Formula 1. This was not in line with the research by Ihromil *et al.* (2018), which stated that the higher the addition of mocaf flour, the color the panelists preferred. In reality, the panelists preferred the color Formula 1 compared to F2 and F3 because the higher the addition of mocaf flour, the paler the color of the vla pie. This is in line with research by Normasari (2010), where the higher the mocaf flour substitution, the lower the panelists' preference for the color of the cookies. In terms of taste indicators, Formula 1 was the most preferred by the panelists because less mocaf flour was used in F1 compared to F2 and F3 with the same ratio of kepok banana peel used in each formula, resulting in a taste that tasted a bit like mocaf flour and banana peels. This is in line with the research of Ihromil *et al.* (2018), which shows that the higher the percentage of substitution of mocaf flour instead of wheat flour, the lower the taste score of the dry cake. The results of this study are also in line with the research of Badriani *et al.* (2020), where the higher the concentration of mocaf flour substitution, the panelist's assessment of the cassava taste decreased.

Based on the results of the calcium test on the vla pie, it is known that Formula 3 has a higher calcium content due to the greater addition of mocaf flour, which is 80%. Based on the results of the analysis of calcium levels, it can be seen that the higher the percentage of use of mocaf flour, the higher the calcium level in the vla pie, although it does not increase much. According to Damayanti (2014), this is because the calcium content in mocaf flour is higher than the calcium content in wheat flour. According to Kemenkes (2018), the calcium content in mocaf flour is higher, namely 60 mg/100 g, while the calcium level in wheat flour is 22 mg/100 g. However, this vla pie product cannot be claimed to be a source of calcium because the requirement is that it must be 15% of the nutritional label reference value, namely 165 mg/100 g.

Based on the results of the potassium test on vla pie, it is known that Formula 1 has a higher potassium content although the addition of less mocaf flour is 50%. Based on the results of the potassium content analysis, it can be seen that the higher the percentage of mocaf flour used, the lower the potassium content in the vla pie, although it does not decrease much because the ingredients used in each formula are not much different, resulting in quite similar potassium levels. According to Kemenkes (2018), the potassium content in mocaf flour is higher, namely 403 mg/100 g, while the potassium content in wheat flour is 0 mg/100 g, so it does not follow existing sources because the higher addition of mocaf flour, the more also high levels of potassium in vla pie. This can be caused by insufficient temperature control and processing time, especially during roasting. This is in line with the research by Damanik *et al.* (2021), where the longer the cooking process, the minerals in the food will decrease because they will evaporate when cooked, and this will result in the potassium content in the tuna fish meal getting lower over time. However, this vla pie product cannot be claimed to be a source of potassium because the requirement is that it must be 15% of the nutritional label reference value, namely 705 mg/100 g.

Based on the results of the water content test on vla pie, it is known that the lowest water content is found in Formula 3 at 40.08%. The water content tends to decrease in Formula 3 due to the increased use of mocaf flour. According to Salim (2011), this is because the water content in mocaf flour is lower, namely 6.9%, while the water content in wheat flour is 13%. This research is in line with the research of Ihromil *et al.* (2018), which shows that the higher the percentage of mocaf flour substitution, the lower the moisture content of the pastries. This research is also in line with the research of Badriani *et al.* (2020), where the higher the substitution of mocaf flour, the lower the water content produced in cassava. However, the water content in vla pie which does not meet standards can also be influenced by the addition of kepok banana peels to vla pie, because the water content in kepok banana peels in 100 grams is 68.90% (Anggraeni & Sian, 2004). In general, the moisture content of vla pie ranges from 5-10% Badan Pengawas Obat dan Makanan Republik Indonesia (2016). In this study, the vla pie produced contained relatively high water content, which might increase the risk of damage. Therefore, recommendations for future research related to this topic are adding foods high in calcium and potassium to vla pie and conducting research related to the effect of consuming vla pie snacks in lowering blood pressure in hypertensive patients.

Conclusions

This research concludes that there is no significant difference between organoleptic characteristics except taste, public

ISSN 3031-0911

acceptability, calcium content, potassium content and water content in vla pie with the addition of mocaf flour and kepok banana peels. Suggestions for future researchers are that it is necessary to carry out tests related to the nutritional content of vla pie (energy, carbohydrates, protein, fat) and carry out shelf life testing to find out how long the vla pie product can last and add color to vla to produce a more attractive color and make additional more varied food ingredients in quite different amounts into the vla pie formulation to increase calcium levels and potassium levels.

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Anti-Proliferative Activity of Fragrant Rice MRQ 74 (*Mas Wangi*) Water Extract Against Colon Cancer Cell Lines (Ht-29)

Nur Anis Aliah binti Azhar[1], Norhayati Abd Hadi[2], Mohd Adzim Khalili Rohin[3], Norhaslinda Ridzwan[4] Ruzaina Ishak[5], Atif Amin Baig[6]

[1,2,3,4]Department of Nutrition and Dietetics, Faculty of Health Sciences, Universiti Sultan Zainal Abidin (UniSZA) Terengganu, Malaysia.
[5]Department of Health and Life Sciences, Teesside University, Middlesbrough, Tees Valley, TS1 3BX, England.
[6]Department of Public Health, University of Lahore, Pakistan.
*Correspondence author : norhayatihadi@unisza.edu.my

Abstract

Fragrant rice MRQ 74 (*Mas wangi*) is one of the rice by the Malaysia Research and Development Institute (MARDI), which is responsible for developing varieties of high-quality rice that is nutritious, suitable for diabetics due to its low glycemic index and low starch content as well as resistant to pest and disease. Colorectal cancer (CRC), also known as colon cancer, is the second most common cancer in the world. This cancer involves many factors, including family history, physical inactivity, high-fat and low-fibre diets, inflammatory bowel disease (IBD), diabetes and other conditions associated with the large intestine. This study aimed to examine the antiproliferative activity of polished rice extract and unpolished rice extract against colon cancer cell line HT-29 using MTT assay. Morphology observation and fluorescence double staining of treated cells were determined using a light inverted microscope and acridine orange/propidium iodide staining (AO/PI). The results showed that unpolished rice extract has better IC50 values (0.46 ± 0.02) mg/mL compared to polished rice extract (0.50 ± 0.06) mg/mL after 72 hours of treatment and showed statistically significant results. Morphological changes at 24, 48, and 72 hours after treatment, the antiproliferative effect was observed, which resulted in a reduction of cell size, cell density, the presence of fragmentation cells and apoptotic bodies. Therefore, unpolished rice extracts have promising natural plants in future as an anticancer properties in the nutraceutical and food industry. These results can provide new information for further in vitro or in vivo biological studies.

Keyword: Fragrant rice MRQ 74, colon cancer, HT-29, MTT assay, anti-proliferative, IC50, morphological changes

Introduction

Around the world, cancer is a terrible disease that causes extreme repressibility and mortality. Due to the need for early illness detection and the fact that the majority of patients are only discovered in the latter stages, administration becomes challenging (Akhtar *et al.*, 2019). Colon cancer is the third most common diagnosis and has become the second deadliest malignancy for both genders (Vettil *et al.*, 2017). According to the Centre for Disease Control and Prevention (2020), colon cancer is a disease which occurs in the colon or rectum. Cells in the colon or rectum grow abnormally, called polyps.

Intake of foods that contain phytochemicals, such as fruits, vegetables, and whole grains, has been linked to a reduced risk of colon cancer (Thanuja *et al.*, 2022). Finding plant food sources and their oxidative stress-targeting bioactive components is, therefore, a crucial component of colon cancer prevention and treatment strategies. According to Malabadi *et al.* (2022), around 50% of people on earth, including a sizable portion of Indians, eat rice as a staple food. This is especially true of cereal grain varieties. Coloured rice varieties are prized in comparison to polished white rice because of their health benefits.

Rice (*Oryza sativa* sp), is an edible starchy food, and the grass plant that comes from the Poaceae family. For more than half of the world, rice is the staple food and boasts the second-highest grain production after corn in 2008 in the human population (Fukugawa *et al.*, 2019). *Oryza sativa* contains two significant variants, which are short japonica rice and long indica rice. Short-grained is a sticky type of rice, usually grown in dry fields that are mainly submerged in East Asia; meanwhile, long-grained rice is a sinica variety and a non-sticky type of rice mainly in lowland rice and grown mostly submerged in Southeast Asia (Wang *et al.*, 2014). Rice comes in a range of colours, types, and origins, such as black, brown, purple, fragrant, or aromatic rice (Saikhrisna *et al.*, 2018).

Fragrance in rice is a crucial component in determining market pricing. It is linked to both local and national identity, making it one of the most essential grain quality attributes in rice. Fragrant rice accounts for an estimated 15-18% of rice trade and is the highest priced in the world market. Countries like the United States, Vietnam and other rice producers and traders are interested in entering this premium segment (Paul *et al.*, 2021). The Malaysian Agricultural Research and Development Institute (MARDI) has introduced four types of fragrant rice varieties: MRQ 50, MRQ 74, MRQ 76 and MARDI Wangi 88.

Fragrant rice MRQ 74 (Mas Wangi) is the second most preferred fragrant rice variety (27%), along with MARDI Wangi 88 (21%), with MRQ 76 (30%) being the most preferred (Ariff *et al.*, 2019). Fragrant rice MRQ 74 was more

ISSN 3031-0911

likely the same as the Basmati type of rice and characterized as a healthy food, especially for diabetic patients. Lengthy, fine grain shape, flaky, non-greasy, high starch content, slightly softer gel consistency, and moderate alkali diffusion value are some of the notable features of fragrance rice. Until now, rice has become the most preferred by consumers that are locally produced (Rahman *et al*., 2022).

Furthermore, new research suggests that edible rice bran varieties may have an anticancer impact, including breast, lung, liver, and colorectal cancer (Sohail *et al*., 2017). The fourth most significant cause of death is colorectal cancer, and it usually begins in the cells of the colon and rectum. The capacity of rice bran-derived bioactive compounds to induce apoptosis, limit cell proliferation, and change the development of the cell cycle in malignant cells has been shown to mediate their anticancer effects (Rohin *et al*., 2020). The bioactive components in rice bran protect against tissue damage by scavenging free radicals and suppressing chronic inflammatory responses. The phytochemicals in rice bran have also been shown to trigger antitumor immune responses and influence the microenvironment of colon tumours to support better colon cancer prevention (Saad *et al*., 2022). However, there still needs to be more detailed research regarding the cytotoxic activity of fragrant rice MRQ 74 water extraction in Malaysia. Therefore, cell morphology observation is one of the methods that have been used to identify cytotoxic activity in plants.

Methods

Plant collection and preparation

Fragrant rice MRQ 74 was collected from MARDI, Malaysia. Rice grain samples were carefully separated from any physical residue before being placed in sealed containers.

Extraction Procedure for Rice Samples

The rice grain samples were extracted based on the method introduced by Acquistucci (2017). Rice grain samples were prepared right before analysis by grinding rice grains into a fine flour using an electronic blender (Warring 2-Speed Blender, 240 VAC-Standard Motor) at maximum speed for about 30 seconds, ensuring that the grains are correctly ground. Then, the soaking process (1:10 w/v) was continued with 50 g of refined flour rice grains by using an aqueous solution (deionized water) at 25℃ for 24 hours. The solvent extract was filtered through a vacuum filtration kit nylon membrane filter (0.22 µm) and was further concentrated using a rotary evaporator under reduced pressure. The concentrated extract was placed in a 40℃ oven to allow for complete solvent evaporation. The extract was stored in a -20℃ freezer until the following analysis.

Extraction Yield Procedure of Rice Extracts

The extraction yield was calculated according to the method of Dey and Rathod (2013) using the formula and expressed as a percentage (%):

$$\text{Extraction method} = W2/W1 \times 100\%$$

W1= original weight of sample, W2= weight of dried extract

Preparation of Rice Extracts

The methods used previously by Rohin *et al*. (2017) with certain modifications were implemented for this study. To make a stock solution of 1 mg/mL, 10 mg of rice grains extract was dissolved in 1 mL of DMSO reagent. The extract solutions were stored at 4℃ until they were used. To generate a working solution of 1 mg/mL, the stock solution for each extract was further diluted in finished RPMI-1640 and McCoy's 5A medium with 10% fetal bovine serum and 1% penicillin-streptomycin.

Cells maintain and harvest.

A colon cancer cell line (HT-29) was used in this study. Cells were collected from UniSZA's Faculty of Bioresources and Food Industry in passage 3 (P3). The cell line was grown in RPMI-1640 media supplemented with 10% bovine serum and 1% penicillin-streptomycin at 37°C in an incubator humidified with 5% Carbon dioxide (CO2) and with a relative humidity of 95%. The cell media was replenished twice weekly to restore the nutrients essential for cell growth.

Determination inhibition concentration of 50% (IC50) by fragrant rice extracts

A colourimetric micro-titration method known as the MTT test, or tetrazolium salt reduction assay, was used to determine the inhibitory concentration (IC50) of rice extracts (Kuete *et al*., 2017). A method used previously by Rohin *et al*. (2017), the cells were harvested from the media, counted with a hemocytometer, and diluted further in RPMI and

ISSN 3031-0911

McCoy's 5A medium (added with 10 % fetal bovine serum and 1 % penicillin-streptomycin). By using 96-well culture plates (SPL Life Sciences, Korea), a total of 100 µL of cell suspension was seeded in triplicates at an optimized density of 1 x 105 cells/cm² for each cell. After 24 hours, each well was received triplicate serial dilutions of rice grains and cooked rice extracts (1.00–0.016 mg/mL) (Rohin *et al*., 2018) as well as 5-fluorouracil (5FU) (a drug) (1.00–0.016 µg/mL). Blank cells (blank) and untreated cells were seeded in each 96-well plate (positive control). After a 48-hour incubation period, 20 µL (5µg/mL) of MTT assay was put into each well of a 96-well plate and left for another 4 hours. The medium was removed, and 100 µL of DMSO reagent was added to each cell. By using a microplate reader, the absorbance at 570 nm was measured with a reference of 630 nm (TECAN, INFINITE M200, Switzerland). The nonlinear regression of the response curves within the same region was used to calculate the IC50 dose concentration. The relative cell viability of the treated cells was described as % of cell viability and will be calculated based on the following formula:

Cell viability (%) = (A570 of treated cells) / (A570 of control cells) x 100%

Cells Morphology Observation

The effects of rice extract on cellular morphological changes were investigated using the Merlin et al. approach (2010). The inhibitor concentration (IC50) value was measured using the MTT assay in this approach to estimate the effective dose concentration of the extract. A light inverted microscope (Nikon, Japan) with a magnification of 10x was used to observe morphological changes at 37°C for 24, 48, and 72 hours.

Fluorescence Microscopy of Apoptosis using AO/PI Doubles Staining

The method according to Hajiaghaalipour *et al.* (2017), was used in this study. Acridine orange (AO)/propidium iodide (PI) staining was used to determine the apoptotic effects of rice extracts on the cancer cell line, which was subsequently observed under a fluorescence microscope. A total of 1 x105 cells/well cell density was seeded into 96-well plates and treated for 72 hours with each extract's IC50 dose concentrations. Then, both untreated and treated cells were incubated with AO and PI staining at a concentration of 10 g/mL, and apoptosis or cell viability was seen at 10x magnification by using an Olympus-BX51 fluorescence microscope (Olympus, Japan) fitted with a Nikon camera (Nikon, Japan).

Data Analysis

All tests for evaluating percentage yield (%) of fragrant rice MRQ 74 water extract sample and IC50 values of HT-29 cell lines at 72 hours treatment were conducted in triplicates. The data were explored using descriptive and inferential statistical analysis by using the Microsoft Excel Spreadsheet 2019 and Scientific Package for Social Sciences version 26.0 software (IBM Corp. US) with $p<0.05$. Descriptive statistics was applied to measure the extraction yield and an IC50 value as means and standard deviation (SD). Based on the variables that were analyzed, this analysis was related to the Independent T-test in percentages of extraction yield. Other than that, a One-way ANOVA test was used to compare an IC50 value of HT-29 cell lines was tested.

Results and Discussions

Percentage Extraction Yield

Table 1 shows the percentage yield (%) of the fragrant MRQ 74 rice water extract sample. The weight of crude extract (g) ± SD in unpolished fragrant rice MRQ 74 extract is (1.12 ± 0.02) higher than polished fragrant rice MRQ 74 extract (0.43 ± 0.03). The percentage of extraction yield in unpolished fragrant rice MRQ 74 extract (2.24%) also had been observed to be higher than polished fragrant rice MRQ 74 extract (0.86%). Overall, the percentage yield was significant between the different types of samples and the percentage of extraction yield ($p<0.05$) with a p-value of 0.00.

Table 1. Percentage yield (%) of fragrant rice MRQ 74 water extract sample

Sample	Weight of crude extract (g) ± SD	Extraction yield (%)	df	F-statistics	p-value
MRQ 74 aged (polished)	0.43 ± 0.03	0.86 ± 0.05	4	2.66	0.00*
MRQ 74 aged (unpolished)	1.12 ± 0.02	2.24 ± 0.03			

Data represent the mean ± SD of three independent experiments.

ISSN 3031-0911

Independent T test, *p<0.05. Extract yield present in percentages (100%).

In this study, the sample extraction is determined for categories, which are fragrant rice MRQ 74, polished rice and unpolished rice. Based on the result, unpolished rice has a higher extraction yield compared to polished rice. This result is similar to Quagliariello *et al.* (2016). The yield of bioactive compounds was increased during the extraction of brown rice with the help of pulsed electrical fields (PEF) and promoted antioxidant activity. Water extraction of rice extract showed lower extraction yield in polished rice extract (0.86%) and unpolished rice extract (2.24%) compared to the study from Sohail *et al.* (2017), the extraction of dried rice bran using ethanol as solvent (9.94%). In vitro antioxidant activity of brown rice extracts varied depending on the extraction method, extract bioactive compound content, brown rice cultivar or variation, and bioactive compound fraction, bound or free (Gao *et al*., 2018; Gong *et al.*, 2017). The extraction yield is based on three types of coloured rice: brown (38.6%), black (33.7%) and red rice (28.5%) (Ziegler *et al*., 2017). It has been shown that brown rice has the highest extraction yield compared to black rice and red rice.

Determination Inhibition Concentration Of 50% (IC50) By Fragrant Rice Extracts

Table 2 shows that unpolished fragrant rice MRQ 74 has a better antiproliferative effect on colon cancer cell lines, which showed the IC50 values (0.46 ± 0.02) mg/mL. This result is similar to the study from Tan *et al*. (2015), which showed that water brewer extracts with IC50 values of 38.33 ± 6.51 µg/mL justified that water brewer extract meets two essential criteria, which are tumour specificity and minimal toxicity against normal cells and become a promising antiproliferative agent for HT-29 colon cancer (p<0.05).

Table 2. IC50 values of HT-29 cell lines after treatment with fragrant rice MRQ 74 water extract at 72 hours.

Sample	IC50 values ± SD (mg/mL)	df	F-statistics	p-value
	72 hours			
MRQ 74 age (polish)	0.50 ± 0.06	7	2.12	0.10
MRQ 74 age (unpolish)	0.46 ± 0.02	7	3.67	0.02*
5-fluorouracil (5FU)	0.33 ± 0.10	6	3.23	0.03*

Data represent the mean ± SD of three independent experiments. One-way ANOVA test, *p<0.05.

The finding from Quagliariello *et al*. (2016) shows that extracts from brown rice observed as the potential of anti-inflammatory or anticancer properties at concentrations 1 and 3 mg/mL. The extracts showed significant toxicity for HT-29 cells after 24 hours of exposure, with an IC50 value at a concentration of 1.3 mg/mL (p<0.05). Additionally, pulsed electric field extracts of brown rice significantly reduced interleukin production and gene expression in colon cancer cells, indicating that brown rice has natural anti-inflammatory substances.

In comparison with different varieties of rice, unpolished rice is categorized as coloured rice. Examples of coloured rice include brown rice, black rice, red rice, purple rice and many more (Priya *et al*., 2019). In a similar method of this study, Thanuja *et al.* (2022) observed the raw black rice that had been ground until it became flour and known as native black rice. Meanwhile, the modified method of native black rice used a buffer of 0.1M sodium acetate and added 0.2 g of alpha-amylase enzyme. This study indicates that by increasing the concentration of black rice from 25–400 µg/mL, the cell viability of HCT-116 clines decreased range from 96.69% to 32.72%. Overall, native black rice flour date-haven the highest anticancer activity concentration centration compared to modified native black rice flour.

A study by Wattayagrn *et al.* (2022) examined the cytotoxic effects of hydrolyzed rice berry rice bran extract on two distinct colon cancer cell lines, SW-620 and HT-29, which are both metastatic cancer cell lines (HRBE). As a result, the hydrolyzed rice berry rice bran extract caused senescence in non-metastatic cancer cells and induced apoptosis in metastatic cancer cells. This finding was similar to this study due to the same method of using ultrapure water at a concentration of 20g/L and concentrated by freeze drying and keeping 20 ℃ until further use. As the results, on HT-29, the half-maximal inhibitory concentrations (IC50) of HRBE were 43,677 µg/mL, 11,164 µg/mL, and 6053 µg/mL, and on SW-620, the IC50 values were 9880 µg/mL, 6870 µg/mL, and 5468 µg/mL during 24 hours, 48 hours and 72 hours, respectively. This can conclude that hydrolyzed rice berry rice bran extract had higher cytotoxic efficiency on SW-620 and HT-29 colon cancer cell lines.

Moreover, a study by Phannasorn *et al.* (2017) examined the impact of purple rice extract on colon cancer cell growth induced by the treatment of proinflammatory cytokines, interleukin (IL-6). This study observed that IL-6 has the power to stimulate the growth and proliferation of HT-29 cells at both 24 and 48 hours. This finding showed that purple rice extract had anti-proliferation effects on HT-29 colon cancer cells that were inflamed. According to a study

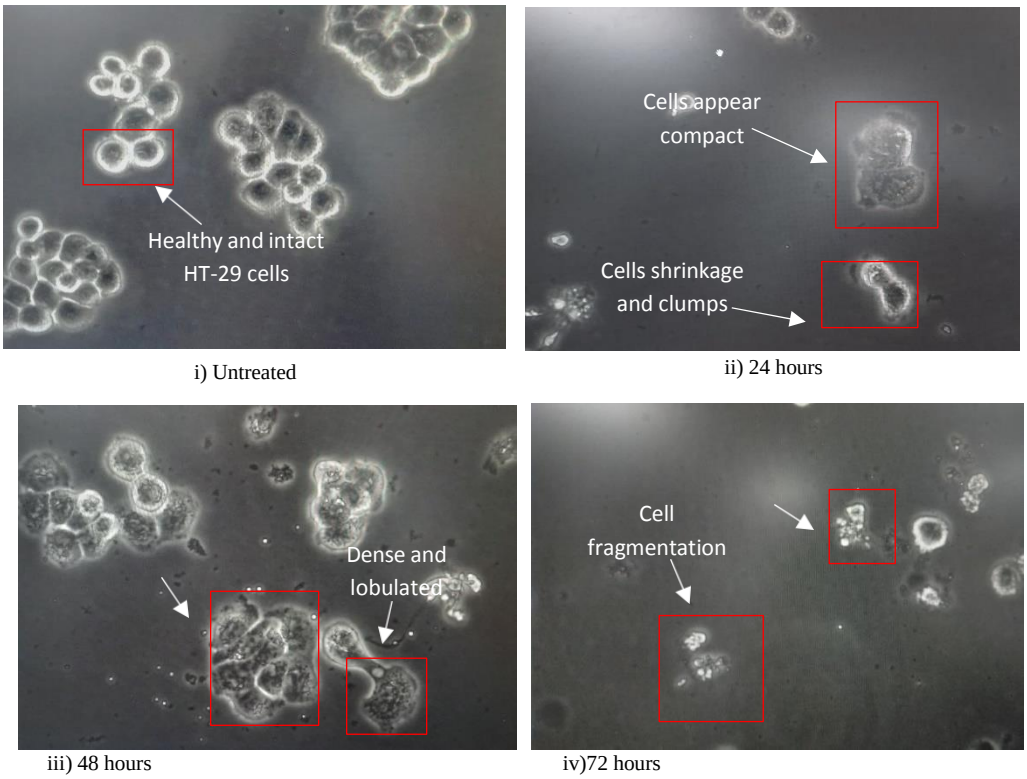
ISSN 3031-0911

conducted by Rao *et al.* (2019), on the capacity of phenolic extracts from coloured rice to induce apoptosis in colorectal cancer, SW480, phenolic compounds found in purple rice may inhibit cancer cell proliferation by activating apoptosis.

A study observed by Moirangthem *et al.* (2021) using human colorectal cancer cells (HCT-116) showed a significant reduction of more than 50% when treated with 200 g/mL of bran extract ($p = 0.04$). In another study by Takashima *et al.* (2013), the rice bran water extract (RBWE) at 1.0 mg/mL, the proliferation of LS174T human colon cancer cells was significantly reduced and showed 37.2% of IC50 values. This showed that rice bran has convincing outcomes for colon cancer cells.

Cells Morphology Observation and Fluorescence Staining Apoptosis of Fragrant Rice MRQ 74 Extract on Colon Cancer Cells Lines (HT-29)

Figure 1, 2 and 3 shows the inhibition of HT-29 by polished rice extract, unpolished rice extract and 5-fluorouracil (5FU) extract. Morphological changes were starting from 0 hours, 24 hours, 48 hours and 72 hours. During 0 hours (untreated cell), HT-29 cells were round, shiny and intact. **[Figure 1,2,3, (i)]**. During 24 hours, HT-29 cells treated with rice extract and 5FU extract observed that the cells appeared compact and started to shrink and clump **[Figure 1,2,3, (ii)].** Then, at 48 hours, the cells become dense and lobulated due to loss of normal cell shape. During this phase, some cells are partially detached from the surface, and some of the organelles become too compact. The volume of the cells decreased as a result of the rounding off of the cells **[Figure 1,2,3, (iii)].** The formation of apoptosis bodies, cell fragmentation, and a lot of debris membrane blebbing occur during the 72 hours, indicating the final stage of apoptosis **[Figure 1,2,3, (iv)]**

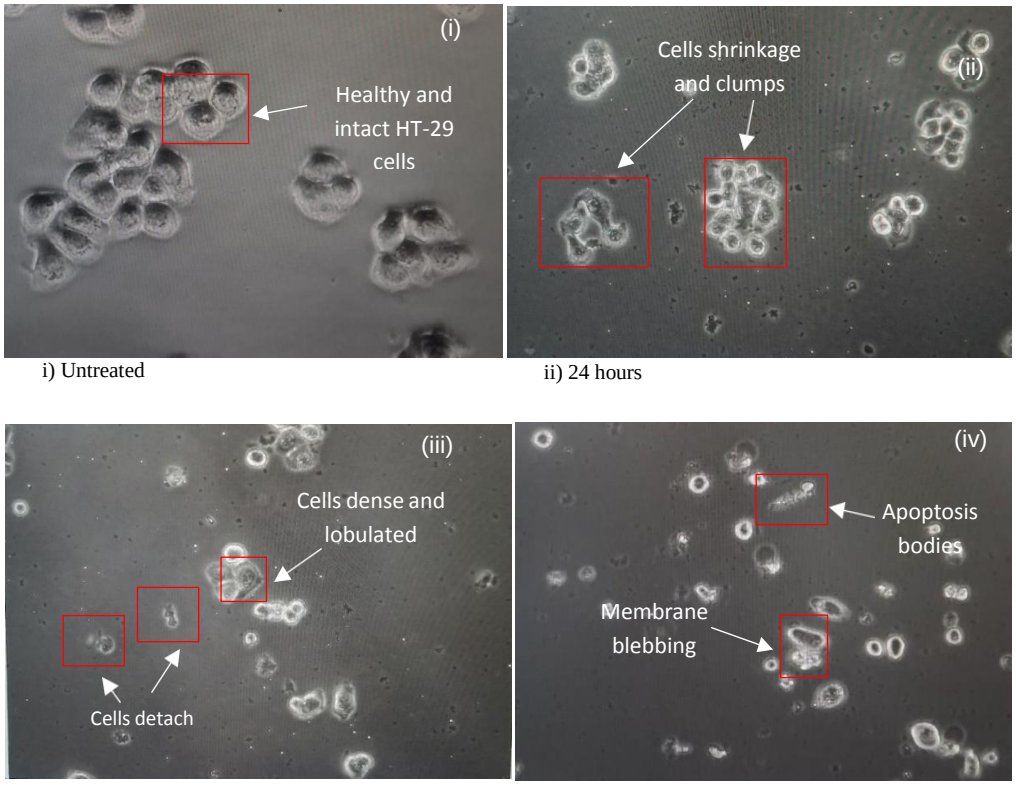


i) Untreated ii) 24 hours

iii) 48 hours iv)72 hours

Figure 1. Inhibition of HT-29 cell line by polished fragrant rice MRQ 74 extract for 3 days. Cells morphology of HT-29 was examined after being treated with IC50 at (i) untreated, (ii) 24 hours, (iii) 48 hours and (iv) 72 hours. The photographs were taken at 20x magnification with inverted microscope (Nikon, Japan).

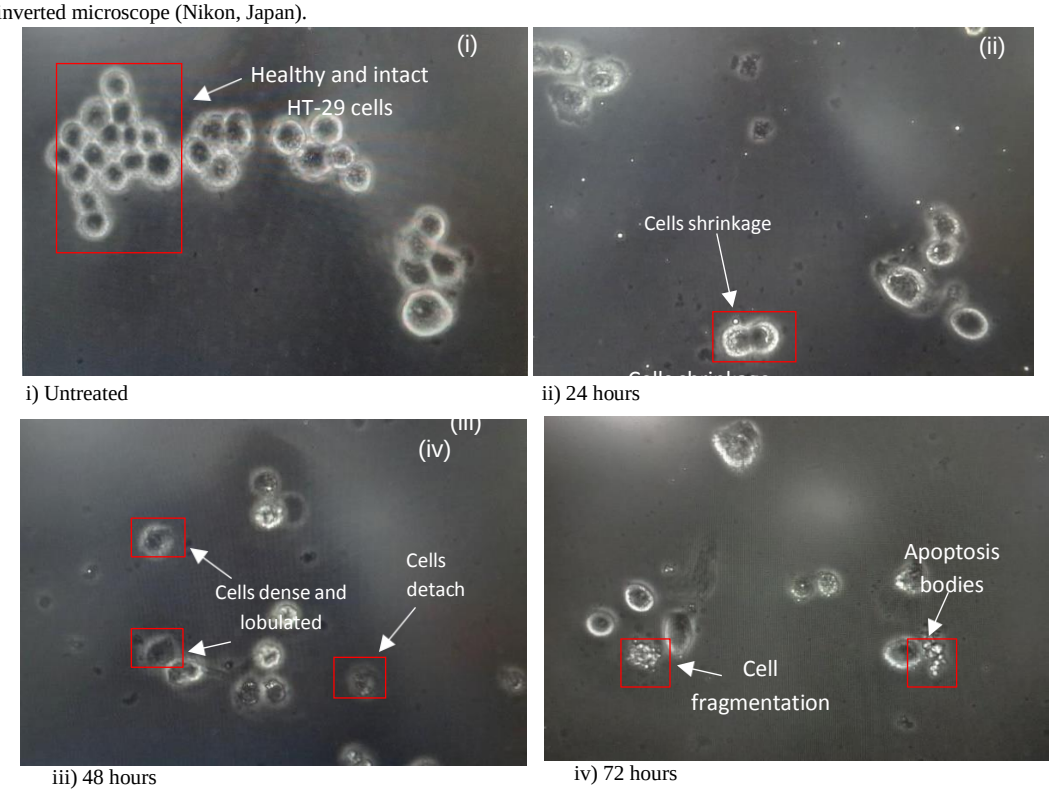
ISSN 3031-0911



i) Untreated ii) 24 hours

iii) 48 hours iv)72 hours

Figure 2. Inhibition of HT-29 cell line by unpolished fragrant rice MRQ 74 extract for 3 days. Cells morphology of HT-29 was examined after being treated with IC50 at (i) untreated, (ii) 24 hours, (iii) 48 hours and (iv) 72 hours. The photographs were taken at 20x magnification with inverted microscope (Nikon, Japan).



i) Untreated ii) 24 hours

iii) 48 hours iv) 72 hours

Figure 3. Inhibition of HT-29 cell lines by 5FU extract for 3 days. Cells morphology of HT-29 was examined after being treated with IC50 at (i) untreated, (ii) 24 hours, (iii) 48 hours and (iv) 72 hours. The photographs were taken at 20x magnification with inverted microscope (Nikon, Japan).

