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TEST BIOACTIVITY OF ANTIBACTERIAL COMPOUND EXTRACT OF RED BETLE LEAF (*Piper crocatum*) AGAINST *Propionibacterium acnes* BACTERIA.

Mita Fatmawati Hidayatullah[1], Reza Anindita[1], Maulin Inggraini[2], Noor Andryan Ilsan[2]

Abstract

Introduction: Skin disease is a disease on the outermost part of the body, which can be caused by chemicals, sunlight, bacteria, viruses, fungi, weak immune systems, and personal hygiene factors. Acne (*Acne vulgaris*) is a skin disease caused by blockage of dead skin cell follicles, excess oil production, and inflammation caused by acne-causing bacteria including *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* in sebaceous follicles. The purpose of this study was to determine the effect of Red Betel leaf extract (*Piper crocatum*) on the growth of *Propionibacterium acnes* with concentrations of 70%, 80%, 90%, and 100%.

Method: This study used an experimental design, to test the inhibition of red betel leaf extract against *Propionibacterium acnes* bacteria with concentrations of 70%, 80%, 90%, and 100%. The data analysis used in this study is descriptive quantitative which shows the size of the growth area of the bacterial colonies of P. acnes expressed in mm (millimeters) around the paper disc.

Results: From the results of the data that has been obtained based on the measurement of the diameter of the inhibition zone of the red betel leaf ethanol extract at concentrations of 70%, 80%, 90%, and 100%, which is 16 mm; 18mm; 18.5mm; and 21 mm. The intermediate category was produced by red betel leaf extract at a concentration of 70%, 80%, 90% and the sensitive category at a concentration of 100%.

Conclusion: The conclusion of this study was that the administration of red betel leaf extract with a concentration of 100% was able to inhibit the growth of P. acnes bacteria with an inhibition zone diameter of 21 mm or included in the sensitive category.

Key words : Antibacterial, *Propionibacterium acnes*, Red Betel (*Piper crocatum*), Kirby Bauer.

INTRODUCTION

Skin disease is a disease in the outermost part of the body, which can be caused by chemicals, sunlight, bacteria, viruses, fungi, weak immunity, and personal hygiene factors (Triana & Fitria, 2019). One of the diseases caused by bacterial infection is acne. Acne (*Acne vulgaris*) is a skin disease caused by blockage of dead skin cell follicles, excess oil production on the skin which causes clogged facial skin pores, triggering bacterial activity and inflammation caused by acne-causing bacteria including *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* in sebaceous follicles (Efrata *et al*., 2018). Acne is not a dangerous skin disease, but it has a big impact on sufferers both physically and psychologically because it can cause anxiety and decrease self-confidence (Saragih et al., 2016). Many of the acne sufferers who use drugs inappropriately and can cause various new problems such as allergic reactions, irritation, resistance and hypersensitivity. In addition, there are complications from increasingly inflamed acne such as comedonal acne, conglobatal acne, papulo-pustular acne and other severe acne (Try *et al*., 2021).

To prevent the use of drugs inappropriately, it is necessary to develop research on the use of medicinal plants as an effort to treat acne. One of the medicinal plants that has the potential as a therapy for acne treatment is red betel leaf (Kumalasari *et al*., 2020). In a study conducted by Syafriana and Rusyita (2017) stated in their research that red betel leaf (*Piper crocatum*) is one type of plant that contains chemical compounds such as alkaloids, flavonoids, tannins, and essential oils. The results of the study explained that the minimum inhibitory concentration value of the ethanolic extract of red betel leaf (*Piper crocatum*) was able to inhibit the growth of P. acnes bacteria at a concentration of 10%. In this study, using 70% ethanol extract of red betel leaf with disk diffusion method (*Kirby Bauer*) with various concentrations of 70%, 80%, 90%, and 100%.

METHOD

A. Research Design

The design of this study was an experiment that tested the inhibition of Red Betel leaf extract (*Piper crocatum*) against the growth of *Propionibacterium acnes* bacteria. Experimental design is a research in which the researcher intentionally provides treatment or intervention to the research subject. The treatment in this study was red betel leaf extract with concentrations of 70%, 80%, 90%, and 100%.

B. Time and Location of Research

This research was conducted in February-March 2022 at Stikes Mitra Keluarga Bekasi Timur.