ISSN 3031-0911

Based on **the figure 4,** fluorescence micrographs of HT-29 were observed in polished fragrant rice extract, unpolished rice extract, and 5-Fluorouracil extract after being treated with IC50 values at 24 hours, 48 hours and 72 hours. For untreated cells, the cells are viable and displayed round and have green fluorescence colour at all hours of treatment **[Figure 4 (a,b,c (i)].** Yellow stain was detected when polished rice extract, unpolished rice extract and 5FU extracts were stained with AOPI at 24 hours and 48 hours **[Figure 4 (a, b (ii,iii,iv)].** At 72 hours, the red fluorescence colour appears **[Figure 4 (c (ii,iii,iv)].**

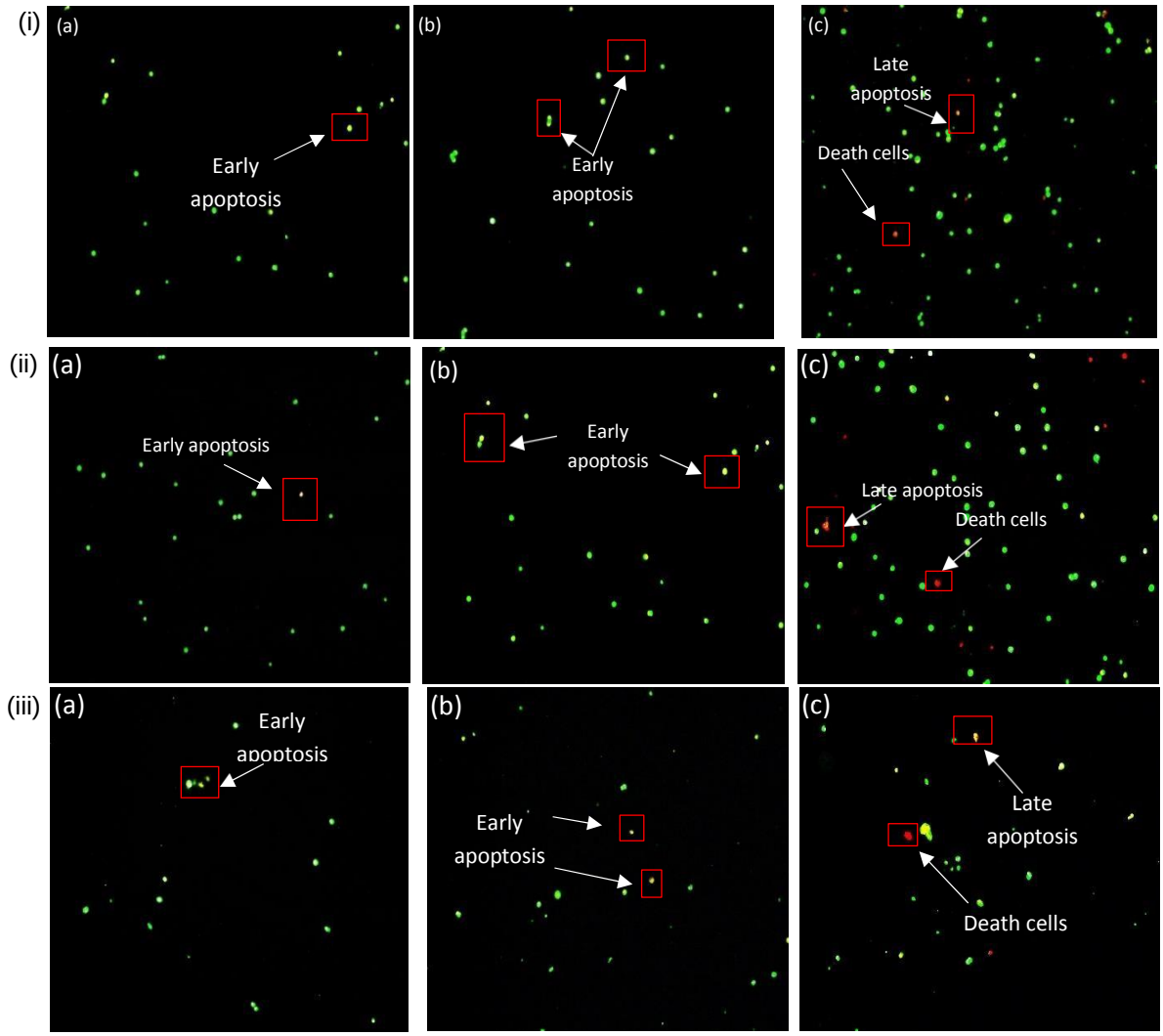


Figure 4. Fluorescence micrographs of rice extracts stained with AOPI. The micrographs of HT-29 were examined after each extract were treated with IC50 values at (a) 24 hours, (b) 48 hours and (c) 72 hours; (i) untreated cells; (ii) fragrant rice MRQ 74 aged (polished); (iii) fragrant rice MRQ 74 aged (unpolished); (iv) 5FU. Magnification 10x were taken with fluorescence microscope (Nikon, Japan).

Cell morphology observation of fragrant rice MRQ 74 extracts on colon cancer cell lines (HT-29) was observed by using the light inverted microscope with a magnification of 10x at three interval times, which are 24 hours, 48 hours and 72 hours. Meanwhile, in determining the apoptotic effect of rice extract on colon cancer cell line, fluorescence staining apoptosis was observed using 10 g/mL concentration of Acridine orange (AO)/ propidium iodide (PI) staining. An Olympus-BX51 fluorescence microscope was used to observe apoptosis or cell viability at a 10x magnification.

Apoptosis is a type of programmed cell death with specific characteristics of morphological and biochemical changes in cells. Apoptosis, or programmed cell death, is essential for the maintenance of tissues, the physiological state of organs, and heritably controlled cell death for the stability of cell proliferation (Majtnerová *et al.*, 2018). Apoptosis has five stages that begin with chromatin condensation, shrinkage and rounding off, nuclear fragmentation, membrane blebbing, the presence of apoptosis bodies, and cell debris (Abou-Ghali *et al.*, 2015). AO is a membrane-permeable cationic colour that reaches living cells' nucleic acids and redirects a green fluorescence. Meanwhile, red fluorescence is produced by all dead nucleated cells after PI enters dead cells with compromised membranes.

Based on **Figure 1,2,3, (i)** of morphological changes, during 0 hours, which was categorized as untreated cells, HT-29 cell lines were round, shiny and intact in polished rice extract, unpolished rice extract and 5-fluorouracil extract.

ISSN 3031-0911

At this phase, the cells were justified as healthy cells because the cells grew normally for a colon cancer cell line and cell confluence up to 90%. For untreated cells, the cells are viable and displayed round and have green fluorescence colour at all hours of treatment **(Figure 4.4 (a,b,c (i)).**

During 24 hours of treatment, HT-29 cells treated with rice extract and 5-Fluorouracil extract observed that cells appeared compact and started to shrinkage and clumps **(Figure 1,2,3 (ii)).** Cells undergo shrinkage, resulting in a smaller size, and the cytoplasm is denser and tightly packed (Baharara *et al*., 2015). Then, at 48 hours, the cells become too compact, dense and lobulated due to loss of normal cell shape. **(Figure 1,2,3 (iii)).** This indicates the phase before the cell completely detached and has a mass with dark eosinophilic cytoplasm (Rahim et al., 2021). Early apoptosis, which indicates a yellow stain, was detected when polished rice, unpolished rice and 5-Fluorouracil extracts were stained with AOPI at 24 hours and 48 hours of treatment **(Figure 4 (a, b, ii, iii, iv).**

During 72 hours, the formation of apoptosis bodies, cell fragmentation and a lot of debris membrane blebbing indicate the final stage of apoptosis. **(Figure 1,2,3 (iv)).** This is consistent with a previous study from Tan *et al.* (2016), using water brewers' rice extract, which displayed apoptotic characteristics such as membrane blebbing, nuclear fragmentation and apoptotic bodies. Based on **Figure 4 (c, ii, iii, iv),** red fluorescence colour and cell undergo late apoptosis and death cell after being treated with polished rice, unpolished rice and 5FU extract.

During 72 hours of treatment, the cell nuclei stained with orange and light red showed cells with an intact shape, indicating that the cells were in the late stages of apoptosis but were not dead (Kntayya *et al.*, (2018). The cells demonstrated as death cells due to the PI dye making cells appear fluorescence red. A recent study from Wattayagorn *et al*. (2022) showed the morphology changes of SW-620, including apoptotic-related features such as membrane blebbing and chromatin condensation. The red colour from PI staining was also observed to indicate late apoptosis.

Conclusions

The results of this study show the IC50 values that indicate anti-proliferative activity against colon cancer cell line (HT-29) after being treated with fragrant rice MRQ 74 water extract. In comparison between the two categories of fragrant rice, unpolished rice extract has promising potential as an anticancer as the IC50 values shows are higher than polished rice after 72 hours of treatment. Moreover, HT-29 cell lines also showed morphological changes after being treated with fragrant rice MRQ 74 water extract. HT-29 cell line shows morphological changes from cell shrinkage to apoptosis of cell in 24 hours, 48 hours and 72 hours. HT-29 also reduce cell viability in AO/PI staining from viable cell to early apoptosis. Orange and red fluorescence colour appeared when the cells were in the phase of late apoptosis and death. It can be concluded that fragrant rice MRQ 74 had an anti-proliferative effect against HT-29 colon cancer cells. Hence, this shows that the fragrant rice MRQ 74 has potential as an anticancer and could deliver new data as well as evidence regarding research on cytotoxic activity on fragrant rice using water extraction.

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The Effect of Online Games Tumpeng Gizi Seimbang to Balanced Nutrition Knowledge, Fat Intake, and Carbohydrate Intake in Overweight Adolescents in High School Surakarta

Nur Sitra Buamona[1*], Sapja Anantanyu[2], Suminah[3]

[1]Department of Nutrition Science, Sebelas Maret University,Surakarta, Indonesia.
[2,3]Department of Agriculture, Sebelas Maret University, Surakarta, Indonesia
*Correspondence author : nursitrabuamona@gmail.com

Abstract

Introduction: Obesity is a chronic health problem in adolescents and can cause various non-communicable diseases in adults. Lack of nutritional knowledge and excessive intake of fats and carbohydrates can cause obesity. Nutrition education is needed through a tumpeng balanced nutrition with innovative methods and media so the information can be effective. The methods of learning by playing and using internet media are familiar to adolescents.
Objective: To know the effectiveness of online games tumpeng balanced nutrition to balanced nutrition knowledge and fat and carbohydrate intake in overweight adolescents.
Methods: Qquasi-experimental research with a pre-test and post-test with a control design. A total of 84 adolescents in Surakarta were sampled in this study using the multistage random sampling technique, which is a gradual sampling process. Sampling technique, namely the process of sampling in stages. The first stage is from senior high school and the equivalent in the Jebres and Laweyan sub-districts, namely SMAN, SMA Swasta, and SMK N. using a simple random sampling technique on each school to obtain SMAN 8 Surakarta, SMA Muhammadiyah. 3 Surakarta, SMAN 1 Surakarta, and SMA Batik 1 Surakarta. Then, the process The sample selection in each group was selected using the purposive sampling technique. The sampling technique is a selection process that is carried out deliberately by setting certain goals and planning. Setting certain goals and planning. The screening was conducted at the start of this study via Google Forms. Adolescents with *z-score* >1 SD were chosen as respondents. Nutrition knowledge was measured with a questionnaire. Fat and carbohydrate intakes were obtained with a food record. The data was analyzed with Wilcoxon and Mann-Whitney.
Results: There were differences in nutritional knowledge between the case and the control group (p=0.001). The results showed that there were differences in carbohydrate intake (p=0.001) in the game group, and there was no effect in the lecture group (p=0.299). There was no effect before and after the intervention in the two groups in the fat intake treatment group, p=0.230 and the control group, p=0.299.
Conclusion: There was a difference in nutritional knowledge and carbohydrate intake between the two groups. Meanwhile, there is no difference in fat intake in all groups before and after the intervention.

Keywords: Adolescents, Overweight, Tumpeng, Nutrition Knowledge, Fat, Carbohydrate

Introduction

Obesity in Adolescents According to a WHO study in 2016, the prevalence of overweight and obesity among children and adolescents aged 5-19 has increased drastically from just 4% in 1975 to more than 18% in 2016 Obesity in adolescents based on WHO studies in 2016 the prevalence of overweight and obesity among children and adolescents aged 5-19 years has increased drastically from only 4% in 1975 to more than 18% in 2016. Adolescents are one of the most susceptible groups to nutritional problems because, at this time, adolescents require nutrient intake as needed for growth and development. If adolescent obesity is not dealt with early, it will have an impact on health and decrease productivity. Obesity is a multi-factor nutritional problem. Nutrition knowledge is an individual's understanding of nutrition science, nutritional substances, as well as the interaction between nutrients and nutritional status and health. It is in accordance with previous research by Muliyati on high school adolescents of Palu, who have good nutritional knowledge, all have normal nutritional status with a percentage of nutrition knowledge of 92.1%. It proves that the knowledge of balanced nutrition influences a person's nutrition status and is also affected by the eating habits of individuals (Muliyati *et al.*, 2019). Individual eating habits that exceed need are factors that cause obesity, i.e. carbohydrate intake and excess fat intake. In Indonesia, Central Java is one of the provinces with a prevalence of obese adolescents above the national figure, with the total number of obesity cases rising from 6,04% in 2017 to 7,9% in 2018 (Hasil Riset Kesehatan Dasar Tahun 2018). Surakarta City is one of the regencies or cities in Central Java Province with a prevalence of adolescent obesity aged 16-18 years of 11.47%, this shows that Surakarta City has a higher overall number of obesity cases when equalized with the number of obesity cases in Central Java Province (Riskesdas Provinsi Jawa Tengah, 2018).

ISSN 3031-0911

Surakarta City Health Service obtained obesity based on IMT by age 15-18 in the highest teens located in the work areas of the Laweyan, Jebres and Banjarsari puskesmas. Nutrition education on food and health then became a priority for adolescent groups through balanced nutrition guidelines. The government, through the Ministry of Health, has issued balanced nutrition guidelines and has a visual form to be easy to understand and implement (Permenkes RI No.41 Tahun 2014. Pedoman Gizi Seimbang, 2014). The educational process requires the media to be tailored to the objectives in order to the educational objectives. Education can be done with the media as a help to facilitate and clarify the students in receiving and understanding the message delivered besides also facilitating educators in delivering education. The education given to adolescents must be through an attractive medium so that adolescents do not feel saturated while the educational process is going on and so that the material is easy to understand (Safitri, 2016). Nutrition education can be done through a variety of media and methods. Conventional methods commonly used are lectures or other methods, one of which is a game method commonly called learning by playing. *(online* games and *offline* games*).* Online game-based learning can be used as an alternative to increasing interest and motivating students in the learning process in the era of the digital native generation. It is in line with previous research that games applied in nutrition education can enhance knowledge, attitudes and healthy eating practices in adolescent groups. When it comes to games with educational content it's called educational games (Ogunsile & Ogundele, 2016). One example of a game-based educational platform with website technology capabilities is Kahoot. This platform can be used through any digital device with a browser and existing infrastructure with good internet connection capabilities Kahoot! aims to make the learning process fun and can be applied virtually to both distance and face-to-face learning (Kalleny, 2020). So, in this study, the visual shape of the balanced nutritional stack was modified into the Kahoot. Platform as a medium of delivering balanced nutrient stack material to adolescents in order to improve the nutritional knowledge of adolescents as well as decrease fat intake and carbohydrate intake in adolescents with obesity nutritional status.

Methods

This design of the study is quasi-experimental with the use of a pre-post control design research plan that aims to find out the impact of online games on nutritional knowledge, carbohydrate intake and fat intake in overweight adolescents before and after getting the intervention. The locations of this research were SMAN 1 Surakarta, SMAN 8 Surakarta, SMA Muhammadiyah 3 Surakarta, and SMA Batik 1 Surakarta. Determination of the number of samples in this study using the Lemeshow formula to calculate the sample size to be used. The Lemeshow formula obtained a sample size of 84 people. The population in this study were students aged 16-18 years at senior high schools and equal in the 2021-2022 school year. The first stage is the sample of the research taken with a multistage sampling technique or the process of gradual duplication. The first phase is the overall research population with the target population of Higher Secondary Schools and equals in Jebres district and Laweyan district: public Senior High Schools, Private Senior High Schools, Public Vocational High Schools and Private Vocational High Schools, then selecting research locations, using simple random sampling techniques in each Senior High School and equivalent so that SMAN 1 Surakarta, SMAN 8 Surakarta, SMA Muhammadiyah 3 Surakarta and SMA Batik 1 Surakarta are obtained. Then, the sample selection process in each group was selected using a purposive sampling technique.

The criteria for inclusion are respondents who are registered as active eleventh-grade students at each study location, respondents aged 16-18 years who are nutritionally obese, have a personal smartphone and are willing to be respondents and participate in research activities. The exclusion criterion is that respondents are absent and participate during the research process. Respondents have a disease that requires a particular diet. The samples were divided into each group, namely the treatment group (intervention with lectures accompanied by online game is Kahoots and the control group (lecture method intervention).

The instruments used in this research are the instruments that have already passed the process of validation, such as a nutrition knowledge questionnaire, a nutritional knowledge questionary made in the form of a google form and filled by the nutrition information respondents consisting of 23 questions such as The instrument used in this study is a questionnaire filled out by respondents using google forms consisting of a nutritional knowledge questionnaire consisting of 23 questions in the form of multiple choice questions interpreted according to the knowledge score, which is said to be good knowledge if the score is 76-100, sufficient if the score is 56-75% and said to be lacking if <56 % (Budiman, 2013). Then fat and carbohydrate intake using food record forms distributed via google classroom and monitoring via Whatsapp is and then grouped according to gender and are categorized into categories do not sufficient with RDA (Recommended Dietary Allowances) (More: 110% RDA and less: <80% RDA) and the

ISSN 3031-0911

comlpiance with RDA (Good: 80 -110% RDA).Interpreted with the nutritional adequacy rate of adolescents said to be less if <80% AKG, good if 80-110 AKG, more if >100% AKG

Results and Discussions

Respondent characteristics

Based on **Table 1.** There were 84 known subjects, mostly of the gender of the subject, with the proportion of the female gender greater than that of the male, being 25 people and 23 people (treatment group 59.5% and control group 54.8%).

Table 1. Subject Demographic Data

General characteristics	Treatment group		Control group	
	n	%	n	%
Gender				
Male	18	42,9	17	40,5
Female	24	57,1	25	59,5
Total	42	100	42	100
Age				
16 years old	9	21,4	12	28,6
17 years old	27	64,3	25	59,5
18 years old	6	14,3	5	11,9
Total	42	100	42	100

Source: Primary data (2022)

This study obtained data characteristic of the subject of the study, including age and gender. Of the 84 subjects of the study, the percentage of female students was higher by 9.5% compared to 40.5% of male students. This is related to the problem of being overweight, which is more common in women than men. In adolescents, women tend to store more of their excess energy in the form of fat storage, so they are more susceptible to nutrition, while in men, the excess energy is used as a process for protein synthesis (Nisa & Rakhma, 2019)

The characteristics of respondents based on knowledge scores, fat intake and carbohydrate intake before and after intervention.

Table 2. shows that there is an increase in knowledge in both groups after the intervention, namely that in the group with game treatment, there was an increase in the percentage in the good category by 14.3% to 71.4% and an increase in the percentage of knowledge in the group with lecture treatment in the good category by 11.9% to 40.5%. Carbohydrate intake in each group also changed to a good category, namely in the group given intervention in the form of online games with a percentage of 23.8% to 78.6 with a percentage of the category 76.2 to 21.4%. As for the group given the intervention in the form of lectures with a good category of 21.4% to 28.6%. From the results of these percentages, the game group has more influence on changing carbohydrate nutrient intake into a good (balanced) intake category. Fat intake of respondents in each group before getting the intervention was in the more category, namely in the online game group of 92.9% and the lecture group of 95.2%. Before getting the intervention, each group was in the good category of 7.1% and 4.8%. After getting the intervention, there was a change in the good category with a percentage of 31.0% in the online game and 7.1% in the lecture group.

Table 2. Distribution of Nutrition Knowledge, Carbohydrate Intake and Fat Intake of Respondents Before and After Intervention

Dependent Variable	Treatment group				Control group			
	Before		After		Before		After	
	n	%	n	%	n	%	n	%
Nutrition knowledge								
Less	28	66,7	3	7,1	29	69,0	7	16,7
Simply	8	19,0	9	21,4	8	19,0	18	42,9
Good	6	14,3	30	71,4	5	11,9	7	40,5
Total	42	100	42	100	42	100	42	100
Carbohydrate Intake								
Less	0	0	0	0	0	0	0	0
Simply	0	0	0	0	0	0	0	0
Good	10	23,8	33	78,6	9	21,4	12	28,6

ISSN 3031-0911

More	32	76,2	9	21,4	33	78,6	30	71,4
Total	42	100	42	100	42	100	42	100
Fat Intake								
Less	0	0	0	0	0	0	0	0
Simply	0	0	0	0	0	0	0	0
Good	3	7,1	13	31,0	2	4,8	3	7,1
More	39	92,9	29	69,0	40	95,2	39	92,9
Total	42	100	42	100	42	100	42	100

Source: Primary data (2022)

In this study, obtained data characteristic of knowledge respondents better improvement of the post-intervention was found in the online game group with a score of 71.4%. If compared with the improvement in knowledge of the respondents in the control group, in the treatment group, it is more effective to improve the knowledge of respondents. This is because the learning process is done by playing (learning by playing), and respondents are not only given one-way education (receiving) but respondents also given the opportunity to train memory related to the material that has been acquired. Hence, it affects the results of the posttest obtained at the end of learning. The increased knowledge of respondents in each group also indirectly affects the eating behaviour that is based on fat intake. Respondents' fatty intake is shown in Table 2. which shows a decrease in percentage in the above category and an increase in the good category, although overall remains in the higher category. This is due to teenagers eating less fibre and more peppers because teenagers prefer to eat foods that are exciting but fast in the presentation process and economical. In the picture of carbohydrate intake, there is a percentage increase in this good category because respondents/teens are already beginning to know the daily portion of carbs they are supposed to consume so that they can be applied. It is consistent with previous research that food selection as satisfaction of nutritional needs is obtained from nutritional knowledge so that it influences the eating behaviour that is implemented as a certain food consumption attitude that affects the individual's nutritional status (Aulia, 2021).

The effect of online games on respondents' knowledge

Based on **Table 3.** Shows the results of the average value of nutritional knowledge before intervention in the treatment group was 60.62, and the control group was 56.43. After the intervention, there was an increase in the average value of 78.38 in the treatment group and 72.40 in the control group. The results also showed that the average value of the difference in nutritional knowledge in the two groups before and after the intervention was greatest in the treatment group, namely the group with online game intervention, which was 17.76. Based on the results of the Wilcoxon test, the results of nutritional knowledge in the treatment group p = 0.001 (p < 0.05) and the control group p = 0.000 (p < 0.05). This value indicates that there is an effect of online games and lectures on the nutritional knowledge of respondents after receiving the intervention. Furthermore, the results of comparative statistical analysis using the Mann-Witney test on the pre-test value of knowledge between groups before treatment showed a value of p=0.052, so there was no difference between the two groups before treatment. After the intervention, the post-test value between groups showed a value of p=0.013, which means there is a significant difference in the knowledge of respondents, so it can be concluded that nutrition education with online game media is more effective in increasing respondents' knowledge.

Table 3. Effectiveness of Online Games on Respondents' Knowledge

	Treatment Group=42					Control Group (n=42)					intra group *p-value*[a]		*p-value* between groups[b]
	Min	Max	Mean	SD	Difference value	Min	Max	Mean	SD	Value Difference	KP	KK	
Pre test	45	93	60.62	11.7	17.76	31	79	56.43	13,6	15.97	0,001[a]	0,001[a]	0,052[b]
Post test	50	94	78.38	9.8		49	87	72.40	10,2				0,013[b]

Source : Primary data (2022)
Description : Treatment group (KP), Control group (KK)
[a]Wilcoxon test, [b]Mann Whitney test; N= 84 ; Significant if p-value < 0,05

The process of nutrition education carried out in this study using the game media is the implementation of one of the forms of intervention to manage obesity, namely, the realization of nutritional education. Nutrition education is implemented as an attempt to improve the knowledge of respondents and is expected to be able to change a person's habits in the fulfilment of daily nutrition (Chiutsi-Phiri *et al*., 2017). Previous research conducted by Sineke has shown

ISSN 3031-0911

that adolescents who have poor nutritional knowledge influence adolescent obesity, where adolescents are obese due to imbalances in energy intake and energy use due to adolescents' knowledge of balanced nutritional intake (Sineke *et al*., 2019). So, the selection of the media in the process of nutrition education is very important as a medium of information with a view to the objectives, objectives and expected results. Learning needs to be carried out with innovative and interactive media so that the message of balanced nutrition can be delivered to adolescents. Based on that, online gaming media is used as an intermediary in the delivery of information, in addition to the method of lectures (conventional method).

Based on the results of the Wilcoxon statistical analysis, it show that there is a meaningful difference in knowledge scores before and after intervention using online games with a value (p=0.001). The results of this study are consistent with the study Amanah *et al.* (2019), which shows that there is a significant difference in the average value of knowledge score of respondents before and after being given education using communicative game media. Other studies have also shown that nutrition education using digital game-based media can improve knowledge in adolescents positively and related to nutrition and healthy eating patterns (Ezezika *et al*., 2018). The effectiveness of learning with digital/online-based game media is also demonstrated by research from Arif *et al*. (2022) that online learning processes can shape respondent thinking more critically because the stimulation given can stimulate the recipient of information to think more critically so that it influences the process of intelligence and is able to apply the information obtained.

The results of the Wilcoxon analysis test in the control group showed that the method of lectures also improved knowledge in adolescents. Although both methods are capable of improving knowledge, significantly higher scores are found in the online game groups with an average difference of 17.76 and the mean value of posttest knowledge **(see Table 3).** The lecture method in the control group has a weakness when the learning process is going on. That is, the learning process is one-way. Whereas in the treatment group, in addition to respondents receiving information through the lecture method, respondents also get the game so that information is easier to accept and easy to learn by respondents. The effectiveness of online media games was demonstrated by the results of different test analyses using Mann-Whitney's statistical test, which obtained results that there were significant differences in knowledge between groups after the given intervention. It indicates that online games are used in research as one of the proper forms of intervention in the nutrition education process. It is supported by other research that the media interactive online quiz game is based on Kahoot. That is used in nutritional education about the importance of effective consumption of fruits and vegetables, improving students' knowledge (Ladiba *et al*., 2021). Other research also suggests that one of the nutritional innovations is the media online interactive quiz game based on the platform of Kahuot. However, the use of these media can boost the interests and learning outcomes of students (Wigati, 2019).

The effect of online games on fat intake and carbohydrate intake

Based on **Table 4**. Interpretation of the Wilcoxon test results obtained data on fat intake before and after the intervention in the treatment group showed a p-value = 0.230 (p> 0.05), and in the control group showed a p-value = 0.299, which means there was no significant difference in intake fat of respondents after being given nutritional education intervention in the form of online games in the treatment group and the control group. Then, a follow-up test was carried out with Mann Whitney, which showed no difference in fat intake before the intervention, indicated by a p-value of 0.385> 0.05 and the results of the test for differences between groups after receiving the intervention showed a significant difference in p-value 0.011 <0.05. Based on the significance of the Mann-Whitney results, it can be stated that the treatment group or the group that was given nutrition education with interventions in the form of online games was more effective in changing the subject's fat intake than the control group, which can be seen in **Table 2** where the percentage of fat intake and carbohydrate intake of respondents compared to daily nutritional adequacy figures.

Table 4. Effectiveness of Online Games on Fat Intake

Fat intake	P-value Treatment Group[a]	P-value[b] Control group	p-value
Pre intervention	0.230	0.299	0,385
Post Intervention			0,011

Source : Primary data (2022)
Description : [a]Wilcoxon test, [b]Mann Whitney Test

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Interpretation of the results of the *Wilcoxon* test obtained by the intervention carbohydrate intake data in the treatment group showed a difference after the intervention, indicated by a value of 0.001 ($p < 0.05$). The same result was also shown in the control group with a value of 0.002 ($p<0.05$), which indicated that there was a significant difference in the carbohydrate intake of respondents before and after receiving a balanced nutrition lecture. Furthermore, further tests were carried out with *Mann Whitney*, which showed that there was no difference in carbohydrate intake between groups before being given the intervention indicated by carbohydrate intake $p=0.520$ ($p>0.05$). The results of the difference test between groups after getting the intervention showed a significant difference with a value of $p=0.001$ ($p<0.05$). Based on the significance of the mann-whitney results, it can be stated that the treatment group or the group given nutrition education with an intervention in the form of an online game is more effective in changing the carbohydrate intake of the subject than the control group, which can be seen in **Table 2** where the percentage of carbohydrate intake of respondents decreased to the good category most in the online game group.

Obesity in adolescents is a serious problem because it continues until adulthood. One of the factors causing this obesity problem is the intake of macronutrients as a source of energy for the body (Kurdanti *et al*., 2015). The imbalance of energy expended or used by the body with excessive intake is generally the cause of overnutrition. Energy imbalance is a habit of eating high calories but not accompanied by physical activity. That this nutritional problem is influenced by a sedentary lifestyle and a diet that is high in calories, and high in fat intake, high fat intake but low in fibre (Budiono *et al.*, 2022).

Based on the results of the study, it is known that the fatty intake of respondents did not experience significant differences between the groups both before and after the intervention. This can happen because respondents are still consuming excess fried foods every day, such as chopped fries, chopsticks, fries and other fried fries. So, even though the food ingredients contain a fairly high amount of protein, these foods also contribute to the fat derived from oil. The results of this study are also similar to the research of Darni (2020), that nutrition education given using the nutrition and nutrition comic media showed no significant difference in the intake of pre-intervention fat sources shown with a $p=0.119$ ($p>0.05$) value in the treatment group and the control group with the $p=0.04$ value ($p >0.05$). It is also due to the fat intake that is easier to burn consumption if it has been processed into food forms such as fries. Because of the intake of fat in a balanced diet of 67 grams/day or 5 tablespoons of oil, this fat consumption is difficult to avoid due to the intake of foods that have to be fried.

Based on the results of the study, it was revealed that the respondents' carbohydrate intake showed significant differences in the treatment group after obtaining the intervention of online games. A significant difference was also shown in the control group with lecture intervention. Although there were significant differences in each group after the intervention was given, the results of the study showed that the carbohydrate intake of respondents after compared with AKG (number of nutritional sufficiency) with the highest percentage of both categories was found in the game group **(see table 2).** Advanced trials also showed there was a significant difference between the groups, so it can be said that online games were effective in reducing the carbs intake in the respondents in the category well faster because of the sources of carbs such as rice, strawberries, potatoes and other things that are easy to restrict their consumption by respondents according to the daily intake recommendations because of their ease of burning. The results of this study are in line with Palupi's research (2022). The nutritional education "EMPIRE" educational media with an online-based psychological approach has an effect on carbohydrate intake in obese indicated by a value of $p = 0.001 (p<0.05)$.

Conclusions

There is an increase in learning after getting an online game education, and there is also an improvement in knowledge after getting a lecture education. However, knowledge improvement is faster if given online games education, so online games are more effective as a balanced nutritional education medium. There is no difference in the intake of fat after getting educational games online. The same thing is also after being given education in the form of lectures. However, online games are more effective in changing carbohydrate intake.

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Syzygium Polyanthum as a Potential Hemoglobin Booster for Iron Deficiency Anemia Due to its High Vitamin C Content

Rhisma Mutiara Devi,[1,*] Adi Magna Patriadi Nuhriawangsa[2], Setyo Sri Rahardjo[3]

[1]Department of Nutrition Science, Sebelas Maret University, Surakarta, Indonesia
[2]Department of Animal Science, Sebelas Maret University, Surakarta, Indonesia
[3]Department of Medical Science, Sebelas Maret University, Surakarta, Indonesia

*Correspondence author : rhismamutiara@gmail.com

Abstract

Anaemia management can be done pharmacologically through Boosting Tablets (TTD) and non-pharmacologically by consuming natural food ingredients such as bay leaves. Bay leaves are one of the sources of iron and vitamin C readily available in the community. This study aims to analyze the nutritional content and vitamin C in bay leaves at Karanganyar. The research design is an experimental study that examines the nutritional content and vitamin C levels in bay leaf powder to facilitate Fe absorption in anaemia management. Vitamin C plays a crucial role in absorbing non-heme iron, which is essential for haemoglobin production in the human body. The vitamin C content in bay leaf powder at Karanganyar, 1294.78 mg per 100 grams, was tested in the Food Chemistry and Biochemistry Laboratory, Food Science and Technology Study Program, Faculty of Agriculture, Universitas Sebelas Maret. A high vitamin C concentration in bay leaf powder is expected to enhance iron absorption and reduce the risk of anaemia-related iron deficiency. This analysis provides insights into the potential contribution of bay leaf powder in addressing anaemia through nutritional approaches.

Keywords: Iron Deficiency Anemia, Iron, Vitamin C, *Syzygium polyanthum* (bay leaves)

Introduction

Iron deficiency anaemia is a common global health problem characterized by a lack of iron in the body, leading to reduced red blood cells and insufficient haemoglobin levels. It affects individuals of all ages and can significantly impact physical and cognitive functions. Adequate iron intake and absorption are crucial for preventing and treating this condition (AlDallal, 2016; Alli *et al*., 2017; Benz, 2018). The prevalence of anaemia worldwide is significant and has a global impact on public health. According to data from the Global Health Observatory (GHO) published by the World Health Organization (WHO) in 2022, approximately 32.8% of the world's population experiences anaemia. The highest prevalence occurs among children under five (42.6%), followed by women of reproductive age (29.1%). In Indonesia, anaemia is also a severe health issue. According to the Basic Health Research (Riskesdas) data from 2018 conducted by the Ministry of Health Indonesia, the prevalence of anaemia among pregnant women is 29.5%, while among children under five years old, it is 35.1%. These figures indicate that anaemia remains a significant concern in Indonesia (Kemenkes RI, 2018; WHO, 2022)

Iron is essential in producing haemoglobin, the protein responsible for transporting oxygen throughout the body. Iron deficiency can result in decreased haemoglobin levels, leading to anaemia. Individuals with iron deficiency anaemia can restore their haemoglobin levels and improve overall health by incorporating iron-rich foods or supplements into their diet. Vitamin C plays a crucial role in enhancing iron absorption. It helps convert non-heme iron from plant-based sources into a more readily absorbable form. By combining iron-rich foods with a good source of vitamin C, individuals can maximize their iron absorption and optimize haemoglobin production (Pdxscholar & Alarcon Basurto 2020; Shubham *et al*., 2020; Zhu *et al*., 2016)

Syzygium Polyanthum, commonly known as "Daun Salam" or Indonesian bay leaf, is native to Southeast Asia. It has been traditionally used for culinary purposes due to its aromatic properties. Beyond its culinary uses, *Syzygium Polyanthum* possesses several potential health benefits, including its high content of iron and vitamin C. *Syzygium Polyanthum* stands out as a possible natural remedy for iron-deficiency anaemia due to its dual advantage of being rich in both iron and vitamin C. By consuming this plant; individuals can increase their iron intake and enhance iron absorption simultaneously, effectively combating anaemia. Moreover, incorporating *Syzygium Polyanthum* into the diet offers a natural and sustainable alternative to synthetic iron supplements, which can sometimes cause digestive discomfort or undesirable side effects. The plant's bioavailability and the synergy between iron and vitamin C may further enhance its potential as a haemoglobin booster (Ismail *et al*., 2019; Silalahi, 2017; Suharni *et al*., 2021).

ISSN 3031-0911

This study aims to comprehensively examine the nutritional content and vitamin C levels in bay leaf powder, particularly in anaemia management. Using an experimental research design, analysis was conducted to understand better the nutritional composition and vitamin C content in bay leaf powder, thus providing a healthy approach to address anaemia.

Methods

This research was conducted in the Laboratory of Chemistry and Biochemistry of Food, Food Science and Technology Study Program, Faculty of Agriculture, Universitas Sebelas Maret. The utilization of laboratory equipment includes tools such as analytical balances, Erlenmeyer flasks, volumetric flasks, beakers, filter paper, glass funnels, burets, filling pipettes, measuring pipettes, dropping pipettes, glassware, aluminium, and stirring rods. The materials used include bay leaf powder, distilled water, Sodium Thiosulfate, 0.001 N iodine solution (I2), and 1% starch indicator. Sample preparation involves mixing bay leaf powder with distilled water to achieve homogeneity, followed by filtration into volumetric flasks (Basir, 2017; Khadijah, 2019).

For the vitamin C analysis, 25 ml of the sample solution is taken and poured into an Erlenmeyer flask, adding two drops of starch indicator. The answer is titrated with 0.01 N Iodine until a dark blue colour change occurs. The vitamin C content is calculated based on this titration. The Proximate Analysis determination of Moisture Content is done by drying a clean porcelain crucible in an oven at 105°C for 1 hour. The crucible is then cooled in a desiccator for 10-20 minutes and weighed to obtain the initial weight. A sample weighing 0.5-1 gram is placed into the porcelain crucible and dried in the oven at 105°C for 12-16 hours. The weight of the crucible and sample is then weighed again after removal from the container and cooling in the desiccator. Determination of Ash Content is carried out with a similar process: a cleaned and dried porcelain crucible is weighed, followed by placing a sample of 3 grams of plant material or 5 grams of concentrate into the container. The model is then burnt in a furnace at 600°C until it turns white. After cooling, the crucible is reweighed (Basir, 2017; Khadijah, 2019).

Determination of Crude Protein is done by weighing 0.3 grams of the sample and placing it into a combustion tube. Approximately 0.2 grams of mixed catalyst and 5 ml of concentrated H2SO4 are added. The mixture is heated in a fume hood, ensuring controlled destruction to avoid overflow. The process is stopped when the solution turns bright green or clear. The resulting solution is transferred to a distillation flask and diluted with distilled water. Distillation occurs until the evolved nitrogen is captured by 0.3 N H2SO4 solution in an Erlenmeyer flask. This methodology aims to determine bay leaf powder's nutritional composition and vitamin C content, particularly in addressing anaemia (Basir, 2017; Khadijah, 2019).

Results and Discussions

The results obtained from the research on *Syzygium Polyanthum* samples have unveiled this plant's chemical composition within the context of various parameters. Specific analytical methods have yielded data depicting certain substances within the model. Based on thermogravimetric analysis of the *Syzygium Polyanthum* sample, the percentage of water content in this plant was recorded at 9.38%. This analysis provides information regarding the proportion of water in the model, which can significantly indicate the physical properties and stability of chemical components within the plant (Basir, 2017; Khadijah, 2019).

Table 1. Analysis of Vitamin C and Proximate *Syzygium Polyanthum*

Analysis	Method	Results
Water	Thermogravimetry	9,38%
Mineral	Dry Method	4,81%
Fat	Soxhlet	4,27%
Proteins	Kjeldahl	9,34%
Carbohydrates	By Different	72,20%
Vitamin C	Spectophotometry	1294,78 mg/ 100 g

The analysis conducted through a dry method indicated a mineral content of 4.81% in the *Syzygium Polyanthum* sample. This analysis is crucial for understanding the mineral content that could contribute to this plant's nutritional value and properties. The fat content in the *Syzygium Polyanthum* sample was analyzed using the Soxhlet

ISSN 3031-0911

method, revealing a range of 4.27%. Information about fat content is essential in exploring the potential energy content and other bioactive components present in the plant. The Kjeldahl method analysis on the sample showed a protein content of 9.34%. Protein content is a crucial indicator for evaluating the nutritional potential of this plant, especially in terms of its contribution to protein intake in the human diet (Basir, 2017; Ismail *et al*., 2019).

A different analysis was employed to assess the carbohydrate content in the *Syzygium Polyanthum* sample, revealing a carbohydrate content of 72.20%. This information holds significant relevance in evaluating the contribution of carbohydrates to the overall nutritional composition of this plant. The results of the spectrophotometric analysis indicated that the vitamin C content in the *Syzygium Polyanthum* sample was 1294.78 mg per 100 grams. Vitamin C plays a crucial role in human health and fitness and enhances the absorption of non-heme iron in the body (Harismah & Chusniatun, 2016; Suharni *et al.*, 2021). Overall, these analysis results provide a detailed overview of the chemical composition of the *Syzygium Polyanthum* sample, including the proportions of water, minerals, fats, proteins, carbohydrates, and vitamin C. This information can offer deeper insights into this plant's potential nutritional and health benefits in its utilization in diets and medical treatments.

The test results prove that the elements vitamin C and Fe in Karanganyar have a high concentration level compared to other ingredients, namely spinach, green beans, Moringa leaves, and bay leaves, in the 2017 Indonesian food composition table. Apart from that, according to Suharni (2021) research, it was stated that the Fe content in 100 grams of bay powder is 1.48 mg. The obtained results from the analyses conducted on the *Syzygium polyanthum* samples offer valuable insights into this plant's botanical composition and nutritional potential. Diverse aspects of the plant's chemical constitution have been scrutinized by meticulously applying distinct analytical methodologies, yielding quantitative data about critical constituents. The thermogravimetric analysis of the *Syzygium polyanthum* sample has furnished a discerning perspective on its water content, indicating a recorded value of 9.38%. This outcome is of paramount significance as it provides essential knowledge regarding the moisture content inherent in the plant material. The information derived from this analysis is pivotal in comprehending the physicochemical attributes and stability of the plant's constituents under varying conditions. In a parallel vein, applying a dry method for mineral analysis has elucidated a mineral content of 4.81% within the *Syzygium Polyanthum* sample. This mineral composition directly relates to the plant's nutritional profile and potential contributions to human health. The insight from this particular analysis enriches our understanding of the plant's mineralogical constitution and its significance in dietary contexts (Wang *et al*., 2021).

An investigation into the lipid content of the *Syzygium polyanthum* sample was undertaken employing the Soxhlet analysis, uncovering a lipid content measuring 4.27%. This lipid analysis offers an intricate portrayal of the plant's fat content, a pivotal aspect in nutritional evaluation. The fat content not only imparts energy density but also serves as a carrier of essential lipid-soluble bioactive compounds, thereby influencing the potential health benefits of the plant. Furthermore, the examination of protein content was achieved through the Kjeldahl method, revealing a protein content of 9.34% within the *Syzygium polyanthum* sample. The protein content is a hallmark parameter for assessing the nutritional value of the plant, especially its capacity to contribute to the dietary protein intake of individuals. This quantitative insight aids in evaluating the plant's suitability as a potential protein source in various nutritional regimens (Hertzler *et al*., 2020).

Table 2. Differences In Ingredient Content

Material	Fe (mg)	Vit C
Bay leaf	44.1	0
Mung beans	7.5	10
Moringa Leaves	6	22
Red spinach	7	62

An alternative approach, "Different Analysis," assessed the carbohydrate content, yielding a carbohydrate composition of 72.20%. The carbohydrate analysis, conducted using a distinct methodology, offers a comprehensive outlook on the plant's carbohydrate constituency, pivotal in deciphering its caloric value and potential dietary applications. Finally, employing the spectrophotometric technique, the analysis of vitamin C content showcased a prominent value of 1294.78 mg per 100 grams within *the Syzygium Polyanthum* sample. Vitamin C, a pivotal water-soluble micronutrient, plays multifaceted roles in human health, particularly in bolstering immune functions and augmenting the absorption of non-heme iron, a vital constituent in haemoglobin synthesis (Pecora *et al*., 2020).

ISSN 3031-0911

In totality, the multi-pronged analyses have collectively furnished an intricate panorama of the chemical makeup of the *Syzygium Polyanthum* sample. These analyses have discerningly revealed the plant's water content, mineral constitution, fat content, protein composition, carbohydrate makeup, and abundant vitamin C. This comprehensive dataset is poised to illuminate potential applications in nutrition, dietary interventions, and healthcare paradigms, substantiating its significance as a promising botanical entity for diverse uses (Kehati, n.d.).

Conclusions

The analyses reveal the *Syzygium polyanthum* intricate chemical composition, including water content (9.38%), mineral content through dry method analysis (4.81%), lipid content through Soxhlet analysis (4.27%), protein content via Kjeldahl analysis (9.34%), distinct carbohydrate content (72.20%), and a notable vitamin C presence (1294.78 mg/100 g) determined by spectrophotometric analysis. These findings provide valuable insights into its potential applications across nutritional and health contexts.

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The relationship between intake of iron, vitamin D, and nutritional status on the incidence of anemia in pregnant women at the Kebak Kramat 1 community health center, Karanganyar

Baarizah Febriana Badri[1]*, Dono Indarto[2], Tonang Dwi Ardyanto[3],

[1] Department of Nutrition science, Sebelas Maret University, Surakarta, Indonesia
[2,3] Department of Medical science, Sebelas Maret University, Surakarta, Indonesia
*Correspondence author : baarizahfebriana@student.uns.ac.id

Abstract

Anemia is one of the World Health Organization's five global health challenges for 2025. The causes of anemia during pregnancy are linked to iron intake and nutritional status, which may result in severe consequences such as miscarriage, preterm birth, pre/postpartum hemorrhage, and low birth weight. This study investigated the association between iron intake, vitamin D, nutritional status, and the prevalence of anemia during pregnancy. This was an analytical cross-sectional study involving a population of 132 pregnancies. The sample included 30 pregnancies. The data was analyzed through the Chi-square, logistic, and multiple linear regression tests. Iron deficiency demonstrated a 15-fold increase in the risk of anemia (p-value 0.016) without correlation. Vitamin D intake is not related to anemia, while nutritional status is not associated with anemia (p-value 0.787). Insufficient iron intake may increase the risk of anemia, whereas a good vitamin D and adequate dietary level may lower this risk. Therefore, further research is needed on anemia knowledge education during pregnancy.

Keywords: Iron intake, Vitamin D, Nutritional State, Anemia, Pregnancy

Introduction

Anemia in pregnant women in Indonesia is still relatively high. According to Riskesdas data (2018), anemia in pregnant women has increased by 48.9%. Meanwhile, in Karanganyar Regency in 2021, 1,163 cases of moderate anemia (8-11 mg/dl) were found in pregnant women in Karanganyar Regency, covering 21 regions (Karanganyar Public Health Service, 2021).

Factors influencing the causes of anemia in pregnant women. Risk factors for anemia in pregnant women are related to iron intake, and the mother's nutritional status also affects cases of anemia (El-Kholy *et al*., 2023; Yang *et al*., 2023). This research by Tadesse *et al.* (2021) said that Fe intake is eight times less likely to cause anemia due to the less varied diet of pregnant women, especially foods that are sources of iron.

The source of iron that pregnant women often consume is vegetable side dishes because they are affordable. Apart from that, you also often consume sweet tea twice a day, which can inhibit iron absorption. Drinking tea has a 2.785 times greater risk of developing anemia compared to pregnant women who never drink tea (Santhakumar *et al*., 2023; Wiafe *et al*., 2023).

An indicator of success in preventing iron anemia is consuming food sources of iron such as animal protein (processed meat, processed chicken, processed fish) (Sukmawati *et al*., 2019). Apart from that, the government has taken several steps to reduce cases of iron deficiency anemia, namely by fortifying minerals and vitamins (such as Fe, zinc, folic acid, thiamine, and riboflavin) which are already on the market (Nugraheni *et al*., 2019).

This study aims to determine the effect of differences in iron intake and nutritional status on anemic pregnant women in the working area of the Kebak Kramat 1 Health Center, Karanganyar Regency.

Methods

This cross-sectional study was conducted in the Kebak Kramat 1 Community Health Center, Karanganyar, for one month in July 2023 (Figure 1). Based on the data of the health report from the Karanganyar public health office in 2022, there were 132 pregnant women with anemia in the Kebak Kramat 1 Community Health Center. Research subjects in research are calculated using the formula from the application http://www.www.openepi.com/SampleSize/SSPropor.htm (Probandari *et al.*, 2020) from which we got at least 30 research subjects. The inclusion criteria included pregnant women in the first and second trimesters of pregnancy, aged 20-35, and hemoglobin level <9-10 g/dl. Pregnant women who had high blood pressure (>120/80 mmHg), parity history >3, postpartum bleeding, and pregnancy history <2 years were excluded from this study. Data on essential characteristics and food frequency were collected by direct interviews using open and SQ-FFQ (semi-quantitative food

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frequency questionnaire) questionnaires, respectively. Data on the nutritional status were collected by measuring MUAC (mid-upper arm circumference). Numerical data were presented as mean standard deviation, and statistically analyzed using a linear regression test. Categorical data were presented as frequency and percentage and statistically analyzed using the chi-square test. Multiple logistic regression test was used to investigate independent and confounding variables against the dependent variable with a significant value <0.05.

Results and Discussions

The sample characteristics consist of maternal age, maternal education level, and family income, which can be seen in the following table:

Table 1. Characteristic sample

Subject Characteristic	Frequency	Percentage
	n	%
Mother's Age		
No Risk (20-35 th)	**21**	**70**
Risk (>35 th)	**9**	**30**
Gestational Age		
First Trimester (4-13 weeks)	**7**	**23**
Second Trimester (14-26 weeks)	**21**	**70**
Third Trimester (27-40 weeks)	**2**	**7**
Mother's Education level		
Elementary School	**3**	**10**
Middle and High School	**24**	**80**
College	**3**	**10**
Family Income		
Below the regionl minimum wage (<Rp2.000.000)	**24**	**80**
Above the regionl minimum wage (>Rp2.000.000)	**6**	**20**

Source : Primer Data, 2023

Based on **Table 1**, information was obtained that the majority of pregnant women were not at risk, as many as 21 people (70%), while nine people (30%) were pregnant women who were at risk (>35 years). Pregnant women with the majority of gestational age in the second trimester (14-26 weeks) were 21 people (70%), while in the first trimester, there were seven people (23%), and in the third trimester, there were two people (7%). Most pregnant women had a secondary education level (SMP-SMA), as many as 24 people (80%). In comparison, the maternal education levels were primary (SD) and higher (university) as many as three people (10%). The majority of the mother's family had an income below the regional minimum wage (<Rp. 2,000,000), as many as 24 people (80%), while the family income was above the provincial minimum wage (>Rp. 2,000,000) as many as six people (20%).

Table 2. Analyzed Univariate Independent and Dependent Variables

Independen and Dependen Variable	Frequency	Percentage
	n	%
Iron Intake		
Not enough	**24**	**70**
Enough	**6**	**30**
Vitamin D intake		
Not Enough	**24**	**70**
Enough	**6**	**30**
Nutritional Status		
Malnutrition	**24**	**70**
Good Nutrition	**6**	**30**
Anemia Occurrence		
Anemic	**25**	**75**
Non Anemic	**5**	**25**

Source : Primer Data 2023

ISSN 3031-0911

Based on **Table 2**. Most pregnant women's food intake is deficient in iron, as many as 24 people (70%), while six people (30%) have sufficient iron intake in pregnant women. Most pregnant women's food intake of vitamin D is deficient, as many as 24 people (70%), while pregnant women with sufficient vitamin D intake are six people (30%). Most pregnant women experienced poor nutritional status, 24 people (70%), while six pregnant women experienced good dietary grade (30%). Most pregnant women also experience anemia, as many as 25 people (75%), while five people (25%) do not experience anemia.

Table 3. Relationship between Iron Intake, Vitamin D and Nutritional Status on the Incidence of Anemia Using Bivariate Analysis

Variable	Anemia Occurrence				**OR**	**CI 95%**		***P*[a]**
	Anemic		**Not Anemic**			**Lower**	**Upper**	
	n	**%**	**n**	**%**				
Iron Intake					15,00	1,652	136,172	0,016*
Not enough	19	45	5	25				
Enough	4	20	2	10				
Vitamin D Intake					0,427	0,033	4,228	0,417
Not enough	19	45	6	30				
Enough	4	20	1	5				
Nutritional Status					0,787	0,165	10,743	0,787
Malnutrition	19	45	5	25				
Good Nutrition	4	20	2	10				

P[a]: *Chi-Square Test* ****) P<0.05 Significant***

Table 3 shows that iron intake is significantly related to the incidence of anemia (OR=15, 95% CI, p=0.016), namely that 19 pregnant women (45%) who experienced insufficient iron intake resulted in anemia. The OR value is 15, meaning inadequate iron intake can increase the risk of anemia by 15 times compared to sufficient iron intake. Vitamin D intake was not significantly related to the incidence of anemia (OR=0.427, 95% CI, p=0.417). Nineteen pregnant women (45%) who experienced insufficient vitamin D intake resulted in anemia. The OR value is 0.427, meaning inadequate vitamin D intake can increase the risk of anemia by 0.427 times compared to sufficient vitamin D intake. Nutritional status was not significantly related to the incidence of anemia (OR=0.787, 95% CI, p=0.787); namely, 19 pregnant women (45%) who experienced poor nutritional status resulted in anemia. The OR value is 0.287, meaning poor nutritional quality can increase the risk of anemia by 0.287 times compared to good nutritional status.

Table 4. Relationship between Mothers'age, Family Income, Mother's Education Level, and Gestational Age on the Incidence of Anemia Using Bivariate Analysis

Variable	**Anemia Occurrence**				**OR**	**CI 95%**		***P*[a]**
	Anemic		**Not Anemic**			**Lower**	**Upper**	
	n	**%**	**n**	**%**				
Mother's Age								
No Risk (20-35 th)	6	30	3	15	0,287	0,048	2,457	0,279
Risk (>35 th)	7	35	4	20				
Family Income								
Below the regionl minimum wage (<Rp2.000.000)	9	45	5	25	0,139	0,616	32,069	0,128
Above the regionl minimum wage (>Rp2.000.000)	4	20	2	10				

ISSN 3031-0911

Mother's Education level								
Elementary School	2	10	1	5	1,009	0,240	4,233	0,419
Middle and High School	9	45	5	25				
College	2	10	1	5				
Gestational Age								
First Trimester (4-13 weeks)	7	35	4	20	2,151	0,346	13,392	0,895
Second Trimester (14-26 weeks)	5	25	3	15				
Third Trimester (27-40 weeks)	1	5	0	0				

P^a : *Chi Square test*

Based on **Table 4**, information was obtained that maternal age was not significantly related to the incidence of anemia (OR=0.287, 95% CI, p=0.279), namely that six mothers (30%) were at risk of developing anemia. Seven pregnant mothers (35%) are not at risk of anemia. The OR value is 0.287, which means that the age of a mother at risk can increase the risk of anemia by 0.287 times compared to that of a mother who is not at risk. Family income was not significantly related to the incidence of anemia (OR=0.139, 95% CI, p=0.128). That is, nine pregnant women (45%) whose income was below the minimum wage (<Rp. 2,000,000) experienced anemia, and as many as four pregnant women (20%) who earned above the minimum wage (>Rp. 2,000,000) experienced anemia. The OR value = 0.139 means that mothers whose income is below the minimum wage can increase the risk of anemia 0.139 times compared to mothers who earn above the minimum wage. The level of education was not related to the incidence of anemia (OR=1.009, 95% CI, p=0.419). Namely, nine pregnant women (45%) had secondary education (middle and high school), and two pregnant women (10%) had essential (primary) and tertiary (college) education levels. An OR value of 1.009 means that pregnant women with a basic education level can increase the risk of anemia by 1.009 compared to mothers with a higher education level. Gestational age was not significantly related to the incidence of anemia (OR 2.151, 95% CI, p=0.895), namely seven pregnant women (35%) in the first trimester (4-13 weeks) and five pregnant women (25%) in the second trimester. (14-26 weeks), as many as one pregnant woman (5%) in the third trimester (27-40 weeks).

Table 5. Multiple Analyzed Independent Variable and Confounding

Variable	**B**	**Wald**	**Sig**	**OR**	**CI95%**	
Nutrition status	-1.544	0.382	0.537	0.214	Lower	Upper
Mother's age	-1.374	0.587	0.444	0.253	0.002	28.616
Gestational age	38.254	0.000	0.998	4.107	0.008	8.509
Level of education	-0.281	0.020	0.889	0.755	0.000	
Family income	36.991	0.000	0.998	1.162	0.015	38.579
Iron intake	-58.885	0.000	0.997	0.000	0.000	
vitamin D intake	16.935	0.000	0.999	22.64	0.000	

P^a : multiple linear regression test

*) p<0.05 significant

Based on **Table 5**, it can be concluded that of all the independent variables (nutritional status, maternal age, education level, family income, iron intake, and vitamin D intake, which is thought not to influence anemia in pregnant women. The most significant OR value was 22.64, meaning that vitamin D intake has a 22.64 times chance of causing anemia in pregnant women.

ISSN 3031-0911

Based on **Table 1**, information was obtained that the majority of mothers were not at risk (20-35 years) as many as 11 people (55%). Meanwhile, there are nine mothers who are not at risk (45%). Maternal age < 20 years or above and > 35 years can cause anemia. This is because iron consumption for 20 years is divided by the fetus in the womb and its own biological growth, which, of course, still requires a lot of iron consumption (George et al., 2021). After the age of 35, they enter an early degenerative stage when body function is not optimal, and they have various health problems. Pregnancies under 20 years and over 35 years are pregnancies with a risk of anemia (Bellakhal *et al*., 2019; Sunuwar *et al*., 2019)

According to Parischa *et al*. (2023), mothers of at-risk ages can reduce the incidence of anemia by 0.68 times compared to mothers of non-risk ages. The results of this study show that there is conformity with the theory put forward by Guo *et al*. (2022) that the ideal maternal age in pregnancy is the 20-35 year age group, and at this age, mothers have healthy reproduction and are less at risk of pregnancy complications. The age group < 20 years is at risk of anemia because reproductive development is not yet optimal, and according to Kumar and Lahiri (2023), pregnancy in the 35-year age group is associated with deterioration and decreased endurance, as well as various diseases that often occur at this age. Pregnancy at >35 years of age is a high-risk pregnancy because, at this age, chronic health problems often occur, one of which is the risk of anemia. Bleeding that occurs during childbirth, if not handled properly, will cause anemia. Apart from that, age is not the only factor that causes anemia; there are other factors, namely socio-economic factors (Kang et al., 2023).

Based on **Table 1**, information was obtained that the majority of mothers had a secondary education level (SMP-SMA), as many as 14 people (70%). Meanwhile, there were three mothers (15%) who had primary (primary) and higher (university) education. According to research by Edison (2019), the relationship between education level and the incidence of anemia in pregnant women shows that the prevalence of anemia in mothers who have a low level of education reaches 90.3% compared to mothers who have a high level of education, which is only 9.7%. The Chi-Square test results obtained a value of ρ= 0.001. This is due to the mother's lack of knowledge on how to process good food so that it does not damage the nutritional content in it and also due to low education, the mother does not work, which reduces household income, and the mother cannot buy nutritious food so that due to this limitation anemia occurs (Panchal *et al* ., 2022). Based on interviews during the preliminary study, mothers only received education from health workers at the Community Health Center only once a month. So, the information obtained is lacking because, during the education class for pregnant women, only one theme is given, such as consuming blood supplement tablets regularly.

Based on **Table 1**, information was obtained that the majority of family income was below the minimum wage (<Rp. 2,000,000), as many as 14 people (70%). Meanwhile, six people (30%) had family income above the minimum wage (>Rp. 2,000,000). The prevalence of anemia is more significant in pregnant women with incomes lower than the Regional Minimum Wage (UMR). This affects the purchasing power of food consumed by families because >57% of family income is spent on purchasing food (Gibore *et al*., 2021; Pasricha *et al*., 2023). According to Septiasari (2019) research, it was found that 25 out of 39 pregnant women (61.0%) of mothers with income < minimum wage experienced anemia, while among pregnant women with income ≥ minimum wage, there were 16 out of 47 people (39.0 %) have anemia. The results of the chi-square statistical test show that the p-value = 0.005 (p ≤0.05), RP 3.460 (95% CI = 1.421 – 8.425), so it can be concluded that mothers with an income <UMR increase the incidence of anemia by 34 times compared to mothers with an income >UMR causes mothers not to get adequate nutrition, thereby risking anemia. The number of families will influence the amount of food distribution within the family. The lack of family income causes a reduction in the location and purchase of daily food, thereby reducing the quantity and quality of the mother's food per day, which has an impact on reducing nutritional status. A common nutritional disorder in pregnant women is anemia. The food sources needed to prevent anemia generally come from protein sources, which are more expensive and difficult to afford for those with low incomes. This deficiency increases the risk of anemia in pregnant women and accelerates the risk of morbidity in mothers (Mekonen & Alemu, 2021; Maugliani & Baldi, 2023). Based on preliminary study interviews, as many as 65% of pregnant women said that they consumed two pieces of vegetable protein (tempeh, tofu, processed nuts) 3-4 times per week.

Based on **Table 1**, information was obtained that the majority of pregnant women with poor nutritional status were 14 people (70%). Meanwhile, there were six pregnant women with good nutritional status (30%). Steven et al. (2022) stated that the relationship between nutritional status influences the incidence of anemia. Nutritional status is also defined as health status resulting from a balance between nutrient needs and input and is a basic need for pregnant women. Nutritional status and the incidence of anemia were also shown by Dewi and Mardiana (2021), who found that the risk of anemia in pregnant women was 2.9 times higher for pregnant women with poor nutritional status than those

ISSN 3031-0911

with good nutritional status. This comparative figure has a relatively significant role in influencing the health of pregnant women. The estimated determinant of R2 is 0.047, meaning that nutritional status contributes 4.7% in influencing the incidence of anemia. Even though the contribution value is small, as long as the regression coefficient β1 is not statistically zero, scientifically, it can be proven that there is an influence between nutritional status and the incidence of anemia (Guo *et al*., 2022)

Based on **Table 1**, information was obtained that the majority of mothers' iron intake was less than 14 people (70%). Meanwhile, six people (30%) had sufficient iron intake, according to research conducted in Jatinangor District regarding the consumption patterns of pregnant women, which shows that the intake of food sources of iron (Fe) in pregnant women is still inadequate, namely 53 people (93%) out of 57 pregnant women (Putri *et al*., 2020). This happens because the source of iron consumed does not come from heme, such as meat and animal food sources, so it is not easy to absorb and does not support the presence of iron in the body. The same thing was done in research in TuaTunu Pangkal Pinang Village regarding the relationship between nutritional intake and the incidence of anemia in preconception women, which showed that the intake of food sources of iron (Fe) in preconception women was still insufficient, namely 98.4% (Devriany & Wardani, 2019). This is because the consumption of foods that are less balanced can interfere with the absorption of iron in the body, and the majority of respondents consume more vegetable protein than animal protein. In addition, the lack of consumption of side dishes and side dishes which are a source of iron that is useful during pregnancy for the formation of new hemoglobin compensates for the small amount of iron that is constantly excreted by the body (primarily through urine, feces, and sweat), and replaces iron losses during pregnancy—lactation for milk secretion (Devriany & Wardani, 2019).

Mothers who are malnourished are at risk of having a difficult or long labor, giving birth to a baby prematurely (not yet full term), bleeding in the mother after giving birth, and usually during labor, the mother also lacks the strength to push during the labor process so giving birth using a high surgical procedure for pregnant women lack nutrition (Xu *et al*., 2022). There are 41% of pregnant women suffer from malnutrition. The emergence of nutritional problems in pregnant women, such as the incidence of CED, cannot be separated from the social, economic, and biosocial conditions of pregnant women and their families, such as education, income level, food consumption, age, parity and so on (Andersen *et al.* 2022). Malnutrition can cause the mother to suffer from anemia. The blood supply that delivers oxygen and food to the fetus will be hampered so that the fetus will experience impaired growth and development. Therefore, monitoring the nutrition of pregnant women is very important (Nuru *et al*., 2021).

Based on **Table 1**, it was found that the majority of mothers' vitamin D intake was less than 14 people (70%). Meanwhile, six people (30%) had sufficient vitamin D intake. Vitamin D is a vitamin that is needed for various metabolic processes in the body. It is fat-soluble and is produced by human skin with energy obtained from food intake. Vitamin D deficiency in pregnant women has an impact on the fetus and newborn. The prevalence of vitamin D deficiency is 63% in Indonesia and Malaysia. According to Smith M. Ellen and Tang Pricha Vin (2019), vitamin D can increase erythropoiesis by increasing the proliferation of erythroid progenitors and reducing proinflammatory cytokines. Additionally, by decreasing proinflammatory cytokines that stimulate hepcidin and through direct transcriptional regulation of the HAMP gene, vitamin D can suppress hepcidin expression. Reducing proinflammatory cytokines and hepcidin may increase iron bioavailability for erythropoiesis and hemoglobin synthesis by restoring iron recycling, preventing iron absorption in macrophages, and eliminating impaired iron absorption, thereby protecting against anemia.

Conclusions

Iron intake is significantly related to the incidence of anemia ($p=0.016<0.05$) and can increase the risk of anemia 15 times. Meanwhile, vitamin D intake is not significantly related to the incidence of anemia ($p=0.417>0.05$). Nutritional status is not significantly related to the incidence of anemia ($p=0.787>0.05$). Factors that have a significant influence on anemia are family income and gestational age. There is a need for further research regarding interviews using recall to determine the food ingredients consumed by respondents and education using questionnaires regarding anemia in pregnant women.

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The Relation Of Nutritional Status And Fat Percentage Towards Blood Pressure Women Aged 45-65 Years Old In Posbindu, Klaten, Central Java, Indonesia

Sophia Denta Prapdika Sari[1*], Ida Nurwati[2], Ratih Puspita Febrinasari[3]

[1] Department of Nutrition science, Sebelas Maret University, Surakarta, Indonesia
[2] Department of Medical science, Sebelas Maret University, Surakarta, Indonesia
[3]Department of Pharmacology, Sebelas Maret University, Surakarta, Indonesia
*Correspondence author : dentasophia@student.uns.ac.id

Abstract

Hypertension is a Non-Communicable Disease (NCD) and is included as the ten main death factor in the world. Hypertension is caused by genetics, gender, age, stress, smoking habits, consuming alcohol, unhealthy eating, obesity, and sedentary lifestyle. Obesity is one of the factors causing hypertension because the body increases the distribution of nutrients and oxygen, which causes dilation of wall vessels and affects the increase of blood pressure. Moreover, obesity causes fat buildup in the body and creates plaques, causing blockage of blood vessels. This research is observational research using the cross-sectional approach. The total number of subjects is 75, chosen from multistage sampling with criteria of women aged 45-60 years old, members of posbindu, having systolic blood pressure ≥120, and agreeing to sign informed consent. Relation analysis used *Kolmogorov-Smirnov* testing because the sample is > 50. If the data is normally distributed, it is proceeded to *Pearson* testing, and if the data is not distributed normally, then it is proceeded to *Spearman testing*. Based on the result of *Spearman testing*, there is a significant relation between nutritional status and blood pressure with the result of p = 0,006 (p<0,05). Meanwhile, the result of *Spearman testing* regarding the relation between fat percentage and blood pressure resulted in p = 0.01 (p<0,05). It can be concluded that there is a significant relationship between body fat percentage and blood pressure. Nutritional status and fat percentage have significant relations to blood pressure.

Keywords: Blood Pressure, Fat percentage, Hypertension, Nutritional Status

Introduction

Non-communicable diseases (NCDs) are currently the leading cause of mortality worldwide. According to WHO (2014), Non-Communicable Diseases account for two of the top 10 causes of mortality (WHO, 2019). As a result, the World Health Organization (WHO) created the Package of Essential Interventions for Non-Communicable (PEN) Diseases program in 2010, effective for treating Non-Communicable Diseases in middle- and low-income countries. With the establishment of WHO's PEN program, Indonesia formed Pos Kesehatan Terpadu (Integrated Health Unit), widely known as Posbindu, in response to said program. Posbindu NCD's primary objectives are healthy, at-risk groups and persons with NCDs over 15. According to the literature, the bulk of Posbindu visitors are women over the age of 50 (Widyaningsih *et al.*, 2022).

Posbindu is an Indonesian Ministry of Health initiative that conducts NCD screening and preventive activities. Diabetes mellitus, hypertension or blood vessels, heart disease, acute obstructive pulmonary disease, and cancer are the integrated Non-Communicable Diseases that the Posbindu program focuses on. Early identification was accomplished through blood pressure, blood sugar, and body mass index measurements, risk behavior interviews, and imparting knowledge on healthy living behavior (Kemenkes, 2019). According to Basic Health Research (RISKESDAS) 2013, the prevalence of NCDs such as hypertension, stroke, diabetes mellitus, and joint disease continues to rise, and these occurrences are expected to rise further (Kesehatan, 2013). Following that, based on statistics from the 2016 National Health Indicator Survey (Sirkesnas), hypertension has risen to 30.9% (Kemenkes RI, 2016). This indicates that one-third of Indonesians are diagnosed with hypertension, but two-thirds are not. Meanwhile, someone with hypertension may not have any symptoms and may be unaware that they have hypertension.

Numerous risk factors, including genetics, gender, age, stress, smoking, alcohol consumption, bad eating habits, obesity, and a sedentary lifestyle, can contribute to hypertension. Meanwhile, age is an unchangeable risk factor for hypertension. A person above the age of 50 is at risk of acquiring hypertension (Zekewos *et al.*, 2019). Aging is a risk factor for hypertension that cannot be addressed because as you become older, changes in the blood vessels occur, resulting in a decrease in the flexibility of the blood vessels (Safitri, 2020). Meanwhile, if a person is overweight at a pre-elderly age, the likelihood of developing hypertension increases. This is because the more weight a person has, the more blood will be utilized to transfer nutrients and oxygen throughout the body, resulting in higher blood volume,

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dilatation of blood vessel walls, and increased blood pressure. (Farahdini 2020) Obesity and overweight are directly related to the excessive amount and kind of food consumed. If the results of the Body Mass Index classed as nutritional status is 25.0, one is considered overweight or obese (Fajar, 2019). Excess body fat has a significant impact on the occurrence of hypertension. According to said notion, this is caused by the buildup of fat in the blood vessels, which causes plaque development and blockages in the blood vessels. Based on this theory, the researchers sought to investigate the efficacy of administering plantains to hypertension patients' blood pressure.

Methods

Field Overview

Klaten is a district in the province of Central Java. Klaten Regency is between 7032'19" South Latitude and 7048'33" South Latitude, and 110026'14" East Latitude and 110047'51" East Latitude. The Regency's administrative boundaries are as follows :

North : Boyolali;
East : Sukoharjo;
South : Gunungkidul (DI Yogyakarta); and
West : Sleman (DI Yogyakarta).

Klaten covers 70,152.02 hectares or 2.15% of the total land area of Central Java Province (3,254,412 ha). Klaten consists of 26 sub-districts, namely: Prambanan, Gantiwarno, Wedi, Bayat, Cawas, Trucuk, Kalikotes, Kebonarum, Jogonalan, Manisrenggo, Karangnongko, Ngawen, Ceper, Pedan, Karangdowo, Juwiring, Wonosari, Delanggu, Polanharjo, Karanganom. , Tulung, Jatinom, Kemalang, South Klaten, Central Klaten and North Klaten (BAPPEDA KLATEN, 2021).

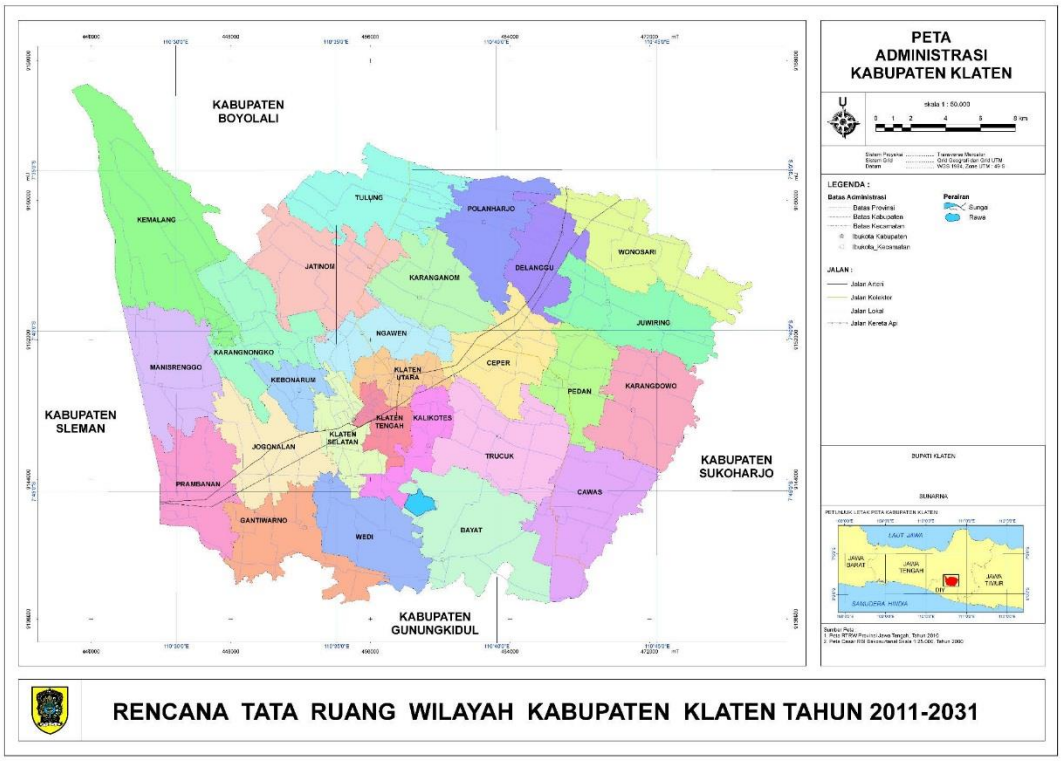

Figure 1. Sampling location

Research's Design

This research is an observational study that employs a cross-sectional approach. This study was carried out in Klaten in July 2023, utilizing three health centers, namely Juwiring Health Center, Bayat Health Center, and Wedi Health Center. The total number of subjects in this study is 75. Subjects were withdrawn using *multistage sampling*, with the requirements being that they were *Posbindu* members, the gender is female, had a systolic blood pressure of ≥120, and were prepared to sign informed consent.

ISSN 3031-0911

Data Analysis

Based on the BMI guidelines for Asia Pacific, nutritional status was divided into three categories: underweight (BMI < 25% results and overweight/obese with ≥ 25 results. According to JNC VII, blood pressure is categorized as usual when the systolic pressure is ≤120 mmHg, prehypertension when the systolic pressure is >120 mmHg, and hypertension when the systolic pressure is >140 mmHg. For data analysis, SPSS version 26 was employed. The features of the subjects were analyzed by descriptive analysis (averages and percentages). Because the sample size was more than 50, bivariate analysis was employed to establish the link between the dependent and independent variables using the *Kolmogorov-Smirnov* test. The Pearson test should be performed if the data are typically distributed —if they are not, the Spearman test should be performed instead.

Results and Discussions

Table 1. Illustrates the characteristics of respondents. The gender in this study was 75 people female. This is because the research criteria state that the research sample is female. The age category in this study was 75 samples aged 45-65 years old. This is also because the research criteria state that the research sample is 45-65 years old. Most of the professions in this research sample are housewives and farmers. The number of research samples with the profession of housewives was 27, farmers were 23, businesswomen were 13, private employees were 7, and government employees were 5.

Table 1. Characteristic of Respondent

Gender	Total	Age	Total	Proffesion	Total
Female	75	< 45	0	Housewife	27
Male	0	45-65	75	Farmer	23
		>65	0	Government employees	5
				Private employees	7
				Business	13
Total	**75**	**Total**	**75**	**Total**	**75**

Table 2. Illustrates the distribution based on nutritional status, percentage of body fat, and blood pressure level. According to the findings, there were 6.7% of respondents who were underweight, 20.0% who were average weight, and 73.3% who were obese. According to the distribution of respondents based on body fat percentage, 75 were overweight/obese. Meanwhile, looking at the findings of systolic blood pressure, it was discovered that 40% of respondents had normal blood pressure, 33.3% of respondents had prehypertension, and 26.7% of respondents had hypertension.

Table 2. Respondents Distribution Based on Nutritional Status, Percentage of Body Fat and Blood Pressure

Variable Nutritional Status	Frequency	%	Variable Precentage of Body Fat	Frequency	%	Variable Blood Pressure Level	Frequency	%
Underweight	5	6,7	Obese	75	100,0	Normal	30	40,0
Normal	15	20,0				Prehypertension	25	33,3
Obese	55	73,3				Hypertension	20	26,7
Total	**75**	100,0	**Total**	**75**	100,0	**Total**	**75**	100,0

Table 3. Illustrates the findings of the *Kolmogorov-Smirnov test* analysis because the study sample had more than 50 respondents. The *Kolmogorov Smirnov test* analysis shows that the three research variables have not a normal distribution, with p = 0,059 (asymp. Sig. 2 sided) ≥ 0,05 for nutritional status, p = 0,075 for percent body fat, and p = 0,080 for blood pressure.

Tabel 3. Test of Normality with *Kolmogorov-Smirnov Test* Analysis

Variable	Kolmogorov-Smirnov
	Statistic
Nutritional Status	0.059
Prencentage of Body Fat	0.075
Blood Pressure	0.080

ISSN 3031-0911

Table 4. Exhibits the *Spearman test* analysis findings since the *Kolmogorov-Smirnov test* results reveal that each variable is not normally distributed. The *Spearman test* study of the correlation between nutritional status and blood pressure produced a p = 0,006 (p<0,05), indicating that the nutritional status variable is significantly linked to the blood pressure variable. The *Spearman test* study of the correlation between the percentage of body fat and blood pressure likewise revealed a significant link with a p = 0.01 (p<0,05).

Table 4. The Correlation Between Nutritional Status and percentage of Body Fat on Blood Pressure Level

Test	Variable	p	Blood pressure
Spearman's rho	Nutritional Status	p	0.006
	Prencentage of Body Fat	p	0.010

The nutritional well-being of the individuals in this study was dominated by those who were categorized as overweight/obese. The Asia Pacific BMI categorization standards were used to assess nutritional status in this study. If the subject has a BMI of ≥23, he or she meets the overweight criterion, and if the subject has a BMI of ≥25, the subject meets the obesity criteria. The high proportion of overweight/obesity in this study might be attributed to several risk factors, one of which was the employment of female individuals aged 45-65 years. Women are more prone to obesity than men because they have higher fat stores than males, resulting in a slower metabolism. Fewer muscles in women contribute to slow metabolism because muscles burn more fat in cells, which causes a woman's body to prefer to store more food as fat (Lubis *et al.*, 2020).

According to the *Spearman test*, there is a substantial correlation between nutritional status and blood pressure, with a p = 0,006 (p<0,05) for the link between the two variables. According to Merdianti *et al.* (2019) study, persons with excessive nutritional status had blood pressure that falls into the high normal range. This incidence occurred due to obesity and high dietary status, which raised cardiac arterial pressure and, in turn, increased sympathetic nerve activity, which raised blood pressure. The findings of this study are also corroborated by Mantuges *et al.* (2018) research showing nutritional status has a link with blood pressure because excess nutritional status has a tight connection with blood fat levels. Increased nutritional status raises blood fat levels and creates deposits on the lining of blood vessel walls. When these deposits accumulate, the blood arteries constrict. Blood flow to the heart is reduced by narrowed blood arteries, resulting in elevated blood pressure (Beck, 2011).

However, other studies report different things, that nutritional status has no relationship with the incidence of hypertension. This is because high nutritional status figures cannot describe the overall proportions of the body. High nutritional status or excess body weight can mean large muscle mass and no measurable fat mass (Yoeniske *et al.*, 2019).

The findings of this study reflect the percentage of body fat in the research participants; it was discovered that all 75 research individuals had an excess body fat percentage. Body fat percentage is measured using a categorization derived from Fauziyah and Afiani (2020) research, which states that women with a body fat percentage of ≥25% are classified as having extra body fat or being overweight. Obese body fat is a body fat proportion of ≥30% (Mukiwanti & Muwakhidah, 2017). Because this study was done on female individuals, the body fat percentage in all subjects was excessive. Women have 10% more subcutaneous fat in the belly and gluteofemoral than males. Furthermore, women retain more fat because they are impacted by a hormone called estrogen, which increases obesity or fat accumulation. Women over 45 will lose 30 to 50 percent of their muscular mass, which will be replaced by body fat, delaying the body's metabolic processes and the accumulation of calories and fat in the body (Sri, 2015).

According to the *Spearman test*, the relation between the body fat percentage and blood pressure is p = 0.01 (p<0,05), indicating a significant relationship. Fat in the body influences blood pressure because fat creates proteins and hormones such as angiotensinogen, adipokines, inflammatory agents, and cortisol, particularly in the intra-abdominal region. *Adiponectin* is a protein that is released by adipose tissue and induces atherogenesis. Adiponectin's role is to prevent leukocytes from adhering to the endothelium and to limit the effect of TNF- α in expressing adhesion molecules on the endothelium, hence slowing the onset of atherogenesis. Adiponectin levels fall in those who are overweight, making atherosclerosis more likely. *Atherosclerosis* is a disorder in which the blood vessels in the artery walls enlarge and stiffen due to fatty lesions in the artery walls. Blood pressure rises when the flexibility of the artery walls declines, making it difficult for blood to flow to the heart (Khairana Sari *et al.*, 2016; Herinasari *et al.*, 2022).

However, other studies report different things, that percent body fat has no relationship with the incidence of hypertension. This is likely due to the accumulation of body fat in the subcutaneous area, not in the abdominal or

ISSN 3031-0911

visceral areas. Apart from that, high blood pressure can also be caused by other things, such as lifestyle and sleep quality (Herinasari *et al.*, 2022).

Conclusions

With a p = 0.006 (p<0,05), nutritional status shows a significant relation with blood pressure level. With a p = 0.01 (p<0,05), body fat content is significantly linked to blood pressure. People are required to regulate their weight, restrict their fat and sodium intake, increase their fiber intake, and attend Posbindu regularly in order to enhance their health, particularly those suffering from prehypertension and hypertension. Researchers intend to conduct further studies on risk factors that may induce hypertension in the future.

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The Effect Of Ethanol Ants Nest Extract (*Myrmecodia pendans*) On Fasting Blood Sugar Levels In Mice Obesity With Type 2 Diabetes

Rizky Alfiqi Nuriza[1*], Setyo Sri Rahardjo[2], Lilik Wijayanti[3]

[1] Department of Nutrition science, Sebelas Maret University, Surakarta, Indonesia
[2]Department of Pharmacology, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia
[3]Clinical Pathology, Department of Faculty Medicine, Sebelas Maret University, Surakarta, Indonesia
*Correspondence author : fikinuriza@gmail.com

Abstract

The Ant's Nest plant (*Myrmecodia pendans*) is known to have the potential to reduce blood glucose in obese patients with T2DM because it contains high flavonoids and tannins that can prevent oxidative stress and inhibit the work of lipase enzymes, pancreatic β cells, Glut-2 and α-glucosidase. This study aims to determine the effect of Ethanol Ants Nest Extract (*Myrmecodia pendans*) on changes in fasting blood glucose with T2D obesity. The type of research is true-experimental with pre-posttest control group design. This study used 30 male Wistar rats aged 8 weeks weighing 150-200 grams. The rats were divided into 6 groups, namely: KNo (normal control), KN (T2D obesity), KP (T2D obesity given metformin), P1, P2, and P3 (T2D obesity given ethanol ants nest extract 150mg/KgBW/day, 300mg/KgBW/day, and 600mg/KgBW/day for 14 days by gastric sonde). Data were analyzed using a Paired t-test and continued by using One-way ANOVA. There was a decrease in fasting blood glucose after 14 days of intervention, which was highest in the P3 group with a fasting blood sugar level of 177,534 ± 4.0 mg/dL ($p < 0.0001$). Giving ethanol ant nest extract has an effect in reducing fasting blood glucose levels.

Keywords: Ants Nest, Diabetes, Ethanol Extract, Fasting Blood Sugar, Obesity

Introduction

Obesity is the primary factor that triggers Type 2 Diabetes Mellitus (T2DM) and its complications. Diabetes mellitus is a chronic metabolic disorder characterized by high blood sugar levels associated with impaired insulin resistance and secretion (Soelistijo, 2021). According to the International Diabetes Federation (IDF), in 2021, there will be around 537 million individuals, or 10.5% of the total number of adults worldwide, suffering from DM. It is estimated that the number of people with DM will increase to around 643 million in 2030 and 783 million in 2045 in the adult age group between 20 and 79 years. According to WHO, the most significant increase in DM sufferers occurred in Southeast Asia, including Indonesia, which ranked 5th at 19.5% (Amalia *et al*., 2022).

The World Health Organization (WHO) estimates that in 2030, diabetes will become the 7th cause of death in the world. According to Suwinawati *et al*. (2020), 90 - 95% of the disease burden that causes 70% of deaths from diabetes cases worldwide is T2DM due to an unhealthy lifestyle. The number of patients is increasing due to the need for more knowledge of T2DM management. Information data, 2020 notes that the prevalence of DM in the 2018 Riskesdas in the Papua region was 1.1%, while compared to the West Java, Central Java, and East Java regions, it was 1.7%, 2%, and 2.5%. According to Soelistijo (2021), a person is categorized as having diabetes mellitus if the fasting blood glucose level is ≥ 126 mg/dl, blood glucose 2 hours after oral glucose tolerance test (TTGO) ≥ 200 mg/dl, blood glucose during ≥ 200 mg/dl with classic complaints and HbA1C ≥ 6.5%.

Current treatments are pharmacological as well as non-pharmacological. Pharmacology is antidiabetic drugs such as glinide, metformin, and others. Non-pharmacology therapy refers to nutritional therapy, namely 3J (schedule, type, and amount) (Pratiwi *et al*., 2018). Long-term use of multiple diabetes drugs has dangerous side effects for sufferers, such as weight gain (BW), insulin allergies, digestive disorders, frequent flatus (farting), and genital and urinary tract infections. It can trigger ketoacidosis (Soelistijo, 2021).

People today prefer alternative medicine, namely traditional medicine. A plant from Papua, the Ant's Nest, is a typical Indonesian plant considered a traditional medicine with various benefits but has yet to be clinically tested. Ant Nest is rich in antioxidants, namely flavonoids and tannins, with an IC50 value of 8.18 ppm, and in the Ethanol Extract of Ant Nest (EANE), the IC50 value is 32.48 ppm (very strong) (Mardany *et al*., 2016). This content can not only be a free radical antidote but also an anti-inflammatory, antimicrobial, and anti-inflammatory (Khairiah *et al*., 2019). Flavonoids and tannins play an essential role in preventing diabetes and obesity. Flavonoids preserve pancreatic β-cells and restore the sensitivity of cellular insulin receptors, maximizing insulin sensitivity. The flavonoid compound quercetin it contains inhibits GLUT2 of the intestinal mucosa, thereby lowering glucose. Other compound levels such as

ISSN 3031-0911

luteolin, rutin, apigenin, kaempferol, α-tocopherol, tannins, and stigmasterol are used to regulate blood sugar, increase insulin production, and inhibit α-glucosidase enzyme activity in the gut (Taebe *et al*., 2012).

Based on the background description above, the purpose of this study is to examine the effect of anthill ethanol extract in reducing fasting blood glucose levels in obesity with T2DM. The benefits obtained are to provide information on the benefits and effective dose of anthill ethanol extract in reducing GDP in obesity with T2DM. It is hoped that it can be used as a reference for further research to be applied in humans.

Methods

The type of research used is true-experimental, which aims to see the effect of giving ethanol extract of ant nest on fasting blood glucose, with a pre-posttest research design using a control group. This study was conducted from May to June 2022 at the Laboratory of the Center for Food and Nutrition Studies (PSPG), Gadjah Mada University, Yogyakarta. This study has received approval from the Research Ethics Committee of the Faculty of Medicine, Sebelas Maret University, with number 108/Un27.06.11/KEP/EC/2023.

The manufacturer of Ethanol Extract Ant Nest goes through the initial process of stripping the ant nest based on the inclusion and exclusion criteria that have been set, then cutting it into pieces to make it easier to dry quickly in the sun until it is not wet and dry to be thin like crackers and then blended to become coarse powder. The next step is the maceration method and modification (Ahmad & Risna, 2011), namely as much as 300 grams of coarse powder of ant nest that has been added with 70% ethanol in a ratio of 1:7. The immersion treatment is tightly closed using a bottle container which is then allowed to stand for 2 days with 1x stirring. Then, the macerate obtained is distilled/evaporated using soxhlet at a maximum temperature of 78oC so that the extract is not damaged.

Wistar rats used in this study were treated and raised in the Laboratory of the Center for Food and Nutrition Studies (PSPG), Gadjah Mada University, Yogyakarta. The sampling technique was purposive sampling with 30 samples, 8 weeks old, 150-200gram weight. Rats were adapted within 7 days with drinking water ad libitum, and comfeed feed. After the rats were adapted for 7 days, the rats were made obese, namely induced with HFHF diet for 14 days given 2 times (morning and evening) ad libitum (Maigoda et al., 2016). After 14 days, it was then measured by the lee index. After the mice were obese, the mice were induced with streptozotocin (STZ) 45 mg/kg BW/day and nicotinamide (NA) 110 mg/kg BW/day for 72 hours. Induction of STZ + NA into the abdominal cavity because in the abdominal cavity, there are many blood vessels, so hyperglycemia will occur. Rats are hyperglycemic if blood glucose is> 250 mg/dl (Maigoda et al., 2016). Experimental animals were divided into 6 groups, namely: KNo (standard control), KN (T2DM obesity), KP (T2DM obesity given metformin), P1, P2, and P3 (T2DM obesity), P1, P2, and P3 (DM given ethanol extract of ant nest 150mg/KgBW/day, 300mg/KgBW/day, and 600mg/KgBW/day.

Blood sampling was performed 2 times after STZ-NA induction to measure post-induction GDP levels in rats in fasting conditions ≥ 8 hours after 14 days of treatment. The number of days required for STZ-NA induction is 3 days. Blood samples were taken by first preparing the rats to be taken blood. Blood was then taken in the orbital sinus through vascularization. Second, prepare supporting equipment to take blood samples (1mL syringe and Eppendorf). Third, the rat is held by the nape of the neck and massaged with fingers. Fourth, the microhematocrit was scraped on the medial canthus. Blood was taken as much as 1mL and collected in Eppendorf (Nugroho, 2018).

The examination of GDP levels was carried out after rats were induced by STZ and NA quantitatively by the GOD-PAP method. Examining GDP levels by preparing rat blood plasma samples, stored in tubes containing 10% EDTA, centrifuged at 4000 rpm for 10 minutes. Plasma sample solution was taken as much as 10 µl added with 1000 µl of GOD-PAP reagent and incubated for 20 minutes, then the results were read on a spectrophotometer λ 500 nm (Nesti & Baidlowi 2017). The formula for calculating GDP levels is below:

Fasting blood sugar = sample absorbance (nm) / standard absorbance (nm) x standard glucose level (mg/dl)

Statistical data analysis using SPSS 26, where the normality test uses paired t-test parametric test, and homogeneity test using Levene. The test used to determine group differences is continued using ANOVA (Analysis of Variance).

ISSN 3031-0911

Results and Discussions

The data in **Table 1** shows that the average Body Weight (BW) value before any treatment was carried out, then after induction of the HFHF di*et, al*l groups of rats increased. Then, in the next group, after the induction of STZ-NA, the weight of the rats decreased in all groups except in the control group, which was not given any treatment at all/healthy. In the table, the average BW after being given EANE in the first week and week 2 increased except in the negative group, namely in the obese group with T2DM, who were not given any drugs. When viewed through paired t-test, results show that in all groups, there are significant differences, with the lowest decrease being in group 1, then group 2, and finally group 3.

Table 2. Blood Glucose Levels of Rats During Intervention

Group	Mean ± SD			P^b
	Initial GDP (mg/dl)	Final GDP (mg/dl)	Δ GDP (mg/dl)	
KNo	71,51 ± 1,88	73, 06 ± 2,61	1,55 ± 0,99	0,025
KN	255,30 ± 3,72	257,93 ± 2,63	2,63 ± 2,15	0,052
KP	257,96 ± 4,27	88,56 ± 2,46	169,40 ± 3,99	0,000
P1	260, 84 ± 8,61	118, 82 ± 3,01	142,02 ± 9,45	0,000
P2	265, 07 ± 2,56	101,85 ± 3,25	163,23 ± 6,41	0,000
P3	261,82 ± 4,39	84,28 ± 1,56	177,53 ± 4,09	0,000
P^a	0,00	0,00	0,00	

KNo : Group of normal/healthy experimental animals.
KN : Type 2 Diabetic obesity trial group.
KP : Type 2 Diabetic obesity trial group + metformin 9mg/200gBB
P1 : Type 2 Diabetic obesity trial group + Ethanol Ants Nest Extract 150mg/KgBW.
P2 : Type 2 Diabetic obesity trial group + Ethanol Ants Nest Extract 300mg/KgBW.
P3 : Type 2 Diabetic obesity trial group + Ethanol Ants Nest Extract 600mg/KgBW.
P^a *: (p<0,05) Uji One Way Anova.*
P^b *: (p<0,05) Uji Paired T-test*

Based on the results of statistical tests to determine the effect of EANE administration in reducing GDP levels. In Table 2, it can be seen that there was a decrease in GDP in the P1 group by 142.02 ± 9.45 ($p < 0.0001$), P2 by 163.23 ± 6.41 ($p < 0.0001$), and in the P3 group by 177.53 ± 4.09 ($p < 0.0001$), in the KNo group where the rats were in normal condition ($p=0.025$) and KN rats negative control group ($p=0.052$) showed no significant decrease in GDP levels. Based on the ANOVA test, it was found that there was a significant difference between the treatment groups ($p<0.0001$).

Table 2. Blood Glucose Levels of Rats During Intervention

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P3	261,82 ± 4,39	84,28 ± 1,56	177,53 ± 4,09	0,000
P^a	0,00	0,00	0,00	

KNo : Group of normal/healthy experimental animals
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P3 : Type 2 Diabetic obesity trial group + Ethanol Ants Nest Extract 600mg/KgBW
P^a *: (p<0,05) Uji One Way Anova*
P^b *: (p<0,05) Uji Paired T-test*

In the results of the study, it was found that the manufacture of obese mice models with T2DM was successfully carried out. The manufacture of obese model rats uses the HFHF diet, while the T2DM manufacture model uses STZ-NA. The administration of STZ-NA can cause an increase in GDP levels as light as the decrease in BW from before in rats (hyperglycemia). Mice are said to be obese and have T2DM if the Lee index is more than 300, and mice are said to be hyperglycemia if blood glucose is> 250 mg/dl. GDP levels in all groups > 150 mg/dL. This is due to previous

ISSN 3031-0911

research that says rats are said to have DM if the GDP value reaches > 150 mg / DL after 72 hours of STZ-NA induction (Ortiz-Andrade *et al*., 2008). Administration of STZ-NA causes damage to pancreatic β-cells, causing a decrease in insulin levels and an increase in blood glucose levels/hyperglycemia. Streptozotocin has a toxic effect, thus the need for Nicotinamide (NA) as a protective substance in manufacturing animal models of type 2 diabetes. The NA-STZ induction type 2 diabetes model has various advantages, namely the existence of stable hyperglycemia without the need for exogenous insulin to survive, insulin stores in the pancreas up to 60%, reducing pancreatic beta cells (-40%) and glucose intolerance, mainly due to impaired insulin secretion. Induction of experimental animals using a combination of nicotinamide (NA) and streptozotocin shows a model of Type 2 DM disease and is more beneficial because it can measure the effects of both antidiabetic and other compounds characterized by a decrease in body weight in obese T2D patients (Ghasemi *et al*., 2014).

The results showed that the administration of EANE using 3 different doses, namely P1, P2, and P3 (150mg/KgBW/day, 300mg/KgBW/day, and 600mg/KgBW/day) had different results. This can be seen from the research data before and after the intervention. The results obtained in the positive group and all treatments significantly have an effect in reducing the blood sugar levels of obese rats with T2DM. The average results of the study in treatment groups 1, 2, and 3 obtained significant results. The results of the GDP value levels 1, 2, and 3 have sequentially significant results according to the increasing dose of EANE. Then, when compared to using a positive group, the results of the difference are higher in the group with the 3rd dose, so it can also be concluded that the effectiveness of the dose of EANE with an amount of 600mg / KgBW is more effective than using metformin drugs in Wistar rats induced with STZ-NA with obesity models with T2D. This is because the anthill plant can reduce blood sugar levels in test animals. After all, it can control blood glucose levels by stimulating pancreatic β-cells to secrete more insulin, inhibiting the work of the α-glucosidase enzyme so that the absorption of glucose in the intestine is inhibited, reducing impaired renal function, and increasing the solubility of blood glucose so that it is quickly excreted through urine (Taebe *et al*., 2012).

The positive control in this study used was metformin. The selection of metformin is a prevalent drug used for people with DM. Metformin has been proven to reduce mortality due to DM because it can maximize insulin sensitivity and reduce the risk of hypoglycemia and cardiovascular disease. It is one of the hypoglycemic agents and can lower blood glucose. The mechanism of metformin in the body is to improve the sensitivity of hepatic and peripheral tissues to insulin without affecting insulin secretion. This effect occurs due to the activation of kinases in the cell (AMP-activated kinase). In addition, metformin increases glucose utilization by intestinal cells, thereby lowering blood glucose, and is thought to inhibit glucose absorption in the intestine after food intake (Mongi *et al*., 2019). The delta results or the difference in the mean of the positive control group are similar. They can be said to be equivalent in function because of the different ways of working, but when compared, the results are better in treatment group 3, which is given EANE with an amount of 600mg / KgBW.

The effectiveness of anthill ethanol extract is because the anthill plant material contains a lot of active compounds, both antioxidants, including flavonoids, phenolic compounds, tocopherols, polyphenols, tannins, and other minerals that are useful as antimicrobials (Sudiono & Revana, 2020). Antioxidants are used as free radical neutralizers in normal cells. Patients with DM easily experience oxidative stress, which causes diabetic complications. Antioxidants are used as free radical neutralizers in normal cells. Patients with DM easily experience oxidative stress, which causes diabetic complications (Putriningtyas, 2013). It was also found that flavonoids contain 313 ppm tocopherol, which can reduce 96% of free radicals at a concentration of 12 ppm (Tatukude *et al*., 2014). Ant nests contain high antioxidants, namely flavonoids and tannins, with an IC50 value of 8.18 ppm, and in the Ethanol Extract of Ant Nests (EANE), the IC50 value is 32.48 ppm (very strong) (Mardany *et al*., 2016).

These results align with research conducted by Kurniawati & Sianturi (2016), which states that anthill plants contain various active compounds, one of which is flavonoids, which are helpful as antidiabetic therapy and can reduce blood sugar levels. Flavonoid compounds are red, purple, and blue pigments commonly found in plants. Flavonoids are protective against β-cell damage as insulin producers and can increase insulin sensitivity. Antioxidants can suppress beta cell apoptosis without altering the proliferation of pancreatic beta cells. The ability of flavonoids, especially quercetin, to inhibit GLUT 2 in the intestinal mucosa can reduce glucose absorption. This leads to a reduction in the absorption of glucose and fructose from the intestine, resulting in lower blood glucose levels. Glucose Transporter-2 is thought to be the primary transporter of glucose in the intestine under normal conditions. In a study conducted by Ayda (2023), it was found that flavonoids can inhibit glucose absorption. When quercetin was ingested with glucose, hyperglycemia significantly decreased. In addition, the mechanism of action of flavonoids in reducing blood sugar levels is through the inhibition of phosphodiesterase, which will then secrete insulin through the Ca2+ pathway by

ISSN 3031-0911

inhibiting phosphodiesterase so that cAMP levels in pancreatic beta cells increase. This will stimulate insulin secretion through the Ca2+ pathway, where the increase in cAMP will cause the closure of the ATP K+ channel in the beta cell plasma membrane. This situation results in membrane depolarization and the opening of the Ca2+ channel so that Ca2+ ions enter the cell and cause insulin secretion by pancreatic beta cells. Another role of flavonoids is to inhibit damage to Langerhans islet cells in the pancreas continuously so that pancreatic beta cells can repair themselves. It can increase the sensitivity of cell insulin receptors by closing and inhibiting K+ channels that stimulate insulin release so that they can cause a decrease in blood glucose levels and hypoglycemic activity. The above is how EANE works to reduce GDP levels in obese rats with T2DM, which can be seen in **table 2.**

Tannin compounds are known to have a hypoglycemic effect by increasing glycogenesis as an astringent and have a protective effect by acting as a free radical catcher and being able to activate antioxidant enzymes (Anggraini, 2020). The role of tannin content can also be seen because it functions as an astringent that can wrinkle the epithelial membrane of the small intestine, thereby reducing the absorption of food juice so that glucose absorption decreases (Raya *et al*., 2016). Another function of tannins is to spur glucose and fat metabolism so that the accumulation of calories in the body decreases, which results in a decrease in cholesterol levels. Tannins work in a direct way, namely the β-subunit insulin receptor, which then activates p13 kinase, which will stimulate the translocation of glucose transporter-4 (GLUT-4), which then stimulates glucose absorption and incorporates glucose into adipocyte tissue (Putriningtyas, 2013). Tannins are compounds that have a role in reducing body fat and weight. Because, in general, tannins can inhibit various aspects of the digestive process, tannins can reduce appetite so that it can lose weight. Tannins easily bind to proteins so that the impact can precipitate proteins that are on the surface of the small intestine, thus suppressing food absorption and being able to inhibit obesity. It was noted that the concentration of anthill ethanol extract with a concentration of 8.4% b/v reduced total blood cholesterol levels in male mice (Taebe *et al.*, 2012). In the research of Sukmawati *et al.* (2018), tannins can inhibit α-glucosidase, which is helpful in inhibiting glucose absorption after eating, thereby slowing down the state of postprandial hyperglycemia.

Conclusions

Ant nest ethanol extract was able to reduce GDP levels significantly. Dose 3 was the most effective dose using ethanol extract at 600mg/KgBB/day. Dose 3 can reduce GDP levels higher when compared to the positive group given the drug metformin. The results showed that ethanol extract could be considered antidiabetic therapy but still needs further research, such as acute cytotoxicity test and chronic cytotoxicity test. Ethanol extract of Ant Nest still needs to be recommended in humans because it still needs longer research.

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Preliminary Test of Edamame Jelly Anti Hypertension: The Analysis Of Acceptability and Nutritional Quality of Edamame Jelly

Yualeny Valensia[1*], Budiyanti Wiboworini[2], Nur Hafidha Hikmayani[3],

[1] Department of Nutrition science, Sebelas Maret University, Surakarta, Indonesia
[2] Department of Nutrition science, Sebelas Maret University, Surakarta, Indonesia
[3]Department of of Medical science, Sebelas Maret University, Surakarta, Indonesia
*Correspondence author : valensia.yv@gmail.com

Abstract

The management of primary hypertension is divided into non-pharmacological and pharmacological therapies. Non-pharmacological therapy can be done by increasing the consumption of nuts. Edamame is a source of protein, amino acids, potassium, calcium, magnesium, and isoflavones. This study used edamame-made jelly with a mixture of carrageenan. This study analyzed the acceptability and content of edamame jelly's potassium, calcium, magnesium, and isoflavones. The research design was experimental by mixing three edamame jelly formulas. The acceptability test was conducted on 30 students, lecturers, and educational staff of the D-IV Clinical Nutrition Study Program, Jember State Polytechnic. The acceptability test includes colour, aroma, taste, firmness, and overall, with five scales. The panellists preferred the formula followed by proximate tests, potassium, calcium, magnesium, isoflavones, and total calories at the Jember State Polytechnic Food Industry Technology Laboratory. The organoleptic test results showed that the most preferred formula was P2 with 25 grams of cooked edamame. The nutritional quality of the selected edamame jelly is 139.5 kcal calories, 9.1 grams protein, 2 grams fat, 21.4 grams carbohydrates, 147 mg potassium, 36 mg calcium, 19.5 mg magnesium, and 8.1 mg isoflavones. There is a need for further research regarding the effect of edamame jelly on reducing blood pressure in sufferers of primary hypertension.

Keywords: Blood Pressure, Edamame, Hypertension, Potassiums

Introduction

Management of primary hypertension is divided into non-pharmacological and pharmacological therapy. One of the non-pharmacological therapies can be done with lifestyle modifications (PDHI 2021). A healthy lifestyle that can be done is to increase the consumption of nuts. Legumes containing bioactive components that benefit patients with primary hypertension are vegetable soybeans or edamame (*Glycine max* (L.) Merril). Edamame is a source of protein, carbohydrates, fibre, amino acids, potassium, calcium, and magnesium. Isoflavones in edamame can also reduce the risk of developing hypertension. Edamame can support the development of functional food ingredients that reduce the prevalence of non-communicable diseases (Fitriyana, 2014).

Consuming edamame for patients with primary hypertension can lower blood pressure because of the potassium, calcium, magnesium, and isoflavones contained in edamame (Rohatin & Prayuda, 2020). Previous research conducted by Sumarni (2020) regarding giving edamame to women of childbearing age (WUS) with hypertension can reduce systolic and diastolic blood pressure. Edamame is provided in the frozen form, consumed directly at a dose of 25 grams/day, accompanied by amlodipine 5 mg/day for 30 days. Another study by Karyadi *et al*. (2022) stated that the freezing treatment of edamame can affect its texture. The panellists' acceptance of the edamame texture (hardness) organoleptic test showed that they did not like frozen edamame because of its hard surface.

Researchers have developed edamame by processing it into jelly as a non-pharmacological therapy for sufferers of primary hypertension. The choice of jelly is because, in previous studies, edamame was given in frozen form with a hard texture. In contrast, jelly is a semi-solid food popular with everyone because of its dense and chewy texture (Prakarsa, 2017). The process of making edamame jelly is by mixing edamame with carrageenan, an ingredient from seaweed that functions as a gel former. Carrageenan has high fibre and potassium content; when consumed, it can help reduce the risk of hypertension (Kusumaningrum & Rahayu, 2018).

Edamame jelly is made in 3 different formulas with different edamame composition ratios. This study aims to analyze the acceptability of 3 edamame jelly formulas and test proximate, fibre, minerals, isoflavones, and total calories in panellists' most preferred formulas.

ISSN 3031-0911

Methods

This research design is experimental with three formulas, namely P1, P2, and P3. The research was conducted at the Nutrition Care Center (NCC) Laboratory of the D-IV Clinical Nutrition Study Program at the Jember State Polytechnic for product manufacture and hedonic testing. In contrast, the proximate, fibre, mineral, isoflavone, and total calorie tests were carried out at the Food Industry Technology Laboratory at the Jember State Polytechnic. This research was carried out from November 2022 to January 2023.

The preparation of the edamame jelly formula refers to previous research with several modifications to the additional ingredients (Afiska *et al*., 2021). The difference between the three formulas lies in the total weight of cooked edamame used, namely P1 30 grams, P2 25 grams, and P3 35 grams. Other ingredients are 16.7 grams of granulated sugar, 2.3 grams of carrageenan, 0.7 grams of vanilla powder, and 450 ml of water, used in the same amount for each formula. Formula P2 is the total weight of ripe edamame, which refers to previous studies (Sumarni, 2020). The three edamame jelly formulas in this study are presented in **Table 1.**

Table 1. Edamame Jelly Formula

Formula	Ripe Edamame (gram)	Sugar (gram)	Carrageenan (gram)	Vanilla Powder (gram)	Water (ml)
P1	30	16.7	2.3	0.7	150
P2	25	16.7	2.3	0.7	150
P3	35	16.7	2.3	0.7	150

In accordance with **Table 1**. making edamame jelly is clean the raw edamame, boil raw edamame for ± 5 minutes. Peel the edamame and weigh according to the weight stated. Blend the edamame with 100 ml of boiled water until smooth and pour into a container. Dissolve carrageenan with 50 ml of boiled water in a separate container and stir thoroughly until there are no lumps, sdd the granulated sugar to the smooth edamame bowl and stir thoroughly. Put the dissolved carrageenan into the edamame container and mix thoroughly. Heat and cook the mixture over low heat while continuing to stir. Add vanilla powder if the mixture explodes and mix well. Remove the dough, pour it into a serving cup, then chill for at least 2 hours. Edamame jelly is ready to serve.

Testing of organoleptic properties aims to determine the response of panellists to the quality properties of organoleptic, including colour, taste, aroma, elasticity, and overall product, using the hedonic method. The organoleptic test was carried out by 30 semi-trained panellists with the following criteria: knowing the sensory properties of the samples to be assessed, not smoking, not hungry or complete, and not sick. Panellists were presented with a selection of each product in a plastic cup and asked to provide an assessment using a hedonic scale and a description of the characteristics of each product on an organoleptic test form with five scales (1: dislike, 2: do not like, 3: neutral, 4: like, and 5: really like). The formula most panellists prefer based on organoleptic testing is continued for nutritional quality testing.

The nutritional quality test of edamame jelly was carried out at the Jember State Polytechnic Food Industry Technology Laboratory in the form of proximate tests, namely water and ash content using the gravimetric method, fat content using the Soxhlet method, protein content using the Kjeldahl method, carbohydrate content using the bay different method by SNI 01- 2891-1992 and SNI 2973:2011. Test potassium, calcium, magnesium, isoflavone, and total calorie levels using the spectrophotometric method by SNI 06-6989.12-2004.

Determination of the best formula is carried out using weighting on the analysis of organoleptic properties, which are then ranked based on the scoring results of the three formulas.

Results and Discussions

Edamame jelly was made in 3 formulas, which the panellists tested. *Edamame jelly* is a processed food made from edamame, carrageenan, granulated sugar, vanilla powder, and water.

ISSN 3031-0911

Table 2. Organoleptic Test

Parameter	Mean±SD*		
	P1	P2	P3
Colour	3,57±0,81	3,60±0,81	3,87±0,81
Aroma	3,33±0,84	3,50±0,86	3,17±0,95
Taste	3,10±0,75	3,70±0,87	3,10±0,92
Elasticity	3,10±0,92	3,73±0,90	3,47±0,90
Overall product	3,30±0,79	3,67±0,84	3,43±0,93

*: ANOVA test

The organoleptic test was determined using the hedonic method with parameters of colour, aroma, taste, elasticity, and overall by 30 semi-trained panellists. *Colour* is the first parameter that can influence the panellists' perception of the product. The most preferred colour parameter is P3, with a hedonic scale 3.87. The colour produced by the P3 formula is light green, while the other two formulas are slightly lighter than P3. The most preferred aroma parameter is P2, with a hedonic scale 3.50. The aroma of the three products is typical of edamame. The results showed that P2 was the most preferred formula regarding taste, with a hedonic scale of 3.70. The delicious taste of edamame is covered by the addition of vanilla powder so that the edamame jelly has a sweet and savoury taste due to the addition of granulated sugar. Formula P2 is a product with the best level of elasticity with a hedonic scale of 3.73. Based on the results of the panellists' assessments, the P2 formula has the highest rating compared to the other two. The correct proportion between the amount of edamame, the jelly powder, and other ingredients resulted in the jelly's elasticity level, which many panellists liked.

The most preferred overall parameter of edamame jelly is P2, with a scale of 3.67. The overall acceptability of the edamame jelly formula is illustrated in **Table 2.** Based on the panellists' overall assessment results, Formula P2 received the highest score compared to the other two. The panellists preferred the P2 formula because the composition of the edamame jelly matched the jelly products on the market that the panellists often consumed. The panellists preferred the P2 formula to the other two formulas regarding aroma, taste, elasticity, and overall, so the formula was continued with analysis of nutritional value, mineral, and isoflavone content at the Jember State Polytechnic Food Industry Technology Laboratory. The test used 100 grams of edamame jelly, while the weight per serving was 150 grams. The results of the analysis can be seen in **Table 3.**

Table 3. Results of analysis of nutritional content of edamame jelly

Parameter	Nutritional Value of Ripe Edamame /100 gr*	Edamame Jelly Test Results /100 gr **	Nutritional Value of Edamame Jelly/serving (150 gr) **
Water content (%)	-	77,58	116,37
Ash Content (%)	-	0,73	10,9
Fat Content (gr)	7,57	1,34	2
Protein Content (gr)	11,5	6,08	9,1
Carbohydrate Content (gr)	8,63	14,28	21,4
Potassium Level (mg)	422	98	147
Calcium Level (mg)	61	24	36
Magnesium Level (mg)	62	13	19,5
Fiber Content (mg)	5	1,06	1,59
Isoflavone levels (mg)	17,92	5,4	8,1
Calorie Content (kcal)	140	93	139,5

Source :
*: USDA (2022)
**: Primary data (2023)

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Table 3 shows that the nutritional value of ripe edamame is higher than the test results per 100 grams of edamame jelly. The nutritional value of edamame jelly is smaller than cooked edamame because only 25 grams of cooked edamame are used in making the jelly. An increase in the nutritional value of edamame jelly can occur if more ripe edamame is used. Previous research conducted by Maulina et al. (2020) stated that giving edamame 25 grams/day effectively reduced blood pressure in women of childbearing age with hypertension.

The results of the organoleptic quality assessment by 30 semi-trained panellists with overall formula P2 weighing 25 grams of cooked edamame was the most preferred formula. The three colour characteristics of the edamame jelly formula are a light green colour, a distinctive edamame aroma, a tending to sweet taste, and a typical suppleness of jelly products. Moreover, the overall characteristics of the desired product are light green, sweet, and chewy, distinct from jelly with a distinctive edamame aroma.

Colour is the first depiction of a product that can affect the panellist's perception. Colour will appear first and often determines the value of a product (Lestari, 2015). Based on the panellists' perception, the more edamame used, the better the resulting colour, so the panellists liked the formula more. The green colour in edamame jelly comes from chlorophyll pigment. *Chlorophyll* is a pigment that gives green colour to photosynthetic plants, algae, and bacteria (Nur *et al*., 2018). The most preferred colour was formula P3 with an edamame weight of 35 grams on a scale of 3.87, and the least preferred was formula P1 with a scale of 3.57 **(Table 2).**

Another parameter that determines the assessment of a product is aroma. *Aroma* is an odour that can affect panellists before enjoying food products because panellists can smell food before consuming it. The aroma of edamame jelly in all three formulas is typical of edamame. Edamame has a distinctive aroma of nuts, namely the unpleasant smell that comes from the oxidation of the lipoxygenase enzyme by linolenic acid (Adinugraha & Michael, 2019). Making edamame jelly uses vanilla powder to reduce the unpleasant aroma of edamame. The most preferred aroma parameter was formula P2 with a hedonic scale of 3.50 **(Table 2).**

Taste is the main parameter for determining the acceptability of a food product (Adinugraha & Michael, 2019). The formula with the most preferred level of taste parameters is P2, with a hedonic score of 3.70 **(Table 2).** Edamame jelly tastes sweet because granulated sugar is added in the making process. The use of granulated sugar can affect the quality of the jelly; the lower the addition of granulated sugar, the more it can cause the jelly to taste bland and the taste response to be heavier, especially the nutty taste (Merta, 2017).

The elasticity of edamame jelly is an organoleptic parameter assessed from the hedonic test. Formula P2 was the panellists' most preferred edamame jelly product, with a hedonic score of 3.73. The process of making edamame jelly is by mixing edamame with carrageenan, an ingredient from seaweed that functions as a gel former (Kusumaningrum & Rahayu, 2018). The results of the overall panellist assessment of the three edamame jelly formulas, namely, P2, had the highest score of 3.66, and the lowest was the P1 formula, with a hedonic score of 3.30 **(Table 2).**

Edamame jelly is a processed product from edamame, a food ingredient containing vegetable protein. The weight per serving of edamame jelly is 150 grams, made from 25 grams of cooked edamame with several additional ingredients. The protein content per 100 grams of edamame jelly is 6.08% or 9.1 grams/per serving **(Table 2).** The vegetable protein in edamame contains essential amino acids, namely leucine, isoleucine, valine, tryptophan, phenylalanine, threonine, lysine, and histidine. Essential amino acids increase the active transport process from the blood into muscle cells and other tissues. Essential amino acids can also increase protein synthesis in muscle and liver cells by inhibiting protein catabolism using insulin. The effect on the cardiovascular system is to increase peripheral blood flow, increasing cardiac output, which influences a decrease in blood pressure (Kusumastuty *et al*., 2016).

Edamame jelly is rich in potassium content because the manufacturing process uses carrageenan addition. Carrageenan has high fibre and potassium content, which can help reduce the risk of hypertension (Kusumaningrum & Rahayu, 2018). The potassium content per 100 grams of edamame jelly is 98 mg per serving, containing 147 mg (Table 2). Consumption of foods high in potassium can have a diuretic vasodilator effect and maintain a balance of extracellular sodium and potassium concentrations so that it can lower blood pressure in people with hypertension (Yuni, 2016). Potassium intake of 2-5 grams/day can lower blood pressure in hypertensive patients because it helps balance sodium (Kusumastuty *et al*., 2016).

Calcium is the most abundant mineral in the human body, primarily found in bones and teeth and a small part in plasma and extracellular fluids. Calcium can improve blood circulation, flex muscles, maintain body fluid balance, and maintain cell membrane permeability (Kurniawan, 2015). The calcium content of edamame jelly is 36 mg per serving **(Table 2).** Adequate calcium intake keeps the calcium balance in the blood maintained. Blood calcium levels are essential because calcium can help flex blood vessel muscles. Plaque or deposits that stick to blood vessels will quickly come off, and blood pressure will become more stable (Rohatin & Prayuda, 2020).

ISSN 3031-0911

Edamame jelly contains 19.5 mg of magnesium per serving **(Table 2).** Hypertension sufferers are advised to consume foods that are sources of magnesium because magnesium can maintain heart health by maintaining heart rhythm and controlling blood pressure to remain normal so that blood circulation remains smooth. Magnesium plays a role in controlling blood pressure and heart muscle contraction. Increasing the magnesium concentration in the blood can make the heart muscle work optimally and decrease blood pressure (Nurmayanti & Kaswari, 2020).

Edamame contains isoflavones that are thought to show antihypertensive activity by increasing NO, reducing angiotensin, and inhibiting the formation of free radical reactions (Maulina *et al.*, 2020). The isoflavone level in edamame jelly is 8.1 mg per serving **(Table 2).** The soy protein consumption of 25 grams per day, equivalent to an isoflavone intake of 37-62 mg, is stated to have met 83% of the recommended daily isoflavone requirements (Yulifianti *et al.*, 2018). Isoflavones work as antihypertensive agents by inhibiting the change of angiotensin I to angiotensin II, thereby inhibiting the release of aldosterone by ACE. Aldosterone impacts the kidneys to retain sodium and water; more water is excreted, and blood pressure will decrease if aldosterone production is inhibited (Maulina *et al.*, 2020).

Conclusions

The hedonic score results show that the most preferred formula is P2 with a cooked edamame weight of 25 grams. The characteristics of the P2 formula are a light green color and sweet, chewy jelly with a distinctive edamame aroma. The nutritional value of the selected edamame jelly is calories 139.5 kcal, protein 9.1 grams, fat 2 grams, carbohydrates 21.4 grams, potassium 147 mg, calcium 36 mg, magnesium 19.5 mg, and isoflavones 8.1 mg. The recommended suggestion is that there is a need for further research regarding the effect of giving edamame jelly on reducing blood pressure in sufferers of primary hypertension

Acknowledgments

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Relationship Between Dietary Diversity And Body Weight Monitoring With The Incidence of Overnutrition in Stikes Mitra Keluarga Students

Vini Andriani Taher[1*] and Guntari Prasetya[2]

[1,2] Department of Nutrition Science, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Indonesia
*Correspondence author : vinitaher09@gmail.com

Abstract

Overnutrition has impacts on the susceptibility to disease and the productivity level of college students. The application of the principle of balanced nutrition is an effort to maintain optimal nutritional status, such as dietary diversity and monitoring weight regularly. This study aimed to analyze the relationship between dietary diversity and body weight monitoring with the incidence of overnutrition. This study is a quantitative method with a cross-sectional design. The total sample was 69 students who were selected using the consecutive sampling technique. Dietary diversity was analyzed with an Individual Dietary Diversity Score (IDDS), which was obtained from 2x24 hours of food recall, and weight monitoring was analyzed with a questionnaire. The Chi-Square test analyzed the data. The results of the analysis showed that the dietary diversity with the incidence of overnutrition p-value = 0.052 and monitoring of body weight with the incidence of overnutrition p-value = 0.005 and Odds Ratio of 4.402. There is no significant relationship between dietary diversity and the incidence of overweight in STIKes Mitra Keluarga students. Still, there is a significant relationship between body weight monitoring and the incidence of overnutrition in STIKes Mitra Keluarga students.

Keywords: College Student, Body Weight Monitoring, Dietary Diversity Score, Nutritional Status, Overnutrition.

Introduction

Students are individuals who are studying to optimize their abilities in Higher Education so that they become intellectuals and professionals (PDDikti, 2020). Many of the students have unhealthy balanced nutrition behaviours, which increase the risk of having nutritional problems. Furthermore, nutritional problems in students can affect health status and productivity levels (Rahmadhani, 2017). According to the Basic Health Research Report (2018), it shows that the prevalence of overnutrition among adolescents in Indonesia had increased from 7.3% in 2013 to 13.5% in 2018. The prevalence of adolescents with overnutrition status in West Java Province is as much as 15.4%, and in Bekasi City, it was 17.4% in 2018. Overnutrition has an impact on disease susceptibility, especially the risk of non-communicable diseases, so it increases morbidity and mortality (Kemenkes RI, 2018).

Optimal nutritional status can achieve individuals who think critically and creatively and have high productivity (Kemenkes RI, 2020a). Optimal nutritional status can be achieved through the implementation of the principles of balanced nutrition as stated in the Guidelines for Balanced Nutrition. The principles of balanced nutrition consist of 4 pillars: diversity of food consumption, physical activity, clean and healthy lifestyle, and routine body weight monitoring (Kemenkes RI, 2014).

Consumption of a balanced nutrition diet can be achieved through a good variety of dietary. Dietary diversity is an indicator of the quality of nutritional intake, which reflects access to and adequacy of nutrition from individual or household food consumption (FAO, 2011). The more you eat a variety of foods, the better your nutritional needs will be fulfilled. Research which had been conducted by Fitriyani (2017) stated that the better the diversity score in food consumption, the better the nutritional status.

The Individual Food Consumption Survey in Indonesia (2014) showed that the food consumption pattern of the Indonesian population is still less diverse; the average consumption of vegetables and fruit is low, food is still dominated by grains, such as rice and wheat and low consumption of tubers. In addition, there is a lower average level of energy and protein adequacy in the adolescent age group (13-18 years) compared to other age groups, namely 72.3% and 82.5%. Ministry of Agriculture, the Republic of Indonesia is targeting to increase food diversification, especially in increasing consumption patterns of tubers by reducing consumption of grains, as well as increasing consumption of animal, plant, vegetable and fruit side dishes in order to fulfil nutritional needs for quality and type of food (Kementrian Pertanian RI, 2021).

Another effort to maintain and achieve optimal nutritional status is through routine body weight monitoring. Monitoring body weight can describe the level of nutritional adequacy of the individual, whether enough, deficiency or excess nutrition (Kemenkes RI, 2014). In addition, research which had been conducted by Rahmadhani's (2017) stated

ISSN 3031-0911

that weight monitoring influences the nutritional status of students in the nutrition study program at the University of Indonesia.

STIKes Mitra Keluarga students are prospective health workers who have an active role in promoting health. Previous research shows that there are still many STIKes Mitra Keluarga students who have more nutritional problems. Furthermore, research which had been conducted by Sagala (2020) showed that as many as 34.8% of STIKes Mitra Keluarga Bekasi City students are categorized as overnutrition. As many as 37% of students who are categorized as overnutrition have bad eating patterns. Moreover, research which had been conducted by Noerfitri, Tivanny and Rizky (2021) showed that as many as 40% of STIKes Mitra Keluarga students are categorized as overnutrition and have low vegetable and fruit intake. Based on the description above, this study aims to analyze the relationship between dietary diversity and body weight monitoring with the incidence of overnutrition in STIKes Mitra Keluarga students.

Methods

This study used quantitative methods with a cross-sectional design. This study has independent and dependent variables. Dietary diversity and body weight monitoring are independent variables, while the incidence of overnutrition is the dependent variable. This study was conducted in March 2023. This research took place at STIKes Mitra Keluarga, East Bekasi District, Bekasi City, Indonesia. The target populations for this study were all STIKes Mitra Keluarga students, while the reachable populations were active students at STIKes Mitra Keluarga. In addition, the sampling technique used consecutive sampling. Exclusion criteria in this study were students who were on a fixed diet and had poor nutritional status (body mass index <18.5 kg/m2). Based on the calculation of the minimum sample size by using the different proportion test formula (Lemeshow *et al*., 1990), a minimum sample size of 62 subjects was obtained. Meanwhile, the total samples in this study were 69 STIKes Mitra Keluarga students.

Dietary diversity was analyzed with an Individual Dietary Diversity Score (IDDS). Dietary Diversity data was obtained from 2x24 hour (weekend and weekday) food recall interviews. Then, it was input by using the Individual Dietary Diversity Score form (IDDS). IDDS classified five food groups, including staple foods, animal side dishes, plant side dishes, vegetables and fruits. Each food group gets a score of 1 if it consumes more than equal to 10 grams, and it is given a score of 0 if it consumes less than 10 grams (Felinda, 2021 & Mahmudiono, 2020). DDS is categorized into two categories: Low (if IDDS score <4 food group) and High (IDDS score ≥ four food group). In addition, an analysis of the average nutritional adequacy of respondents was conducted by using the NutriSurvey software.

Body weight monitoring data was obtained from filling out questionnaires by respondents in the form of the habit of weighing regularly every month. The results of measuring body weight monitoring are in 2 categories, namely Not Routine (if you do not weigh yourself every month) and Routine (if you regularly weigh yourself at least 1x/month) (Kemenkes RI, 2014). Overnutrition data was obtained based on measurements of Body Mass Index (body weight in kg divided by height in m2) for students aged 18 years are classified as overnutrition if the results of BMI/Age > 1 SD, while those aged > 18 years are classified as overweight if the results of BMI > 25.0 kg/m2 (Kemenkes RI, 2014). The results of measuring the incidence of excess nutrition are in two categories, namely, Yes and No.

The analysis conducted included univariate and bivariate analysis. The statistical test used was the Chi-Square test in order to determine the difference in proportions and the relationship between the independent and dependent variables. The ethics component of this study was submitted to the Research Ethics Commission Muhammadiyah University Prof. Dr. Hamka with Research Ethics Approval number 03/23.03/22347.

Results and Discussions

Respondents Characteristics

Table 1. shows that the highest number of research respondents aged 20 years old (37%), all respondents are female (100%), the majority of respondents live at home (98.6%), most of the respondents are nutrition students (52.2%), the total of overnutrition in the study respondents are 34.8% (overweight is 13.1% and obesity is 21.7%), the dietary diversity is low by 63.8% and non-routine weight monitoring is 47.8%.

ISSN 3031-0911

Table 1. Distribution of Respondents based on Respondent Characteristics

No	Variable	Frequency (n)	Percentage (%)
1.	**Age (year):**		
	18	2	2,9
	19	20	29
	20	26	37
	21	16	23,2
	22	3	4,3
	23	2	2,9
2.	**Gender:**		
	Female	69	100
3.	**Place to live:**		
	Boarding house	1	1,4
	Home	68	98,6
4.	**Study Program:**		
	Nutrition	36	52,2
	Medical Laboratory Technology	11	15,7
	Pharmacy	14	20,3
	Nursing+Ners	8	11,6
5.	**Nutritional Status:**		
	Overweight	9	13,1
	Obesity	15	21,7
	Normal	45	65,2
6.	**Dietary Diversity Score:**		
	Low	44	63,8
	High	25	36,2
7.	**Body Weight Monitoring:**		
	Non routine	33	47,8
	Routine	36	52,2
	Total (N)	**69**	**100**

The incidence of overnutrition in this study reaches 34.8%, with the proportion of overweight at 13.1% and obesity at 21.7%. This study is in line with research conducted by Sagala (2020), which shows that 34.8% of STIKes Mitra Keluarga students experience overnutrition. Furthermore, research conducted by Noerfitri *et al.* (2021) showed that as many as 40% of STIKes Mitra Keluarga students experience overnutrition. Factors contributing to nutritional problems in college students include inadequate nutritional intake, poor diet, low food quality, and lack of physical activity. It can be caused by students' busy schedules, stress, and . not having enough time to cook for themselves (Permana *et al.*, 2020).

Dietary Diversity

Table 2. Distribution of Respondents Based on Food Group Consumed

No.	Food Group	Frequency (n)	Percentage (%)
1.	Staple foods (carb)	69	100
2.	Animal side dishes	68	98.6
3.	Plant side dishes	25	36.2
4.	Vegetables	45	65.2
5.	Fruits	16	23.2

Based on **Table 2**., the results of the number of food groups of DDS instrument which are consumed the least by respondents are the fruit group, only 23.2% of respondents, followed by 36.2% of respondents who consume plant side dishes.

ISSN 3031-0911

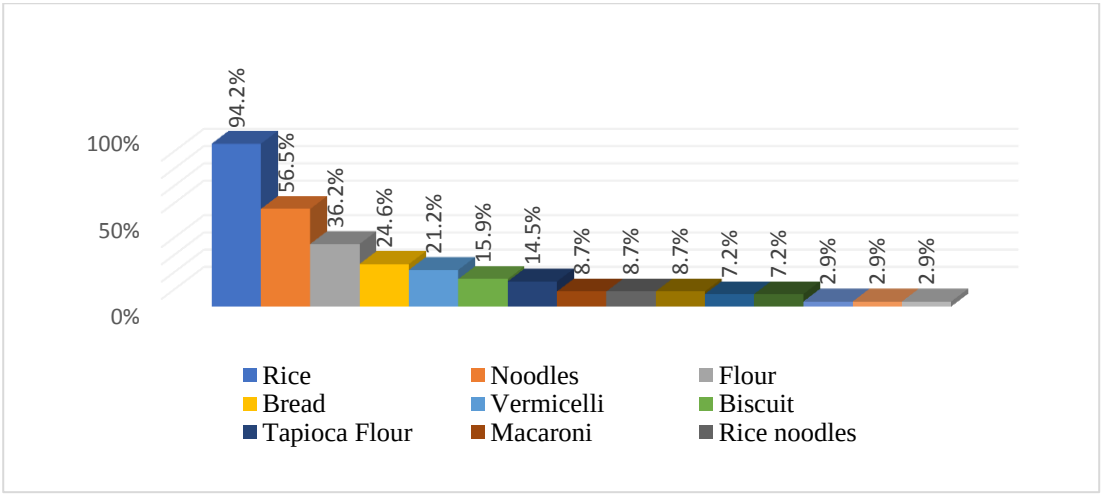

Figure 1. Percentage of Staple Foods Most Consumed by Respondents

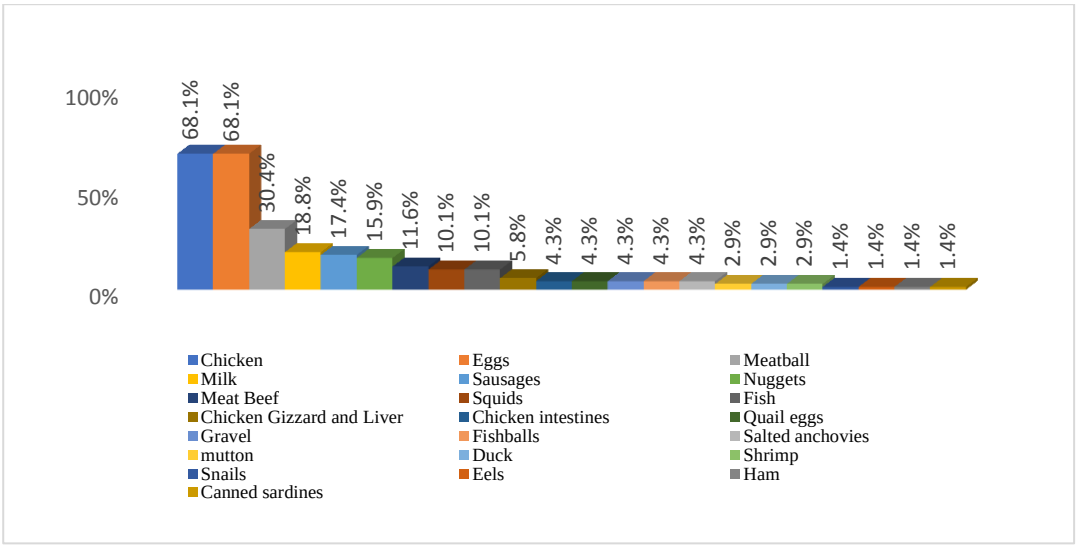

Figure 2. Percentage of Animal Side Dished Most Consumed by Respondents

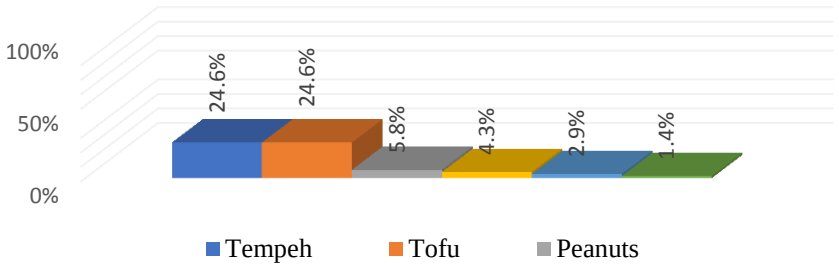

Figure 3. Percentage of Plant Side Dished Most Consumed by Respondents

ISSN 3031-0911

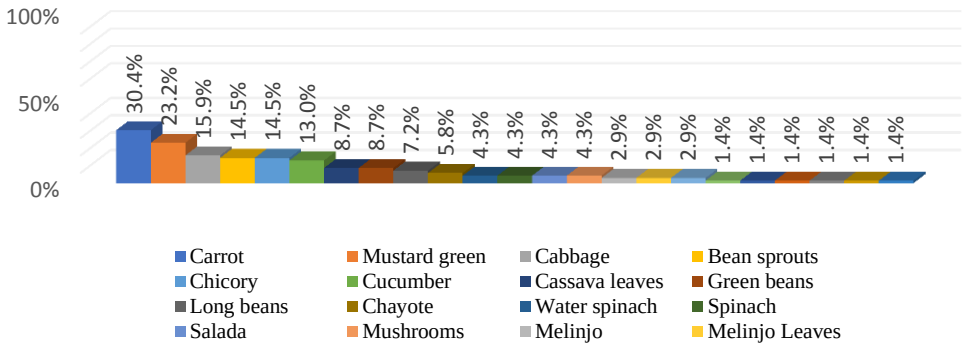

Figure 4. Percentage of Vegetables Most Consumed by Respondents

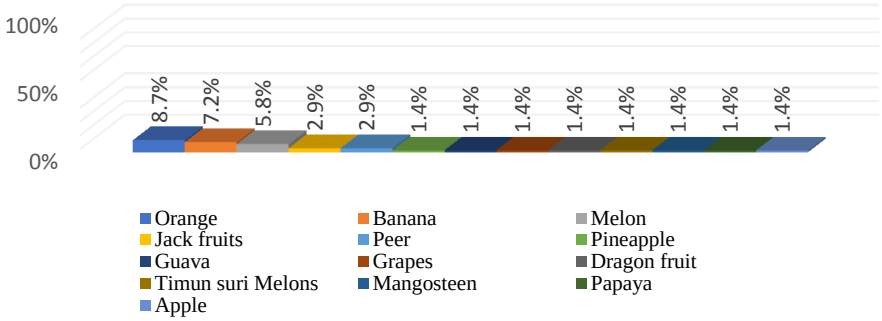

Figure 5. Percentage of Fruits Most Consumed by Respondents

The list of food types based on food groups consumed by respondents based on 2x24 hours food recall analysis is shown in Figures 1, 2, 3, 4, and 5. It is known that the staple food groups most consumed by respondents are rice and noodles, the most consumed animal side dishes are chicken meat and chicken eggs, the most consumed plant side dishes are tempeh and tofu, the most consumed vegetable group are carrots and mustard greens. The most consumed fruit groups are oranges and bananas.

Nutrition Intake

Table 3. Average Intake of Macronutrients in Respondents

No.	Nutrition Intake[a]	Nutrition Intake[a]			RDA[b]
		Weekday	Weekend	Average	
1.	Energy	1375 (61.1%)	1607 (71.4%)	1491 (66.3%)	2250
2.	Carbs	177 (49.1%)	205 (57%)	191 (53%)	360
3.	Protein	52 (86%)	55 (91.6%)	53 (88%)	60
4.	Fat	51 (78.5%)	63 (97%)	57 (87.7%)	65

Description:
[a]unit= Energy (kcal), Carbs (gram), protein (gram), fat (gram)
[b]RDA for the age 19-29 years for women (Republic of Indonesia's Ministry of Health, 2019)

Table 3. shows that nutritional intake on college days (weekdays) is lower than on weekends. The average energy intake of respondents is lower than the recommended RDA for the age group of women, which is 2250 kcal. The average of all macronutrients (carbohydrates, protein, fat) consumed by the respondents is below the RDA.

ISSN 3031-0911

ISSN 3031-0911

Body Weight Monitoring

Table 4. Distribution of Respondents by Frequency of Body Weight Monitoring in a Month

Body weight monitoring/month	Frequency(n)	Percentage (%)
Never	33	47.8
One time	13	18.8
Two times	10	14.5
Three times or more	13	18.8
Total	**69**	**100 %**

Table 4. shows that the frequency of body weighing in a month in respondents is one time and three times or more each by 18.8%.

Relationship between Dietary Diversity and the Incidence of Overnutrition

Table 5 shows the results of the proportion of respondents who are included in overnutrition in respondents with a low dietary diversity score, namely 43.2%. Moreover, the chi-square test shows the value of p = 0.052 (p> 0.05). It can be concluded that there is no significant relationship between dietary diversity and the incidence of overnutrition in STIKes Mitra Keluarga students.

Table 5. Analysis of the Relationship between Dietary Diversity and the Incidence of Overnutrition

Dietary Diversity	Overnutrition				Total (N)		*p-value**
	Yes		No				
	n	%	n	%	n	%	
Low	19	43.2	25	56.8	44	100	0.052
High	5	20	20	80	25	100	

N = 69 (*The data was significant if p-value <0,05)

This study used DDS based on five food groups consisting of staple foods, side dishes of animals, plants, vegetables, and fruits. The diversity of food consumption is in the less category if you consume <4 food groups. The proportion of respondents with less diversity in food consumption is 43.2%. Many respondents consume less or do not consume the plant side dishes, vegetables, and fruits, marked by the lower percentage of respondents who consume plant side dishes, vegetables, and fruits. It is in line with research conducted by Fitriyani's research (2017), which shows that respondents with overnutrition status tend to score less diversity in food consumption, as well as lower vegetable and fruit intake. Consuming less fiber from vegetables and fruits can risk nutritional problems such as overnutrition and obesity in adolescents and young adults. Consumption of adequate amounts of vegetables and fruits can reduce the risk of obesity and non-communicable diseases such as diabetes, metabolic syndrome, and heart disease (Singh *et al.*, 2019).

In the difference in nutritional intake of respondents on college days and weekends, respondents' energy intake and macronutrients on weekends tend to be higher than on weekdays. It is presumably because the respondents skip meals during a busy class schedule, and it may affect the amount of nutritional intake. It is in line with research conducted by Lestari (2017) that students tend to skip meals due to busy schedules, so nutritional intake on college days is lower than on weekends, and consumption of vegetables and fruit is very low.

The results of the statistical test analysis show that there is no significant relationship between dietary diversity and the incidence of overnutrition. However, the proportion of nutritional events tends to be smaller in the group of respondents with good food consumption diversity. These results align with research conducted by Swamilaksita & Sa'pang (2017) and Melani (2016), which stated that there is no relationship between diversity in food consumption and the nutritional status of obesity in adolescents. It is presumably because the respondents consumed a lot of high-calorie foods, so even though the diversity of food consumption is good, their calorie intake exceeds nutritional needs, which leads to overnutrition. An individual consumes excess intake and lacks physical activity in the long term, so there will be a risk of overnutrition (Praditasari & Sri, 2018).

ISSN 3031-0911

The analysis showed that the low food consumption diversity group in the normal nutritional status group was more than the overnutrition group at 56.8%. Therefore, respondents with normal nutritional status also needed a better food consumption diversity score. Based on the analysis results and linkages with previous research, the incidence of overnutrition is not only influenced by the diversity of food groups consumed, but the multifactorial causes of the incidence of overnutrition also influence it. The causes of overnutrition can be influenced by various factors, including energy imbalances and poor eating patterns, poor sleep patterns, stress, and lack of physical activity (Amrynia & Prameswari, 2022). In addition, dietary diversity needs to pay attention to food with balanced proportions; the amount is not excessive, and it is consumed regularly (Kemenkes RI, 2014).

Relationship of Body Weight Monitoring and the Incidence of Overnutrition

Table 6. shows that the proportion of respondents who are included in overnutrition are respondents in the group who do not routinely monitor body weight, namely 51.5%. Furthermore, the statistical test results show the p-value = 0.005 (p <0.05). Therefore, it is concluded that there is a significant relationship between monitoring body weight and the incidence of overweight in respondents. The Odds Ratio (OR) test results are known to be 4.4. It shows that respondents who do not monitor their weight routinely have a 4.4 times greater risk of experiencing overnutrition compared to respondents who monitor their weight routinely at least once a month.

Table 6. Analysis of the Relationship of Body Weight Monitoring and the Incidence of Overnutrition

No.	Body Weight Monitoring	Overnutrition				Total		*Odds Ratio* (95% CI)	*p-value*
		Yes		No					
		n	%	n	%	n	%		
1.	Non routine	17	51.5	16	48.5	33	100	4.402 (1.508-12.847)	0.005
2.	Routine	7	19.4	29	80.6	36	100		

Regular weight monitoring is one of the four pillars of balanced nutrition. Monitoring body weight is an effort to maintain normal weight (Kemenkes RI, 2014). In this study, routine weight monitoring is associated with the incidence of overnutrition. Based on the study results, it is known that most respondents have implemented routine weight monitoring, which is characterized by the proportion of respondents who weigh at least once a month as much as 52.2%. The results show that the proportion of respondents who do not weigh themselves regularly and are categorized as overnutrition is 51%. Based on the results of the statistical test analysis, it shows that there is a significant relationship between monitoring body weight and the incidence of overnutrition. Respondents who do not routinely monitor their weight have a 4.4 times greater risk of experiencing overnutrition compared to respondents who monitor their weight regularly.

These results are in line with research which had been conducted by Rahmadhani (2017) that there is a relationship between routine weight monitoring and nutritional status in college students. Students who do not monitor their weight have a 2.4 times greater risk of experiencing overnutrition. Monitoring body weight independently and routinely is a strategy for weight management in individuals with obesity (Carpenter *et al*., 2022). In addition, routine monitoring of body weight can provide information on weight deviations that may be experienced by individuals so that it can be useful in weight management (Kemenkes RI, 2014).

Conclusions

STIKes Mitra Keluarga students in this study experience 34.8% of overnutrition, 63.8% of low dietary diversity, and 47.8% of non-routine weight monitoring. There is no significant relationship between dietary diversity and the incidence of overnutrition in STIKes Mitra Keluarga students. However, there is a significant relationship between body weight monitoring and the incidence of overnutrition in STIKes Mitra Keluarga students. Students who do not routinely monitor their weight have a 4.4 times greater risk of experiencing overnutrition compared to students who regularly monitor their weight.

ISSN 3031-0911

It is expected that future researchers will examine the factors of overnutrition that are not present in this study in more depth. There are research limitations in this study, namely that there is no data on the history of respondents' conditions before the study, such as a family history of obesity and a history of obesity since childhood. In addition, there are areas for improvement of the food recall instrument that cannot describe the eating habits of individuals in the long term. Meanwhile, one of the factors affecting overnutrition status is long-term eating habits. Is there any limitation of the study? It may help to be added as a messages to future studies.

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Relationship Between Knowledge and Physical Activity with Nutritional Status Among High School Teachers in Surakarta

Laras Eka Nur Hasanah*, Yulia Sari , Ratih Puspita Febrinasari

[1] Department of Nutrition science, Sebelas Maret University, Surakarta, Indonesia
[2] Department of Nutrition science, Sebelas Maret University, Surakarta, Indonesia
[3]Department of of Medical science, Sebelas Maret University, Surakarta, Indonesia
*Correspondence author : larasenh@student.uns.ac.id

Abstract

Overweight is a condition in which excess fat accumulates in the body, which can pose a risk to health. The prevalence of overweight in Surakarta City is 24.03%. Government employees have the highest weight of 20%, while the most public employees with teaching qualifications amounted to 69.23%. Teachers tend to feel that they have a lot of work to do and feel pressured at work. Stress, a lack of physical activity, and low nutritional knowledge lead to nutritional problems such as being overweight. Research is needed on the relationship between knowledge and physical activity and overweight among teachers in high schools in Surakarta. This study aims to obtain information related to the relationship between nutritional knowledge and physical activity and nutritional status among teachers in public high schools in Surakarta. The method used was a cross-sectional research design. The study population was teachers in SMA Negeri 1–8, Surakarta, with a sample of 96 respondents. Knowledge data was obtained from the Multiple Choice Questionnaire, physical activity data was collected using a Physical Activity Questionnaire with Global Physical Activity, and nutritional status was obtained based on anthropometric measurements. The statistical test used was the chi-square test. The results showed that most were in the good knowledge category. Most do light physical activity. There is no significant relationship between knowledge and nutritional status among teachers in public high schools in Surakarta. There is a significant relationship between physical activity and nutritional status among teachers in public high schools in Surakarta. In conclusion, there is no significant relationship between knowledge and nutritional status because knowledge has an indirect effect on nutritional status. There is a significant relationship between physical activity and nutritional status. High health awareness leads to health-related activities, including eating habits and physical activity

Keywords: Knowledge, Physical activity, Nutritional status, Teachers

Introduction

Overweight is a condition in which there is an accumulation of excess fat in the body that can pose a risk to health (WHO, 2020). The prevalence of overweight among adults in Indonesia itself has increased by 2.1% per year from 2013 to 2018. The province with the highest prevalence of obesity nationally is Central Java with 14.3%, while the highest prevalence by district or city in Central Java is Surakarta City with 24.03% (Riskesdas, 2018). In Riskesdas (2018), the highest number of overweight government officials was 20%. According to the Basic Education Data (2022), the number of civil servants with teacher status is 69.23%. According to the Central Bureau of Statistics (2022), the highest number of teachers is in Central Java Province, and the number of teachers in Surakarta City itself is 9,752 teachers, while the number of high school teachers in Surakarta City is 1,136 teachers. As for civil servant teachers in public high schools, there are 380 teachers, consisting of 8 schools (Ministry of Education and Culture, 2022).

High school teachers have a workload in terms of preparing their students for the next level, such as choosing to go on to university or work. Students are expected by teachers to be able to think critically and logically and to have the ability to solve the problems they encounter (Nurrahmawati, 2018). Teachers are also referred to as catalysts, people who are listened to and obeyed by their students (Fitrah et al., 2020). High school teachers tend to experience stress due to various factors such as working conditions, work performed, work pressure, and work environment. Teachers tend to feel that they have a lot of work to do and feel pressured at work (Waloni, 2022).

ISSN : 3031-0911

According to Tomiyama (2019), a person may eat more than usual when under stress, choosing foods that are high in calories, sugar, and fat. A survey conducted by the APA (Association of Psychology America) reported that up to 39% of people overeat in response to stress. According to a study conducted by Isramilda (2019), there is a significant relationship between stress and obesity (p-value = 0.022). The higher the stress level, the greater the tendency to overeat. Overeating is one way to relieve stress (Syarofi & Muniroh, 2020).

Stress, overeating, an unbalanced diet, poor sleep, and low nutritional knowledge lead to nutritional problems such as overweight (Wulandari et al., 2021). The higher a person's nutritional knowledge, the more attention they pay to the type and quality of food they consume (Ni Wayan, 2018). In addition, those who are physically inactive have a three-fold higher risk of obesity than those who are physically active (Telisa et al., 2020). With this background, the researcher is interested in conducting a study on the relationship between nutrition knowledge and physical activity and the nutritional status of teachers at Surakarta State High School.

Methods

This type of research is quantitative with a cross-sectional research design. This research was conducted in public high schools in Surakarta City in March 2023. There are 8 public high schools in Surakarta City, namely Public High School 1 Surakarta, Public High School 2 Surakarta, Public High School 3 Surakarta, Public High School 4 Surakarta, Public High School 5 Surakarta, Public High School 6 Surakarta, Public High School 7 Surakarta, and Public High School 8 Surakarta. There are 380 civil servant teachers teaching in public high schools in Surakarta City (DAPODIK, 2022). The sample size was calculated using the minimum sample formula (Sugiyono, 2017) for as many as 96 respondents. using the cluster random sampling technique. Data collection consists of data on age, gender, most recent education, nutritional status, nutrition knowledge, and physical activity.

The variables studied were the identity of respondent characteristics (age, gender, and most recent education), nutritional status, nutritional knowledge, and physical activity. The research instrument used for the nutritional knowledge variable was a multiple-choice questionnaire (MCQ) consisting of 15 questions. The knowledge classification was divided into three levels: poor knowledge < 56%, adequate knowledge 56-75%, and good knowledge 76-100%. Physical activity data were collected using the Global Physical Activity Questionnaire (GPAQ), with questions covering activities at work, travel to and from work, leisure activities, and habits. The classification of physical activity is divided into three levels: low-intensity physical activity > 3000, moderate-intensity physical activity 600–3000, and light-intensity physical activity ≤ 600. Nutritional status is obtained from the results of anthropometric measurements, namely body weight using a weighing scale, followed by body height using a microtoise device.

Data analysis is divided into univariate and bivariate analyses. In this analysis, frequency distribution tables and cross-tabulations (chi-square) with percentage or proportion measures are used.

Results and Discussions

Characteristics Of The Respondents

The sample used in the study consisted of 96 respondents from 8 public high schools in the city of Surakarta, namely Public High School 1 Surakarta, Public High School 2 Surakarta, Public High School 3 Surakarta, Public High School 4 Surakarta, Public High School 5 Surakarta, Public High School 6 Surakarta, Public High School 7 Surakarta, and Public High School 8 Surakarta. The distribution of respondents' characteristics is shown below.

ISSN : 3031-0911

Table 1. Characteristics Of The Respondents

Characteristics Of The Respondents	n	%
Gender		
Male	19	19,8%
Female	77	80,2%
Total	96	100%
Highest level of education		
Bachelor's degree	64	66,7%
Master's degree	32	33,3%
Total	96	100%

In the following table, it is known that the characteristics of respondents in public high schools throughout Surakarta city show more female gender, namely 77 respondents (80.2%). There are 19 male respondents (19.8%). According to the characteristics of the last education, the last education of a bachelor's degree is more, namely 64 respondents (66.7%), and the last education of a master's degree is 32 respondents (33.3%).

Table 2. Distribution Of Respondents According To Their Nutritional Status, Knowledge Of Nutrition And Level Of Physical Activity

Variables	n	%
Nutritional Status		
Overweight	34	35,4 %
Pre-Obesity	46	47,9 %
Grade 1 Obesity	15	15,6 %
Grade 2 Obesity	1	1,1 %
Nutrition Knowledge		
Poor Knowledge	0	0 %
Adequate Knowledge	18	18,8 %
Good Knowledge	78	81,2 %
Physical Activity		
Low intensity physical activity	8	8,3 %
Moderate intensity physical activity	30	31,3 %
Light intensity physical activity	58	60,4 %

The World Health Organization's 2017 report provides information on sources of nutritional status.

Based on the table above, it is known that out of eight high school teachers in Surakarta city, the most nutritional status is pre-obesity, namely 46 respondents (47.9%), and the least is obesity level 2, namely 1 respondent (1.1%). Based on the table above, it is known that out of eight high school teachers in the city of Surakarta, none of them have insufficient nutrition knowledge. While those who have sufficient nutrition knowledge are 18 respondents (18.8%), those who have good nutrition knowledge are 78 respondents (81.2%). Based on the table above, it is known that among the eight high school teachers in the city of Surakarta, the lowest number of respondents engaged in low-intensity physical activity was 58 (31.3%). While the number of respondents with moderate-intensity physical activity was 30 (31.3%), the least number of respondents had heavy-intensity physical activity, as many as 8 (8.3%).

ISSN : 3031-0911

Table 3. Relationship Between Nutrition Knowledge And Physical Activity With Nutrition Status Among High School Teacher

No	Variables	Nutrition Status								Total		*p value*
		Overweight		Pre-Obesity		Grade 1 Obesity		Grade 2 Obesity				
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	
Nutrition Knowledge												
1	Adequate Knowledge	10	29,4	7	15,2	1	6,7	0	0	18	18,8	0,205
2	Good Knowledge	24	70,6	39	84,8	14	93,3	1	100	78	81,2	
Physical Activity												
1	Low intensity physical activity	19	55,9	31	67,4	7	46,7	1	100	58	60,4	
2	Moderate intensity physical activity	8	23,5	14	30,4	8	53,3	0	0	30	31,2	0,029
3	Light intensity physical activity	7	20,6	1	2,2	0	0	0	0	8	8	

Note. There are 96 samples used. This result uses the chi square test as part of its statistical analysis. Physical activity and nutritional status are the variables that are correlated, with a p value of 0.029.

Based on the table above, it can be seen that most respondents have good knowledge of pre-obesity nutritional status, as many as 39 respondents (84.8%), and out of 96 respondents, there are no respondents who have adequate knowledge of level 2 obesity nutritional status, or as much as 0%. Based on statistical tests, a value of $p = 0.205$ ($p \geq 0.05$) was obtained. Based on the table above, it is stated that most respondents do low-intensity physical activity with pre-obesity nutritional status, as many as 31 (67.4%) respondents out of 96 respondents, and out of 96 respondents, there are no respondents doing moderate-intensity physical activity with level 2 obesity nutritional status, or as much as 0%. There are still respondents who do not do moderate-intensity physical activity with nutritional status level 1 obesity and level 2 obesity, or as many as 0 respondents. Based on statistical tests, a value of $p = 0.029$ ($p < 0.05$) was obtained.

The results of the study of the characteristics of the respondents showed that out of half of the respondents, 77 (80.2%) were female. This is because women are less physically active than men, so more energy is converted to fat. Women also have less muscle mass (Gustiranda et al. 2020). Because of gender differences in body composition, women are at higher risk of obesity. The percentage of body fat is higher in women than in men (Gifari, 2020). Another cause of obesity risk in women is hormonal factors. The effects of hormones on the female body are not limited to the menstrual cycle. According to Qoirinasari (2018), the influence of hormones also increases the risk of obesity in women. Gender affects nutritional status because men and women have different energy and iron needs, posture, and muscles (Pindobilowo, 2018).

According to the characteristics of the last degree, the proportion of respondents with the last degree was higher. According to Fajrin (2021), the higher the level of education, the easier it is to receive information. The higher the level of education, the better the nutritional status. However, according to research by Lubis (2020), there is no relationship between the respondent's level of education and nutritional status. This is because education is one of the factors that influences how a person shapes their own behavior. The behavior induced by education is based on knowledge and awareness formed through the learning process, and because the behavior is based on awareness, it is expected to last over time (Inaya, 2019).

Respondents with pre-obese nutritional status were higher because teachers as educators have more responsibilities and duties (Purwanto et al., 2020). The role of teachers in educating students is very important because it requires knowledge, skills, and experience. In addition, teachers motivate students to be enthusiastic about learning and help them achieve good grades (Muthmainnah, 2023).

The results of Wiranti's research (2022) showed that most of the teachers who experienced fatigue were female teachers over the age of 40. The cause of fatigue at work is due to meeting energy requirements that do not match the needs and nutritional status of central. The study revealed that 81.2% of respondents had good nutritional knowledge, because an educator must have good knowledge. Besides that, he also has

ISSN : 3031-0911

the ability to make students interested in learning and be able to encourage students to be able to improve their mastery of various skills they have in the teaching and learning process in classroom activities that can create learning conditions for improving these skills (Pujianto et al., 2020).

According to Notoatmodjo (2010) in Fitriani Rika et al. (2020) nutritional knowledge is about food and nutrients, sources of nutrients in food, foods that are safe to eat without causing illness, and how to prepare food safely without losing nutrients in it. Healthy lifestyles and a lack of understanding of the contribution of nutrients in different types of food lead to problems with intelligence and daily physical activity (Khoiroh, 2022).

Physical activity burns calories in the body. Therefore, if calorie intake is too high and not balanced by physical activity, the body becomes obese (Fitri et al., 2018). Activities that involve physical activity are work, domestic work (e.g., caring for children, housework), mobility (e.g., walking or cycling to work), and leisure physical activity (e.g., dancing or swimming) (WHO, 2018).

In the results of the study, the highest number of respondents with low-intensity physical activity was 31.3%. This means that low physical activity tends to be associated with high obesity and nutritional status, and physical activity is associated with body fat percentage. Light physical activity causes fat accumulation (Wahyuningsi, 2019).

Based on data analysis using the Chi-Square test on the relationship between nutritional knowledge and nutritional status, it shows that there is no relationship between nutritional knowledge and nutritional status in teachers at SMA Negeri Kota Surakarta obtained a p value of 0.205 or p value ≥ 0.05. This is consistent with Pantaleon's (2019) statistical finding that there is no significant relationship between nutrition knowledge and nutritional status. This is because knowledge has an indirect effect on nutritional status, while the direct effect on nutritional status is nutritional food intake and infectious diseases. Again, Saleky's research (2022) explains that there is no relationship between knowledge of a balanced diet and nutritional status. The results of research conducted in Ghana also showed no statistically significant relationship between nutritional knowledge and nutritional status (p = 0.253) (Issahaku, 2021).

The role of nutritional knowledge in influencing food intake can affect nutritional status, as consuming the right foods also affects nutritional status (Lestari, 2020). However, health concern is needed as a form of individual willingness to take health actions (Destianty and Caninsti, 2021). A person with higher health concerns is more likely to engage in health-related activities, including those related to eating habits and physical activity (Janetius & Krithika, 2020). For example, in order to stay healthy, individuals may engage in preventive health behaviors such as healthy eating, dieting, and increasing physical activity (regular exercise) (Destianty and Caninsti, 2021).

Physical activity is any movement of the body produced by skeletal muscles that requires energy. Low physical activity is an independent risk factor for chronic diseases and is estimated to cause all-cause mortality worldwide (WHO, 2018). The results showed that out of 96 respondents, 31 respondents (67.4%) had low physical activity in pre-obese nutritional status and 19 respondents (55.9%) had obese nutritional status. Meanwhile, out of 96 respondents, there were no or 0 respondents (0%) who performed moderate intensity physical activity in nutritional status level 2 obesity and heavy intensity physical activity in nutritional status level 1 obesity and level 2 obesity.

Based on data analysis using the Chi-Square test to test the relationship between physical activity and nutritional status, it shows that there is a relationship between physical activity and nutritional status among teachers at SMA Negeri Kota Surakarta, obtained a p value of 0.029 or p value <0.05. The results of this study are consistent with Izhar's research (2020), which states that there is a significant relationship between physical activity and the incidence of overnutrition in Jambi 1 State High School students. 68.9% of students who have moderate activity have an overnutrition status. The results of this study are supported by research by Sumael (2020), where respondents with low levels of physical activity obtained 75% results for obesity. The chi-square results obtained a p value of 0.02 or p value < 0.05. Therefore, it can be concluded

ISSN : 3031-0911

that there is an association between obesity and physical activity. Physical inactivity and sedentary behavior are the main causes of obesity. Physical activity affects the degree of obesity.

The low level of physical activity among the respondents is due to a lack of time for physical activities such as exercise because they are busy taking care of the family, preparing meals for the family, and most of them are busy going to work. They also tend to use motorbikes rather than walking or cycling when doing activities around them (Basri, 2020). The lower the level of physical activity, the higher the risk of obesity. An overweight body has difficulty supporting its body when actively moving, so an obese person will have limited mobility and relatively reduced activity (Ibrahim, 2018).

Conclusions

Based on the results of the research conducted, it can be concluded that most teachers' nutritional knowledge is in the good knowledge category (70.6%). Most teachers engage in low physical activity (67.4%). There is no significant relationship between nutritional knowledge and the nutritional status of teachers in public high schools in Surakarta City (p-value = 0.205), because knowledge has an indirect effect on nutritional status. There is a significant relationship between physical activity and the nutritional status of teachers in public high schools in Surakarta City (p value = 0.029). High health awareness leads to health-related activities, including eating habits and physical activity.

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Identification of *Vibrio sp.* in Green Clam (*Perna viridis*) at West Bekasi Traditional Market

Andien Endina[1], Nadia Laura Agustin Simanjuntak[1], Maulin Inggraini[2*] Noor Andryan Ilsan[2]

[1,2] Department of Medical Laboratory Technology, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Indonesia
*Correspondence author : maulin.inggraini@stikesmitrakeluarga.ac.id

Abstract

Indonesia is an archipelagic country with the potential for an abundant wealth of marine products such as various types of fish, shellfish and others. Communities who live far from coastal areas can obtain fishery products that are sold in the market. Market conditions could be better organized, clean enough and have better environmental sanitation. This condition can cause other organisms to multiply, such as bacteria. Green clam (*Perna viridis*) is one of several types of shellfish widely consumed by the public. Green clam (*Perna viridis*) contains much protein. However, the processing of the mussels could be better, and they could be consumed without going through the cooking process. In that case, it can be a source of transmission and contamination from microorganisms and chemicals in the waters. This study aims to obtain information on the cleanliness and safety of green mussels, foods often eaten by the community. The method used is the spread plate method, Gram staining, and biochemical tests. Sampling was done by grinding raw green mussels and then adding sterile aquadest with a ratio of 1:1. 0.1 mL of water was taken and placed in TCBS media, then spread with drugal sky, then incubated for 24 hours at 37 degrees. The growth of colonies on TCBS selective media concluded that they were *Vibrio sp.* colonies. It was also found that gram-negative bacteria in the form of bent bacilli, like commas on gram staining, were the strongest reference for identifying *Vibrio sp.* In conclusion, in this study, four green clams from traditional markets in the Kranji area, West Bekasi, were used to contain *Vibrio sp*. bacteria caused by faecal contamination containing these bacteria in seawater. Communities must be more careful in choosing green clams to eat with their families, make sure the seller's stalls are clean, clean the shells properly, and process the shells by applicable recommendations.

***Keywords**:* Green clam, Traditional market, *Vibrio sp.*

Introduction

According to Yuhantaka (2018), Indonesia is an archipelagic country with the potential for an abundant wealth of marine products, such as various types of fish, shellfish, and others. Communities who live far from coastal areas can obtain fishery products that are sold in the market. In general, market conditions could be better organized, cleaner, and better environmental sanitation. This condition can cause other organisms to multiply, such as bacteria. Green clam (*Perna viridis*) is one of several types of shellfish widely consumed by the public. Green clam (*Perna viridis*) contains much protein. However, the processing of the mussels could be better, and they could be consumed without going through the cooking process. In that case, it can be a source of transmission and contamination from microorganisms and chemicals in the waters. Shellfish live in water, so they absorb seawater and nutrients that settle on the seabed to clean seawater so that materials such as heavy metals can also be absorbed into their bodies. If seen from the process of shellfish filtering water to get food, shellfish are vulnerable to contamination by harmful microorganisms.

Dangerous microorganisms, such as pathogenic bacteria in food, can trigger foodborne disease (Devi *et al*., 2019). According to the Regulation of the Head of the Food and Drug Supervisory Agency (BPOM, 2009) RI No.HK.00.06.1.52.4011 of 2009, which confirms the limit in consuming seafood for fishery product types contaminated with *Vibrio sp.* bacteria to reduce the occurrence of Foodborne disease, namely negative every 25 grams (Suliyaningsih, 2020). According to Hikmawati, Susilowati, and Ratna (2019), Foodborne disease is a disease caused by consuming food that has been contaminated with bacteria. The incidence of foodborne disease is caused by consuming seafood. As much as 10-20% of cases are caused by the bacterium *Vibrio sp. Vibrio sp.* are pathogenic or harmful bacteria for humans that will cause gastroenteritis. Gastroenteritis is the increase and decrease in the frequency of stool consistency compared to the subject with colon disease. *Gastroenteritis* is loose stools (feces), liquid / semi-liquid (semi-solid), and much water. This lasts less than seven days and occurs spontaneously. The main causes of acute gastroenteritis are bacteria, viruses, *Helicobacter pylori*, and *Vibrio cholerae*. Other parasites can also cause gastritis. Symptoms that often arise are vomiting, abdominal pain, stomach cramps, and frequent diarrhea, so sufferers will lose a lot of fluids and

ISSN 3031-0911

electrolytes, which will cause severe dehydration (Suliyaningsih et al., 2020). Meanwhile, according to SNI No.7388:2009, clams cannot contain *Vibrio sp.* If this happens, it indicates that the shellfish has been contaminated and is not good for consumption because it can cause foodborne diseases such as diarrhea (Annisa, 2017).

Vibrio sp. has characteristics including a short rod shape, is a Gram-negative bacteria, has a flagellum, does not have spores, does not have a capsule, is facultatively aerobic and reproduces by binary fission, grows on Thiosulfate Citrate Bile-salt Sucrose Agar medium (Muna, 2021). Kranji Market is an area near where we live. Several times, we bought food ingredients (including green clams) there. So, for that reason, we finally chose Kranji market as the place where we took samples of green clams, which were suspected to contain *Vibrio sp.* bacteria.

Methods

The research method used is descriptive observation. Data were collected from the West Bekasi Traditional Market on June 8, 2023. The results of the data were obtained after practical learning. Green clam samples were extracted by crushing 2 grams and giving them distilled water in a ratio of 1:1, then 0.1 mL of distilled water was taken and spread on TCBS media..

Results and Discussions

The research results on green mussels or *Perna viridis* in TCBS media were yellow colonies with small round shapes and convex elevations. The results for colonies on MCA are pink colonies with a rounded shape, convex elevation, and wavy edges. Gram staining shows the shape of bent rod bacteria like commas with red color, which indicates Gram-negative bacteria according to the literature. The MR and VP results show positive MR results, as seen from the formation of a red color change after dropping the Methyl Red reagent and negative VP because the media does not change color. It remains yellow. The SIM test showed positive sulfite and motile results following the literature showing that *Vibrio sp.* has a flagellum to move.

In contrast, the indole test was not carried out due to the unavailability of indole reagents. The TSIA test showed negative slant and positive butt results, as seen from the change in the color of the media, which was previously red to yellow. The SCA test shows a negative result because the media remains green. The results of the sugar test showed that the results for glucose, mannitol, and maltose were negative. The results for lactose and sucrose were positive because the media changed color from yellow to orange. The last one is the catalase test. The catalase test showed a positive result because bubbles formed after dropping H_2O_2.

TCBS media (Thiosulphate-Citrate-Bile Salts-Sucrose), spread with green clam water and incubated for approximately 24 hours at 37°C, resulted in a single colony growing yellowish. According to research conducted by Hikmawati, Susilowati, and Setyaningsih (2019) states that the yellow colonies on TCBS media are *Vibrio sp.* bacteria. Green clams are used as samples in practicum because *Vibrio sp.* is often found in seawater and seafood, especially green clams. *Vibrio sp.* can give a yellow color to TCBS media because *Vibrio sp.* ferments sucrose in the media, so a yellow color is formed. TCBS media is a selective and differential medium for the growth of *Vibrio sp.* bacteria because it contains bile salts, NaCl, thymol blue, ox bile, agar, sucrose, bromothymol-blue, yeast extract, sodium trisulfate, bile-salts function to inhibit bacterial growth besides *Vibrio sp.* NaCl is the optimal medium for halophilic growth, and sodium trisulfate, a source of sulfur and ferric citrate, is used to detect H2S production (Muna, 2021).

Vibrio sp. obtained from a sample of green clam gave a red color with a bent rod shape (like a comma) on Gram staining, classified as a Gram-negative rod-shaped bacteria. Gram stain has two types, namely Gram-positive and Gram-negative. Cappuccino and Sherman (2013) suggested that Gram staining was carried out to distinguish between Gram-positive and Gram-negative bacteria. Gram-negative bacteria do not have a thick peptidoglycan, so the primary dye (crystal violet) will fade when given a bleach (Lugol's iodine), and the bacteria will be stained red by the secondary dye (safranin).

The MR (Methyl-Red) test carried out on samples of green mussels gave the result of a change in the color of the media to red after being given the Methyl-Red reagent so that it was identified as positive in the MR test. These results are to the statements of Suliyaningsih, Arifin, and Ismunanti (2020) through their research, which stated that the MR test was carried out to determine the ability of bacteria to oxidize glucose and produce acid as the final product so that the media will turn red.

ISSN 3031-0911

The VP test (Voges-Proskauer) on green clam samples suspected of containing *Vibrio sp.* gave negative results, which were indicated by the absence of a color change in the medium from yellow to reddish after adding *alpha-naphthol* and KOH reagents. This is by the statement of Suliyaningsih, Arifin, and Ismunanti (2020), which stated that a negative result on the VP test was marked by no change in the color of the media to reddish because the VP test was carried out to determine the ability of bacteria to produce acetyl methyl carbinol from organic acids in glucose metabolism. Furthermore, *Vibrio sp.* does not produce diacetyl or acetoin.

Vibrio sp. that had been grown on SIM (Sulfide Indole Motility) media gave positive indole and motility results but negative sulfide, which was indicated by the presence of a blackish-red ring on the surface of the media and visible branching colonies from the loop needle puncture marks, but no black precipitate on the surface of the media. Media, after incubation, for approximately 24 hours. Cappuccino and Sherman (2013) stated that SIM media contains peptone and sodium thiosulfate as sulfur substrates, $FeSO_4$ acts as an H2S or sulfide indicator so that there is no reaction between $FeSO_4$ and H_2S, and the media is not blackish. The reaction between *p-dimethylaminobenzaldehyde*, butanol, and *hydrochloric acid* by *Vibrio sp.* produces a red-black layer/color. It shows movement with the growth spread from puncture marks because *Vibrio sp.* is motile.

The SCA (Simmon's Citrate Agar) media test on the green clam sample showed no change in the media color to blue after being incubated for approximately 24 hours at 37°C, so it was indicated as negative. This can happen because the sodium carbonate in the SCA medium does not change the *bromothymol-blue* indicator in the media. After all, it does not ferment citrate into a carbon source to precipitate energy due to the unavailability of glucose (Cappuccino & Sherman, 2013).

The sugar test that was carried out gave positive results on the sucrose and lactose tests but negative on the glucose, mannitol, and maltose tests, which were indicated by a change in color from orange to yellow on mannitol, maltose, and lactose media and no color change on the media. Glucose and sucrose (stay orange). This shows that *Vibrio sp*. cannot ferment glucose and sucrose but can ferment lactose, maltose, and mannitol (Cappuccino & Sherman, 2013).

Vibrio sp. from a sample of green clam grown on MCA (Mac Conkey Agar) media gave purple colonies, and there were other yellow colonies. *Vibrio sp.* produces purple colonies because *Vibrio sp.* can ferment lactose on MCA media and grows well because MCA media is a differential selective medium for Gram-negative bacteria, so *Vibrio sp.*, a Gram-negative bacteria, can grow well. However, yellowish colonies are thought to be Gram-negative bacteria that do not ferment lactose because these bacteria cannot lower the pH of the media, which is detected by a neutral red indicator due to lactose fermentation (Cappuccino & Sherman, 2013).

Conclusions

Based on the research that has been carried out, it can be concluded that four samples of green clams purchased at the Kranji traditional market, West Bekasi, were positive for containing *Vibrio sp.*

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Examination of Nail Fungus in Garbage Transporters using Saboraud Dextrose Agar Culture Media

Gefira Syahrotul Aini[1], Maulin Inggraini[2*], Noor Andryan Ilsan[3]

[1,2,3] Department of Medical Laboratory Technology, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Indonesia
*Correspondence author : maulin.inggraini@stikesmitrakeluarga.ac.id

Abstract

Onychomycosis, a fungal infection affecting both toenails and fingernails, can lead to nail discoloration, yellowing, blackening, deformation, sponginess, and brittleness. This condition is typically caused by different types of fungi, such as dermatophytes, non-dermatophyte molds, and yeasts. Onychomycosis has an estimated prevalence of 10% worldwide. The prevalence of dermatomycosis cases in Indonesia is in the low range of 3.5-4.7%. This study aims to determine the prevalence of onychomycosis (nail fungus) and explore the relationship between personal hygiene, length of work, and the use of boots in waste transporters at the UPTD cleanliness Rawalumbu area, Pengasinan Village. This type of research is descriptive. This research design is cross-sectional with ethics code 218/EC/KEPK/STIKES-PI/II/2023. The sample in this study amounted to 30 garbage transporters at the UPTD cleanliness Rawalumbu region, Pengasinan Village. The study included garbage transporters in the UPTD Rawalumbu area of Pengasinan Village who were willing to complete the Informed Consent sheet. Garbage transporters with mental disorders or difficulty participating in interviews were excluded. The sampling method used in this research is purposive sampling. The sampling place in this research was conducted at the UPTD cleanliness Rawalumbu area, Pengasinan Village. The results of this study obtained 20 samples (67%) positive for onychomycosis and ten samples (33%) negative for onychomycosis. The positive results were two nail samples detected *Trichophyton* sp. fungus type (15%) and 18 other samples found *Aspergillus* sp. fungus type (85%). This research concludes that the examination of 30 nail samples of garbage transporters found 20 samples (67%) positive for onychomycosis and 10 samples (33%) negative for onychomycosis. Of the 20 positive samples, two nail samples detected the fungus type *Trichophyton* sp. (15%), and 18 other samples found the fungus type *Aspergillus* sp. (85%). The results of the statistical test performed coefficient contingency showed a significant relationship between personal hygiene, length of work, and using boots with the incidence of onychomycosis in garbage transporters at the UPTD cleanliness Rawalumbu area, Pengasinan Village, which was assessed by statistical tests where the p-value was obtained <0.05.

Keywords: Onychomycosis, Personal Hygiene, Garbage Transporters

Introduction

Onychomycosis, a fungal infection affecting both toenails and fingernails, can lead to nails thickening and becoming uneven, cracked, and dull while the color changes to yellow, brown, or black. This condition is typically caused by different types of fungi, such as dermatophytes, non-dermatophyte molds, and yeasts. Gupta *et al.* (2020) estimated the worldwide prevalence of onychomycosis to be about 10%. Dermatomycosis has a low prevalence in Indonesia, estimated at 3.5-4.7%. The age group most susceptible to onychomycosis infections is 25-44 years, with a prevalence of 23.8%. Men have a higher incidence rate of onychomycosis (65%) compared to women (35%). The high infection rate in men may result from frequent nail injuries due to daily and sports activities (Bitew & Wolde, 2019; Gupta *et al*., 2017; Mamuaja *et al*., 2017). Nail changes resulting from onychomycosis infection can significantly decrease the quality of life and comfort for those with the condition. Onychomycosis predominantly affects individuals who work in wet and humid environments, such as garbage collection professionals. Poor personal hygiene practices may contribute to developing fungal infections (Artha & Oktasaputri, 2020; Elewski & Tosti, 2015; Hidayat, 2018). According to Lolowang *et al.* (2020), waste pickers are highly susceptible to onychomycosis because they work in humid and unhygienic environments.

A study conducted by Mulyati and Zakiyah (2020) revealed that out of the 57 scavengers at the Bantar Gebang landfill, 50 samples were positive for onychomycosis (87.72%), and seven samples were negative (12.28%). This high risk of infection is corroborated by Sari *et al.* (2016), who found that scavengers are prone to various diseases due to work conditions. This is unchanged because it already adheres to the principles and lacks context. This study aims to determine the prevalence of onychomycosis (nail fungus) and explore the relationship between personal hygiene, length of work, and the use of boots in waste transporters at the UPTD cleanliness Rawalumbu area, Pengasinan Village. Researchers chose to study waste transporters due to their increased risk of nail fungus infection from their unclean and

ISSN 3031-0911

humid work environment. The study's findings will serve as an informative resource for the Bekasi City government to educate garbage transporters at the UPTD cleanliness Rawalumbu area, Pengasinan Village, regarding the significance of personal hygiene during their duties.

Methods

The study included garbage transporters in the UPTD Rawalumbu area of Pengasinan Village who were willing to complete the Informed Consent sheet. Garbage transporters with mental disorders or difficulty participating in interviews were excluded. A total of 30 garbage transporters from the UPTD of cleanliness in the Rawalumbu area of Pengasinan Village were sampled for this study. This study was conducted under a research permit obtained from STIKes Mitra Keluarga. Additionally, an ethics letter was secured from STIKes Prima Indonesia. The research received informed consent from the garbage transporters, who participated as research subjects, after being approved by the ethics committee (registration number 218/EC/KEPK/STIKES-PI/II/2023). This research was conducted during February and March of 2023 at the UPTD Cleanliness in the Rawalumbu area of Pengasinan Village. The samples were analyzed at the Mycology Laboratory of STIKes Mitra Keluarga without deviations from standard procedures.

Sabouraud Dextrose Agar (SDA) Media Preparation Procedure : It weighed 29.25 grams (6.5%) of SDA media in a weighing cup. Transferred the weighed media into an Erlenmeyer and dissolved with 450 mL of distilled water. Then, Erlenmeyer was heated until it evaporated so that it mixed perfectly. Erlenmeyer was sterilized with an autoclave for 15 minutes at 121°C with a pressure of 2 atm. Waited until cool, added 0.225 grams (0.05%) chloramphenicol while shaking until dissolved. Then, put it into a petri dish and homogenize it. Sterile Distilled Water Making Procedure : 10 mL amount of Aquadest was added to a test tube. The test tube was then covered with cotton and sterilized using an autoclave at a temperature of 121°C for 15 minutes (Zulneti, 2020).

The nail is cleansed using an alcohol swab, followed by scraping with a scalpel. The resulting nail-scraping sample is placed into a container for examination at a laboratory. Nail samples are collected using a sterile cotton swab moistened with sterile distilled water. The swab is then used to spread the sample on the center of the surface of the SDA media. The Petri dishes are wrapped in paper and incubated at room temperature for 5-7 days. Fungal growth is observed and recorded. Observations were macroscopically conducted to identify fungal colonies that grew on SDA media by evaluating their colony shape, color, and surface, as reported in the literature. Microscopic observations were conducted to identify fungal colonies that grew on SDA media with LPCB reagents. The glass object was cleaned to remove fat residues, and LPCB was applied droplet by droplet to the glass object. Then, the fungal colonies were aseptically taken using an ose needle, and the colony was stretched and mixed with LPCB. Technical abbreviations are explained when first used. Then, the fungal colonies were aseptically taken using an ose needle, and the colony was stretched and mixed with LPCB. It was covered with a glass object and observed under a microscope at a magnification of 10x10, then continued with a magnification of 40x10 (Zulneti, 2020).

Results and Discussions

This study was conducted at the UPTD cleanliness Rawalumbu area, Pengasinan Village, Bekasi City. The study sample amounted to 30 nail scrapings from garbage transporters. The samples were then examined by two methods, namely by culturing using Sabouraud Dextrose Agar (SDA) media. Then microscopic observations were made in the mycology laboratory of STIKes Mitra Keluarga

Table 1. Percentage incidence rate of onychomycosis

Result	Amount	Percentage
Positif	20	67%
Negatif	10	33%
Total	30	100%

Table 1. illustrates that 67% of 30 nail scraping samples from garbage transporters yielded positive results for nail fungus, while 33% were negative. Confirmation tests were performed to verify whether or not the garbage transporters were infected with nail fungus. Scraping samples were taken from the toenail, planted on SDA media, and incubated at room temperature for 5-7 days. The resulting figures are depicted below.

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Figure 1. Colony growth result on SDA media

Figure 1. displays the findings of fungal colony growth in nail scraping samples on SDA media. Figure 2 displays the findings of fungal colony growth in nail scraping samples on SDA media. The samples were incubated at room temperature for 5-7 days. The following figure illustrates the results of the microscopic examination obtained in this study.'

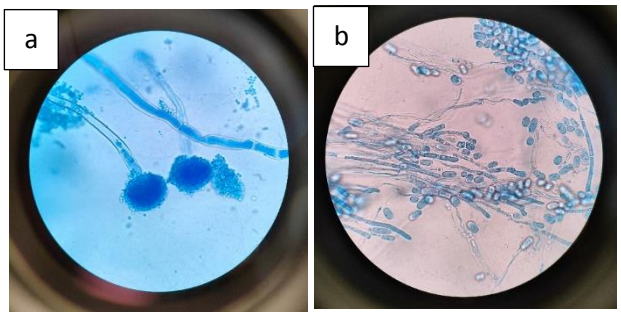

Figure 2. a. *Aspergillus* sp. b. *Trichophyton* sp. (40x10 magnification)

Figures 2. display the microscopic observations of colonies grown on SDA media for 5-7 days at room temperature. The percentage of the genus is determined based on these results and the macroscopic observations.

Table 2. Percentage of genus against positive samples

Result	Amount	Percentage
Aspergillus sp.	18	85%
Trichophyton sp.	2	15%
Total	20	100%

Table 2. displays that 18 samples (85%) were positive for *Aspergillus* sp. and 2 samples (15%) tested positive for *Trichophyton* sp. Furthermore, a survey was conducted to determine the factors that affect the incidence of onychomycosis, as shown in **Table 3.**

Table 3. Statistical Test Performed Coefficient Contingency of Factors Affecting Incidence of Onychomycosis with Sample Size of 30 Respondents

Factors affecting	Incidence of onychomycosis	p-Value < 0.05
Cutting nail frequency		
Often	8	0.038
Rarely	12	
Shower frequency		
Not shower	0	

ISSN 3031-0911

Shower 1x	1	0.542
Shower 2x	16	
Shower ≥2x	4	
Personal hygiene		
Hand and foot washing	23	0.033
No hand and foot washing	7	
Work duration	5	0.011
≤ 5 years	15	
≥ 5 years		
Boots wear	9	0.032
Wearing boots	11	
Not wearing boots		
Age	11	0.429
16–30 years	9	
31–45 years		

Table 3 displays the factors that impact the occurrence of onychomycosis. The statistics were then subjected to the coefficient contingency test to determine if there was an association between these factors and the incidence of onychomycosis. Of the four factors, cutting nail frequency, personal hygiene, work duration, and use of boots have a significance value <0.05, indicating a relationship with the incidence of onychomycosis. Meanwhile, the frequency of bathing and age do not show statistical significance (p>0.05), indicating no significant relationship between the frequency of daily bathing and age with the incidence rate of onychomycosis.

Based on the study results, garbage transporters took 30 nail-scraping samples. Table 1 displays the findings, with 20 samples (67%) testing positive and ten (33%) testing negative. Positive results are observed by the presence or absence of fungal growth on SDA media and the subsequent examination using LPCB under a microscope. LPCB is a dye applied to stain the cellular structure of fungal cells (Elsavira, 2021).

The macroscopic research yielded two genera, *Aspergillus* sp., and *Trichophyton* sp., as shown in **Figure 2.** To confirm the fungal genus, LPCB was used in conjunction with microscopic examination, as discussed in Figure 3. The microscopy results indicate *that Aspergillus* sp. exhibited hyphae that were difficult to discern, visible vesicles, and conidia that were not easily visible. This aligns with previous research by Ounleye and Olaiya (2015). The characteristics of *Aspergillus* sp. include skeptical hyphae, round vesicles, round conidia, and conidiophores made up of long stalks that support bulging vesicles. Conversely, *Trichophyton* is characterized by straight and smooth hyphae, with no macroconidia but abundant slightly oval round microconidia. This is in line with Kidd *et al.* (2016) study found that microconidia were present in abundant oblong, round shapes along the hyphae, while macroconidia were rare. If present, they were clavate, smooth, thin-walled, and varied in size.

According to the percentages presented in **Table 2,** the microscopic results identified *Aspergillus* sp. in as many as 18 samples (85%) and *Trichophyton* sp. in 2 samples (15%). *Aspergillus* sp. was the most frequently occurring finding in the study. This is due to *Aspergillus* sp. being one of the non-dermatophyte fungi responsible for causing onychomycosis, and its spores are present in the air as a contaminant, making it ubiquitous in the environment. This fungus can be found in soil, decaying plants, and other forms of organic waste (Ekasari & Nahlia, 2020). The high percentage of *Aspergillus* sp. fungi found in this study is in line with the results of research by Mulyati and Zakiyah (2020). The report indicated that the culture examination yielded nine fungi species, with *Aspergillus* sp. being the most prevalent, accounting for 48.72% of the total.

Garbage transporters who frequently trim their nails had an onychomycosis incidence of 8 people, whereas those who rarely trim their nails had an incidence of 12. The entry of dirt into the nails is a possibility for a person not trimming their nails regularly. The statistical test in Table 3 shows a significance value of 0.038 <0.05, indicating a relationship between nail trimming and onychomycosis incidence. This study aligns with Khotimah's (2020) research, which states that individuals who do not maintain proper hand and foot hygiene, including failure to cut and clean nails with soap, may be more susceptible to developing onychomycosis fungal infection.

The study examined 30 garbage transporters and found that one person who showered once a day, 16 people who showered twice a day, and four people who showered two or more times a day had onychomycosis. According to the statistical analysis in Table 3, the significance value was 0.542, greater than the threshold of 0.05, indicating no

ISSN 3031-0911

significant correlation between the frequency of daily showers and the incidence of onychomycosis. However, even if a person showers regularly, neglecting proper nail hygiene can still result in uncomplicated onychomycosis infection. According to Kamil *et al.* (2021), proper personal hygiene, including showering, can affect the occurrence of onychomycosis. It is essential to maintain good personal hygiene by keeping nails short and tidy.

Personal hygiene can be observed when garbage transporters clean their hands and feet after picking up and transporting garbage. Of the workers, 23 tested positive for onychomycosis and promptly washed their hands and feet, while seven did not. According to Andarmoyo (2012), proper hand and foot care involves maintaining cleanliness by rinsing with clean water, using soap to clean, and properly drying. According to statistical tests in Table 3, the significance value of 0.033 < 0.05 indicates a relationship between personal hygiene and the incidence of onychomycosis.

The most prevalent onychomycosis infection was observed in individuals who had been working for ≥ 5 years, with 15 people affected, compared to 5 people who had worked for ≤ 5 years. This study is by the research of Amalia *et al.* (2018). The duration of work reflects the length of time an individual is exposed to dermatophyte infection agents. The longer the duration of exposure to fungal infection risk factors, the higher the likelihood of contracting an infection. **Table 3** suggests a significant relationship between the duration of work and the incidence of onychomycosis, with a statistical value of 0.011 <0.05.

Onychomycosis infection was found in 10 individuals who wore boots and ten who did not wear boots. The risk of contracting onychomycosis is equal for those who wear boots and those who do not wear boots while working. This study is in line with the research of Laksono *et al.* (2020). Wearing closed-toe shoes, specifically boots, for more than six hours daily and poor foot hygiene can lead to increased foot moisture and fungal colonization. Statistically, the results show a significant relationship between wearing boots and the occurrence of onychomycosis with a value of 0.032 <0.05.

Age is considered among various risk factors that contribute to fungal infections as the immune system typically weakens with age. The age group between 16-30 years exhibited the highest prevalence of nail fungal infections in this study. Because the majority of participants in this study fell within this age bracket, the data could have been more varied. The statistical tests yielded a significance value of 0.429, indicating no clear association between age and the prevalence of onychomycosis. The results demonstrate that onychomycosis can affect individuals of all ages, indicating that age plays a relatively minor role in the risk of developing onychomycosis.

The study's limitations include the absence of 20-40% KOH reagent examination as an initial screening for nail fungus detection and the lack of identification at the species level. Future research should consider performing examinations using 20-40% KOH reagents as an initial screening for nail fungus detection while also analyzing data results to the species level.

Conclusions

This study concludes that among 30 nail samples from garbage transporters, 20 samples (67%) were positive for onychomycosis, while ten samples (33%) were negative. Of the 20 positive samples, two (15%) detected the fungus type *Trichophyton* sp. and 18 (85%) identified *Aspergillus* sp. The statistical tests revealed a significant correlation between onychomycosis incidence and personal hygiene habits, duration of work, and the use of boots among garbage transporters at the UPTD cleanliness Rawalumbu area, Pengasinan Village, with a p-value less than 0.05. It is suggested to maintain good personal hygiene practices, limit work duration, and wear appropriate boots to prevent onychomycosis.

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Potential of Mangosteen Peel Extract (*Garcinia mangostana*) as Alternative Dye to Eosine in Papanicolaou Buccal Smear Method

Vanesa Mariana[1], Ria Amelia[2]*, Siti Nurfajriah[3]

[1,2,3] Department of Medical Laboratory Technology, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Indonesia
*Correspondence author : ria.amelia@stikesmitrakeluarga.ac.id

Abstract

Introduction: Cytological staining using the *Papanicolaou* method combines hematoxylin and eosin staining. Eosin gives a red or orange color to acidophilic elements. Mangosteen peel extract has anthocyanin compounds, which turn orange in an acidic environment. This study aims to determine the effect of mangosteen peel extract as a substitute for eosin on buccal smear cytology using the *Papanicolaou* method.
Method: The location of this study was conducted at the STIKes Mitra Keluarga cytohistotechnology laboratory on February 13-March 3 2023 with an experimental research design. The sample is a buccal smear from 10 students. The sampling technique uses purposive sampling, and the mangosteen peel extract uses the maceration method.
Results: The results showed that dilutions of 1:1 (average: 2.8) and 1:5 (average: 2.85) yielded the lowest results. In contrast, the results were closest to control (average: 4) is retail of 1:3 (average: 3.4). The Normality Test obtains Sig. 0.0001, which shows <0.05, means that Ha is rejected or the data is not normally distributed. So, the non-parametric test was continued, namely the Kruskal-Wallis test. Kruskal Wallis test results sig. 0.0001 event <0.05, which means that Ho is rejected or there is an effect of mangosteen peel extract as an alternative dye to replace eosin in the *Papanicolaou* method on buccal smear cytology.
Conclusion: Mangosteen peel extract has the potential to stain the cytoplasm as an alternative dye to eosin in buccal smear cytology using the *Papanicolaou* method. The optimum concentration of mangosteen rind extract solution as a substitute for eosin is 1:3, with the highest average value of 3.4.

Keywords: Buccal smear, Mangosteen Peel Extract, Eosin, and *Papanicolaou*.

Introduction

Staining in cytology plays an essential role in producing quality preparations. This staining plays a role in giving color to cells or tissues so that a contrasting shape is observed under a microscope (Noor *et al*., 2020). Cytological staining can use the *Papanicolaou* method, a combination of hematoxylin and eosin staining. Hematoxylin will stain the cell nucleus, and eosin will stain the cytoplasm. Staining such as hematoxylin and eosin are synthetic dyes or chemicals (Kaban & Gigi, 2021).

Hematoxylin is a natural dye obtained from extracting American logs (*Haematoxylon campechianums*) with the chemical formula $C_{16}H_{14}O_6.xH_2O$. The alkaline nature of hematoxylin is capable of staining basophilic elements, making it useful for cytological and histological staining. Hematoxylin in cytological staining plays a role in coloring the cell nucleus, which is acidic so that it gives a blue color (Merck KGaA, 2021). Eosin is a dye for cytological synthesis that has acidic properties and is capable of coloring acidophilic elements with the chemical formula $C_20H_6Br_4Na_2O_5$.

Material Safety Data Sheet, according to regulation (EU) no. 1907/2006, states that eosin is a cytological solution that has an acute inhalation toxicity effect, which can give symptoms of irritation to the respiratory tract and severe eye irritation based on testing on rabbits. This threatens laboratory staff and users of eosin as a cytohistological dye (Smart Laboratory, 2019). Another weakness of synthetic dyes is that they are expensive, and the long storage time will damage the material. Based on the problem of the impact caused by synthetic dyes such as eosin, it is necessary to search for natural materials that have the potential as cytological stains to replace eosin, one of which is mangosteen rind. This is due to the mangosteen rind, which contains anthocyanins. When in a very acidic environment, it can give the maximum red color (Ernawati & Rahayu, 2017). This type of anthocyanin is pelargonidin, which appears freely in the form of a red pigment but gives an orange color to flowers and a red color to fruit.

This research refers to several previous studies. Research on the potency of red dragon fruit juice as an alternative stain for eosin in *Papanicolaou* staining by Permatasari *et al.* (2020) used chicken oral epithelial smear preparations from 7 treatments with varying concentrations of 1:1, 1:2, 1:3, 1:4, 1:5, pure or mother liquor and *Papanicolaou* solution as a comparison. Results 1:1 get the lowest value, while the results closest to the reference

ISSN 3031-0911

solution are 1:2, 1:3, 1:4, 1:5, and mother liquor or pure. However, this study stated that the maximum point of red dragon fruit squeezing occurred at a shelf life of 4 days because the fruit had undergone a ripening and ripening process. Then, there will be a decrease in conditions followed by damage during aging (8 days of storage) (Permatasari *et al*., 2022).

As for research on the study of cytoplasmic staining on buccal smears using *Curcuma longa* (turmeric) extract as a substitute for eosin by Sankar *et al.* (2022) stated that the primary coloring pigment in *C. longa* which is acidic, gives a yellow color to the components of the cell cytoplasm. However, in this study, only centrifugation was carried out so that the supernatant was obtained, and pure extraction was not carried out (Sankar *et al*., 2022).

Based on the search for references, research has yet to be found regarding using natural dyes for mangosteen rind. This research will utilize mangosteen peel waste for cytological staining using the *Papanicolaou* method, so researchers are interested in conducting research on the potential test of mangosteen peel extract as an alternative dye to replace eosin on the *Papanicolaou* method on buccal smear cytology. Suppose the results of this study prove to be effective in substituting eosin. In that case, it is hoped that it can become a source of information for future researchers to develop the use of mangosteen rind as a cytological dye using the *Papanicolaou* method.

Methods

This type of research is descriptive quantitative. The research design used is experimental. The sampling method was carried out by purposive sampling. This research was conducted on 13 February-3 March 2023. Sampling in the form of buccal smears was carried out at STIKes Mitra Keluarga. Sample testing was carried out in the Pharmacognosy Laboratory (Room 214) and the Cytohistotechnology Laboratory (Room 305) STIKes Mitra Keluarga. The sample in this study was a buccal smear from STIKes Mitra Keluarga students. Buccal smear samples were taken from several 10 students. The samples will be stained with *Papanicolaou* as a control and mangosteen peel extract with concentrations of 1:1, 1:3, and 1:5, repeated twice to obtain a total sample of 80 preparations.

The test dye was obtained from 100 grams of powdered mangosteen rind. Mangosteen peel powder was dissolved in 495 ml of 96% ethanol and 5 ml of concentrated HCl. Leave it for one day, then strain it to get the extract. The extract is concentrated using a rotary vacuum evaporator until the volume is approximately one-fifth of the initial extract volume or until it becomes a paste. The extract results were made by making a soaking solution with a concentration of 1:1 (17 gr paste: 17 gr ethanol), 1:3 (8.5 gr paste: 25.5 gr ethanol), and 1:5 (5.6 gr paste: 28, 3 grams of ethanol). The density of the ethanol solution is 0.814 gr/ml. Ethanol is converted from grams to milliliters with the formula V = mass: density. The conversion calculation results are 1:1 (17 gr paste: 20.88 ml ethanol), 1:3 (8.5 gr paste: 31.32 ml ethanol), and 1:5 (5.6 gr paste: 34.76 ml ethanol).

The sample used is a buccal smear, which is scraped using a sterile spatula or wooden stick to obtain epithelial cells. The results of the smears were made preparations on the object glass. Preparations were stained with the *Papanicolaou* method. Eosin dye was used as a control, and mangosteen rind extract solution was used as a test dye. Buccal smear smear results were fixed with 95% alcohol for 3 minutes. The next step is rehydration with 90%, 80%, 70%, 60%, 50%, 40% and 30% alcohol for 3 minutes in each solution. Dip with distilled water—core staining stage with hematoxylin for 15 minutes. Run under running water for 1 minute. Blueing stage as much as three dips. Dehydration step with 30%, 40%, 50%, 60%, 70%, 80% and 90% alcohol for 2-3 minutes in each solution. G-6 (OG-6) orange staining stage for 1-2 minutes. Dip with 95% alcohol, as much as four dips. The glass objects to be immersed in the test dye solution and the control dye solution are separated. The slides for the control were soaked in an eosin dye solution for 3-5 minutes. The object glass for testing is immersed in the test solution (mangosteen peel extract 1:1, 1:3, and 1:5) for 3-5 minutes. Dip with 95% alcohol for four dips. They are clearing stage with alcohol-xylol for 3-5 minutes and xylol for 3-5 minutes. The object glass is dripped with mounting and then covered with a cover glass.

Staining results were observed microscopically to see cell shape and color intensity using a 100x10 magnification light microscope and oil immersion. The results of the observations will be processed and analyzed using Microsoft Excel 2019 and SPSS. The data obtained in tabular form are the results of observations, while the observed are the intensity of the core color, the intensity of the color of the cytoplasm, and the shape of the cell. The assessment of each point is in the form of ordinal data indicating the level of coloring quality. The meaning of ordinal data to be given is 1: unclear, 2: not clear, 3: clear, 4: very clear. After the data is obtained, it will be continued with the normality test. If the results are normally distributed, it will be tested with one-way ANOVA. If the data results are not normally distributed, then the Kruskal Wallis test will be continued. The value of the error rate of the data test results is 0.05, which means that the maximum data error rate is 5% and 95% of the data is correct.

ISSN 3031-0911

Results and Discussions

Illustration of buccal smear preparations in test solutions 1:1, 1:3, and 1:5 with quality comparison results in **Figure 1. (A)** Researchers used the test solution, namely mangosteen peel extract as a substitute for eosin with a dilution of 1:1 with information on the quality of the cell shape The cytoplasm and nucleus are visible, the color intensity of the cytoplasm is clear with a thin orange color. In contrast, the nucleus is a faint grayish orange. **Figure 1. (B)** shows buccal smear epithelial cells using *the Papanicolaou* stain as a comparison/control. The quality of the comparison/control shows that the shape of the cytoplasm and epithelial cells is apparent, the intensity of the cytoplasmic color is apparent with a purplish pink color, the epithelial cell fragments are evident because the background of the epithelial cells in the preparation is visible faintly, the intensity of the nuclear color is apparent with a dark purple color.

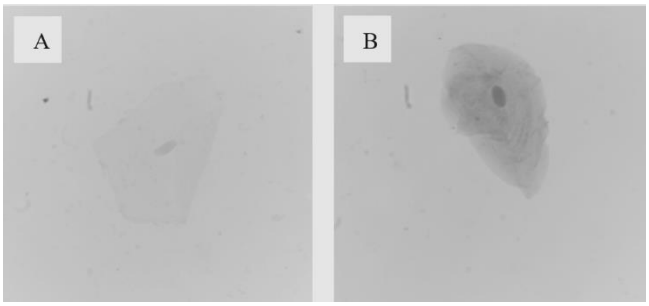

Figure 1. Buccal smear staining results, (A) mangosteen rind extract staining results at a dilution of 1:1, (B) buccal smear staining results using the *Papanicolaou* method

The results of the comparison of the quality of the preparations are in Figure 2. (A) the researcher used a test solution, namely mangosteen peel extract, as a substitute for eosin with a dilution of 1:3, with the description of the quality of the shape of the cyto-plasma and epithelial cells being evident, the intensity of the cytoplasm color being apparent with a slightly dark orange color, the epithelial cell fragments are evident because the background of the epithelial cells in the preparation is visible faintly, the intensity of the nuclear color is apparent with a grayish orange color.

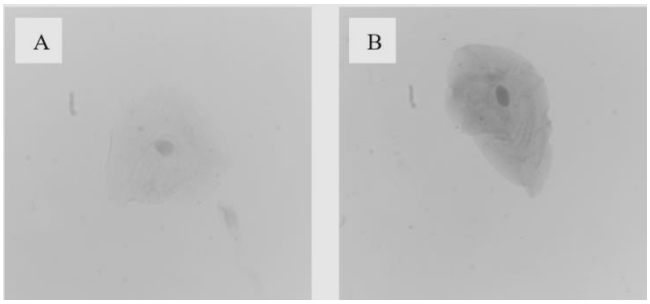

Figure 2. Buccal smear staining results, (A) staining results of 1:3 dilution of mangosteen peel extract preparations, (B) buccal smear staining results using the *Papanicolaou* method

The results of the comparison of the quality of the preparations are in **Figure 3. (A)** The researcher used a test solution, namely mangosteen peel extract, as a substitute for eosin with a dilution of 1:5 with a description of the quality of the cell shape, the presence of cytoplasm and nuclei was visible, the intensity of the color of the cytoplasm was clear with a thin orange color while in the nucleus faint grayish orange.

ISSN 3031-0911

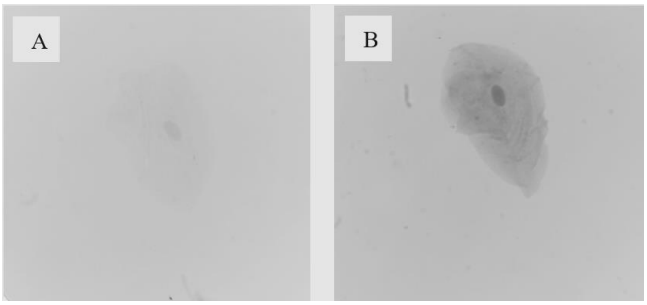

Figure 3. Buccal smear staining results, (A) staining results of 1:5 dilution of mangosteen peel extract, (B) buccal smear staining results *Papanicolaou* method

They were testing mangosteen peel extract as a substitute for eosin dye, which can color the cytoplasm so that it is orange. Testing was carried out on 80 buccal smear preparations, which had been assessed for quality based on a score of 1-4 for each preparation. The assessment data is searched for the sum, mean, and mode. The sum value of the 1:1 concentration test was 56, the 1:3 concentration was 68, the 1:5 concentration was 57, and the comparator (control) was 80. The mean value from the 1:1 concentration test was 2.8; a concentration of 1:3 was obtained at 3.4; a concentration of 1:5 was obtained at 2.85, and the comparator (control) was obtained at 4. The mode value of the test concentration 1:1 was obtained at 3, the concentration at 1:3 was obtained at 3, the concentration 1:5 was obtained at 3, and the comparator (control) was obtained at 4.

The results of the normality test and the non-parametric data test in this study were statistically tested, namely the regular distribution test, using the Kolmogorov-Smirnov test. Based on the **table 1**, a significant value is obtained, namely 0.0001. Sig. The table shows <0.05, which means Ha is rejected or the data is not normally distributed. So, the test cannot be continued with the one-way ANOVA test. This is because one-way ANOVA usually requires distributed data. So, the data will be continued with a non-parametric test, namely the Kruskal-Wallis test.

Table 1. Normal distribution test results using the Kolmogorov-Smirnov Test

Normality test

		Kolmogorov-Smirnov[a]		
	Treatment	Statistic	df	Sig.
Quality Value	1:1	0.327	20	0.0001
	1:3	0.387	20	0.0001
	1:5	0.420	20	0.0001
	Control	.	20	.

Testing continued with the Kruskal-Wallis test. Based on the Kruskal Wallis test on the data, the Asymp value was obtained. Sig. 0.0001. Asymp value. Sig. shows <0.05, which means Ho is rejected or there is an influence of mangosteen peel extract as an alternative dye to replace eosin in the *Papanicolaou* method on buccal smear cytology.

Table 2. Kruskal Wallis test results for each treatment of buccal smear preparations using the *Papanicolaou* method

Rank

	Treatment	N	Mean Rank
Quality Value	1:1	20	26.15
	1:3	20	44.00
	1:5	20	26.85
	Control	20	65.00
	Total	80	

Statistic test

	Quality Value
Kruskal-Wallis H	45.154
df	3
Asymp. Sig.	0.0001

ISSN 3031-0911

Mangosteen rind extract contains anthocyanin compounds, which tend to give a red color in very acidic pH conditions (pH <3). If the anthocyanin is in an alkaline solution (pH >10), it will affect the anthocyanin pigment to a green color. The solvent used is ethanol-HCl. Research by Ernawati and Rahayu (2017) states that ethanol and HCl can extract the anthocyanin content of mangosteen rind. This is because anthocyanin compounds dissolve in polar solvents (Ernawati & Rahayu, 2017). The addition of polar HCl aims to provide an acidic atmosphere because anthocyanins are stable in an acidic environment. The pH test was carried out using a pH meter, and the measurement results obtained the pH of the extract, which was 2. However, the pH was measured once before mixing with the immersion solution. This can trigger the color results obtained by the extract needing to be more optimal (Lestari et al., 2014). Maceration is carried out for a day. Too extended maceration will damage the quality of the compound. According to research by Kurniawati (2020), soaking time for one day can extract mangosteen skin optimally. If the soaking time exceeds the optimum limit, it can cause the compound to decompose (Kurniawati, 2020).

The results of research with dilutions of 1:1, 1:3, and 1:5 obtained an average of 2.8 at a dilution of 1:1, 3.4 at a 1:3 dilution, 2.85 at 1:5 dilution, and four at control. The average value at the 1:1 dilution is the lowest; this is because during the process of making the test solution, the solution was already crusty, and the ethanol had evaporated so that the staining results often found cell shapes and the intensity of the cytoplasm color was less clear. The highest average value is a dilution of 1:3. This is because the observed staining results found that many cell shapes and cytoplasmic color intensity were clear, epithelial cell fragments were apparent. After all, the epithelial cell background was visible faintly, and the nuclear color intensity was evident. In line with the research of Permatasari *et al.* (2022), concentrations yield good results at dilutions of 1:2, 1:3, 1:4, and 1:5 concentrations and mother/pure solution, while at 1:1 dilution, the results are not good (Permatasari *et al*., 2022). The results of the study are by the usefulness of *Papanicolaou*: epithelial cells appear more transparent so that overlapping cells can be more easily distinguished, the color of acidophilic cells varies from red to orange, and basophilic cells are blue-green, stains that have partially or even wholly dried can be stained with entirely satisfactory, and the differential staining does not completely disappear (Chantziantoniou *et al.*, 2017).

The *Papanicolaou* method consists of several stages, namely fixation, rehydration, blueing, dehydration, eosin, clearing, and mounting. These stages have different goals. Fixation was carried out using 95% alcohol to adhere the epithelial cells to the glass slide firmly. The rehydration stage uses alcohol with a decreasing concentration to introduce water into the cavities to facilitate the hematoxylin level. If the rehydration stage is not carried out correctly, it will affect the results of staining with hematoxylin. Hematoxylin will give a reddish-purple color to the cell nuclei and will turn blue after blueing (Beandrade *et al.*, 2020). The dehydration stage uses an alcohol solution for 1 minute with an increasing concentration to remove water from the preparation. If the immersion time for the dehydration stage is less than 1 minute, it will cause the water carried not to be lost to the maximum; if it is more than 1 minute, it can cause epithelial damage. Orange G-6 aims to counterstain cytoplasmic keratin to bright orange, making it easier to diagnose cancer cells. Eosin aims to dye the cytoplasm red or orange. The clarification stage using xylol aims to remove the solution used in the dehydration stage. The final stage is an attachment with Entelan, which aims to protect it from contamination by microbial growth. However, the final stage was not carried out, so it could be a factor of error during observation (Khristian & Inderiati, 2017; Dey, 2019).

Testing the quality of mangosteen peel extract compounds was influenced by irradiation, storage temperature, and storage time. Mangosteen peel extract is recommended to be stored in a dark bottle. This is because irradiation can affect the quality of the mangosteen peel extract. The anthocyanin contained can absorb light, causing damage to the anthocyanin structure, which results in the color changing to colorless. However, at the time of the study, mangosteen peel extract was not stored in dark bottles. The color results given by the mangosteen peel extract could be more optimal. Apart from being stored in a dark bottle, mangosteen peel extract also needs to be stored in the refrigerator. The refrigerator can help the extract be stable rather than stored in an open room. An increase in temperature can cause the decomposition (dissociation) of anthocyanin molecules, resulting in colorless compounds. According to Kurniawati (2020), storage time has a significantsmall effect. Storage must be in a dark bottle and at a low temperature so that it is not easily degraded. Based on research that has been carried out, it is necessary to pay attention to the storage and temperature of mangosteen peel extract to obtain maximum results.

Conclusions

The results of the buccal smear preparations were stained. However, not the same as the controls, with a significant value using the Kruskal Wallis test method of 0.0001 <0.05, which means that there is an effect of mangosteen peel

ISSN 3031-0911

extract as an alternative dye for eosin in the Papanicolaou method on buccal smear cytology. Concentration results are good at 1:3 dilution with an average value of 3.4. Mangosteen peel extract with 1:3 dilution can replace eosin dye in the Papanicolaou method. However, further research still needs to be done regarding the resistance to staining levels of mangosteen peel extract.

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Correlation Between Neutrophil Lymfocyte Ratio (Nlr) And Platelets Values in Dengue Hemorrhagic Fever Patients at Private Hospital Jatiasih Bekasi Period 2019-2021

Yuniar Rohma Maulida[1], Ria Amelia[2*], Elfira Mayasari[2],

[1,2,3] Department of Medical Laboratory Technology, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Indonesia
*Correspondence author : ria.amelia@stikesmitrakeluarga.ac.id

Abstract

Introduction: Dengue Hemorrhagic Fever (DHF) is an infectious disease caused by dengue virus infection. This disease can cause symptoms of thrombocytopenia due to the extensive use of platelets to prevent plasma leakage. Apart from that, predicting the critical period for plasma leakage can be seen from the results of the NLR examination. This study aimed to determine the relationship between the NLR value and the platelet value in DHF patients at one of the Jatiasih Bekasi Private Hospitals for the 2019-2021.
Method: This study uses a quantitative descriptive—retrieval of secondary data by purposive sampling and the Spearman correlation test analysis method. The research was conducted in February - March 2023 in one of the Jatiasih private hospitals—data collection for the period June 2019 to December 2021. The research data will be analyzed using SPSS with the Spearman correlation test method.
Results: The research results obtained data on 74 dengue fever patients. Male gender with an average age of 20-44 years. The results of the Spearman correlation test showed a significant p-value of 0.026, indicating a relationship between NLR and platelets in DHF infection. There is a positive relationship between the NLR test and platelets. This is thought to influence the active cellular immune response to DHF, characterized by an average platelet count.
Conclusion: There is a relationship between the neutrophil-lymphocyte ratio value and the platelet count in DHF patients with a weak correlation level.

Keywords: DHF, Jatiasih, Neutrophil, NLR and Platelets.

Introduction

Dengue virus infection is a common health problem in tropical and subtropical areas worldwide, including in Indonesia. Dengue Hemorrhagic Fever (DHF) is an infectious disease caused by the dengue virus. This virus can be spread through the mosquito vector of Aedes albopictus and Aedes aegypti. Factors that cause the spread of dengue fever include climate factors, environmental conditions, population density, community behavior, and others (Kemenkes RI, 2020).

According to Kemenkes RI (2022), Over the past four years, there has been a drastic increase of more than eight times in reported cases of DHF in Indonesia. The number of DHF cases increased from 505,000 cases to 4.2 million cases in 2019. However, in 2021, 73,518 cases of DHF were recorded, with 705 deaths. There has been a decrease in cases and deaths from DHF compared to 2020 when there were 108,303 cases and 747 deaths.

The number of cases of morbidity and mortality can be described by indicators of Incident Rate (IR) per 100,000 population. The impact of dengue fever in 2019 was 51.53 thousand per 100 thousand population. In West Java Province, the IR is 47.62 thousand per 100 thousand population (Kemenkes RI, 2020). According to Dinas Kesehatan Kota Bekasi (2019), the spread of DHF cases in 2019 had increased (2,484 cases). The highest increase in the incidence was in the Jatiasih area, with 408 cases.

Dengue infection can cause plasma leakage, which occurs due to the activation of capillary endothelial cells. Plasma leaks that occur can result in loss of intravascular fluid, even shock, until death. Therefore, it is necessary to support laboratory blood test results such as platelet values (Kemenkes RI, 2020). However, in estimating the course of dengue fever, laboratory blood test results such as the neutrophil and lymphocyte ratio (NLR) value need to be supported. According to Java *et al.* (2018), in viral infections, leukopenia generally occurs, namely a decrease in the number of white blood cells. In addition, changes in the NLR value (neutrophils < lymphocytes) also play a role in determining the critical period for plasma permeation.

Several previous studies, namely research on the relationship between platelet, hematocrit, and hemoglobin levels with the degree of dengue hemorrhagic fever in hospitalized pediatric patients at BRSU Tabanan (Made *et al*., 2022). Subsequent research by Java *et al.* (2018) compared NLR values in children with dengue fever and DHF. Based on previous research, there has never been any data regarding the relationship between NLR levels and platelet values in dengue fever patients at one of the Jatiasih Bekasi Private Hospitals for the 2019-2021 period, which were discussed simultaneously and tested for correlation. Therefore, researchers are interested in conducting research on the

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relationship between NLR levels and platelet values in dengue fever patients at one of the Jatiasih Bekasi Private Hospitals for the 2019-2021 period. The hope is that the results of this research can be a source of helpful information for the community regarding the health and prevention of dengue infection in the area.

Methods

This type of research is quantitative descriptive. The research design used is *cross-sectional*. The method of data collection is done by *purposive sampling*. The research was carried out from February - March 2023. This study used data from medical records at one of the Jatiasih private hospitals, namely patients with dengue virus infection who were taken from June 2019 - December 2021 (for two years). This research was conducted at one of the Jatiasih private hospitals.

The population in this study were patients suffering from dengue fever who had checked platelet counts and leukocyte counts at one of the Jatiasih private hospitals in the period June 2019 – December 2021, namely 74 people out of 233 people. The samples used in this study had to meet the inclusion criteria, namely patients who had confirmed dengue fever by a doctor and underwent a hematological examination and leukocyte count. The exclusion criteria are patients confirmed by a doctor for dengue fever and undergo a hematological examination but suffer from bacterial infections such as urinary tract infections, pneumonia, and so on. Two variables are used in this study, namely the independent and dependent variables. The independent variable in this study was patients with dengue virus infection. Meanwhile, the dependent variables in this study were NLR values and platelet counts.

Results and Discussions

Data on patients with DHF at one of the Jatiasih private hospitals collected from June 2019 - December 2021 obtained 74 patients. Data from 74 research subjects who completed the predetermined exclusion and inclusion criteria. Patient data collected by gender and age obtained from the medical records of one of the Jatiasih private hospitals was obtained as follows:

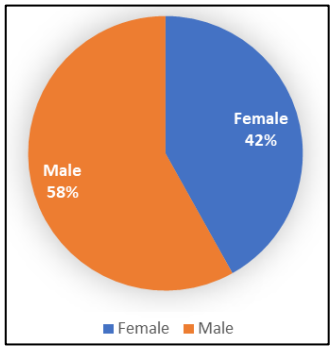

Figure 1. Characteristics of DHF Sufferers Based on Gender

Based on **figure 1** shows that the data on patients with confirmed dengue fever mainly were men, 43 people (42%) out of 74 samples, and 31 women (58%).

Table 1. Data on Patients Infected with Dengue Based on Age

Age	Amount	Percentage (%)
Toddler		
1 – 5 years	9	12
Chidren		
6- 10 years	10	14
teenager		
11 – 19 years	13	18

ISSN 3031-0911

Mature 20 – 44 years	35	47
Elderly 45 – 60 years	7	9
Total	74	100

Based on **Table 1**, the results show the distribution of patients with confirmed dengue fever, totaling 74 patients, with nine people in the toddler age group (1-5 years), ten children (6-10 years), 13 teenagers (11-19 years) people, adults (20-44 years) numbering 35 people, elderly (45-60 years) numbering seven people in patients suffering from dengue fever infection starting from the age of 1 year to the age of 59 years.

Table 2 NLR Value in Patients Suffering from DHF

NLR value	Amount	Percentage (%)
<3,13	45	61
>3,13	29	39
Total	74	100

Based on **Table 2,** it can be seen that the patients with DHF had normal NLR values in 45 people (61%), while there were 29 people with abnormal NLR values (39%).

Table 3 Platelet Count in Patients Suffering from DHF

Platelet value	Amount	Percentage (%)
<150.000	59	80
150.000-450.000	15	20
>450.000	0	0
Total	74	100

Based on **Table 3**, it is illustrated that the majority of patients with DHF had a below-normal platelet count in 59 people (80%), while 15 people (20%) had normal platelet results. Analysis of the relationship between NLR values and platelets was carried out using multiple linear regression tests. This analysis can be seen based on **Table 4**:

Table 4 Value of the level of relationship between variables (r), sig value = 0.026

Inspection	R Value	Interpretation
Platelets and NLR	0,259	Weak Relationship

Based on **Table 4**, a significant value was obtained of 0.026 <0.05 (5%), so it can be concluded that H0 was rejected, which shows a relationship between the number of platelets and the NLR value in patients suffering from dengue fever. The relationship between platelet count and NLR value shows a value of 0.259, which means the relationship is weak.

Based on the study's results, data were obtained from 74 patients with confirmed DHF at a private hospital in Jatiasih with a minimum age of 1 year and a maximum of 59 years with an average age of 24. The age of DHF sufferers is adults aged 20-44 years, as many as 35 people (47%). The age range of 20-44 years is the productive age category. This is similar to research conducted by Kafrawi *et al.* (2019), who said that the most DHF sufferers were adults aged 18-40 years, as many as 43 people (69.4%), and research by Hidayat *et al*. (2017) obtained the most results at the age of 20-40 years, namely 69 people (50%). Age is one of the factors that affect susceptibility to dengue virus infection. One reason is because of the activities they do outside the home. Adults tend to be more active outside the home, including in work, travel, or social activities. This increases their chances of being bitten by Aedes mosquitoes, which carry the dengue virus. In addition, awareness and self-protection factors can also affect the infection rate. Some adults may be less aware of the threat of *Aedes* sp. Mosquitoes. and paying little attention to preventive measures such as using mosquito nets, wearing protective clothing, or using insecticides. This can increase their risk of getting bitten by

ISSN 3031-0911

mosquitoes and possibly being infected with the dengue virus (Hidayat *et al*., 2017). Awareness and self-protection factors can also influence infection rates. Some adults may be less aware of the threat of Aedes Sp. Mosquitoes. and less attention to preventive measures such as using mosquito nets, clothing that protects the body, or using insecticides. This can increase their risk of getting bitten by mosquitoes and possibly being infected with the dengue virus (Hidayat *et al*., 2017). Awareness and self-protection factors can also influence infection rates. Some adults may not be aware of the threat of the Aedes Sp mosquito. Furthermore, less attention to preventive measures such as using mosquito nets, clothing that protects the body, or using insecticides. This can increase their risk of being bitten by mosquitoes and possibly infected with the dengue virus (Hidayat *et al.*, 2017).

Gender characteristics in the data obtained show that there are more men than women, where there are 43 men (58%) while there are 31 women (42%). This is similar to research by Hidayat *et al*. (2017) that the male sex was more numerous, namely 81 people (58.7%), while 57 women (41.3%). In general, men have a higher risk of infection than women because the production of immunoglobulins or antibodies in women is genetically and hormonally more efficient than in men (Hernawan and Afrizal, 2020)

Based on **Table 2**, the results of the NLR values are dominated by the interpretation of normal results. This is presumably because the respondent has received medical action, so that the condition of the respondent is good. However, it is different from the results of the platelet values contained in Table 4, which are dominated by decreased interpretation results. It is suspected that the patient is experiencing a critical or acute phase of DHF. In the critical phase of DHF, there is a horse's saddle curve, characterized by a decrease in platelet values. Based on research by Ishaque *et al.* (2022) and Cahyani *et al.* (2020), an increase in NLR values occurs in the early phase of fever, which is characterized by a decrease in the number of neutrophils (neutropenia) and an increase in lymphocytes (lymphocytosis). Neutropenia occurs due to neutrophil apoptosis triggered by dengue infection and bone marrow depression caused by viruses directly or through proinflammatory cytokines. In dengue infection, there is an increase in the relative number of lymphocytes, which indicates the important role of lymphocytes in the body's defense mechanism against the dengue virus. The mean time to decrease in NLR values is usually seen more frequently between the 3rd and fifth day after the onset of infection. A decrease in NLR values indicates the late febrile phase of dengue infection.

The decreased platelet count can be caused by increased platelet activation due to the large number of blood vessels being damaged due to activation of the body's immune response to eliminate the dengue virus by forming antigen-antibody bonds. The antigen-antibody bond can trigger activation of the complement system, thereby causing an inflammatory reaction in the blood vessels. As a result, blood vessels lyse and cause increased use of platelets to close wounds. Therefore, thrombocytopenia occurs. Thrombocytopenia in dengue infection can also occur due to bone marrow suppression (Hidayat *et al.*, 2017). Therefore, from the research results, there is a weak relationship between the number of platelets and the NLR value on the incidence of dengue fever.

Conclusions

The results of this study indicate that there is a weak relationship between the value of the Neutrophil Lymphocyte Ratio and the value of platelets in DHF patients at one of the Jatiasih Bekasi Private Hospitals for the 2019-2021 period.

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