C. Research Sample

The samples in this study were Red Betel Leaf (*Piper crocatum*) and pure culture of *Propionibacterium acnes* ATCC 11827.

D. Research Variables

The variables in this study are independent variables. Independent variables are independent variables, unlike independent variables which are always paired with the dependent variable (Sugiyono, 2018). The independent variable in this study was the diameter of the inhibition zone on the media containing *P. acnes* bacteria after administration of red betel leaf extract with varying concentrations.

E. Tools and Materials

The tools used are glass and non-glass tools, analytical balance (IKA, Ohaus MB 120), incubator (Memmerth IN-30), autoclave (AGGRAM), Laminar air flow (UK 1200 MM), rotary evaporator (IKA-RC 2), oven (IKA 125), and P100 micropipette (Acura digital 825).
The materials used were red betel leaf (*Piper crocatum*), 70% ethanol, distilled water, NA (*Nutrient agar*) and MHA (*Mueller hinton agar*) medium, *Propionibacterium acnes* ATCC 11827 bacteria culture.

F. Making Red Betel Leaf Extract

Wash the red betel leaves and then air dry them until they are completely dry. Once dry, puree with a blender. Weigh the fine powder as much as 100 grams and given 500 ml of 70% ethanol and stored in a place that is protected from light for 3 days while stirring occasionally. Then filtered, the residue was re-maceration with added 250 ml of 70% ethanol and stored for 2 days. Then evaporation is carried out using a *rotary evaporator*.

G. Qualitative Phytochemical Screening

1. Alkaloid Test

   Weigh 1 gram of red betel leaf simplicia powder, add 5 ml of chloroform and 10 ml of 10% ammonia. Strain into a test tube and add 10 drops of 2N H2SO4 to the filtrate. Shake until two layers are formed, then transferred to 3 test tubes as much as 1 ml. To each tube, a few drops of *Mayer*, *Wagner* and *Dragendorff* reagents were added. Positive results of alkaloids are indicated by the formation of a precipitate with *Mayer's* reagent forming a white precipitate, with *Wagner's* reagent forming a brown precipitate and *Dragendorff's* reagent forming an orange precipitate (Trian, 2020).

2. Tannin Test

   Weigh as much as 20 mg, then add 96% ethanol. Then 2-3 drops of 1% FeCl3 solution were added. Positive results are indicated by the formation of a bluish black or greenish color (Eka, 2017; Trian, 2020).

3. Flavonoid Test

   Weigh as much as 200 mg, then add 5 ml of 96% ethanol and heated for 5 minutes. Then add a few drops of concentrated HCl and 0.2 g of Mg powder. Positive results are indicated by the appearance of a dark red color for three minutes (Trian, 2020).

4. Saponin Test

   Weigh as much as 2 grams then put into a test tube, add distilled water and boil for 2-3

minutes, and then cooled, then shaken vigorously. Positive results are indicated by the formation of stable foam (Trian, 2020).

5. Essential Oil Test

The 0.5 gram thick extract was diluted with 1 ml of 96% ethanol solvent and then heated on a hot plate using a watch glass until the residue was obtained. Positive results are indicated by the characteristic odor produced by the residue (Rukmini *et al*., 2020).

H. Resistance Test

The diameter of the inhibition test was carried out using the Kirby Bauer method, in which a sterile cotton swab that had been dipped in a suspension containing P. acnes bacteria was rubbed on the MHA medium evenly. Then attached to each paper disk that has been soaked in the extract according to the concentration for ± 30 minutes and sterile distilled water. Paper disks were placed on the surface of the MHA media and incubated for 24 hours at 37°C. The test material is categorized as positive if the laboratory test results on Red Betel leaf extract can inhibit the growth of P. acnes which is characterized by the formation of an inhibitory zone formed around the paper disk (Basarang, 2019).

RESULTS

A. Plant Determination

Determination of a plant aims to find out the truth of the identity of the plant, whether the plant is really the desired plant. Thus errors in the collection of materials to be studied can be avoided. The determination was carried out at the Bogor Research Center-Indonesian Institute of Sciences (LIPI), Cibinong, West Java. The results of the determination can be seen in the following table:

Number	Kol Number	Type	Family
1	Red Betle Leaf	*Piper crocatum* Ruiz and Pav.	*Piperaceae*

B. Plant Organoleptic

Organoleptic tests are carried out as an initial introduction to the plants that will be used for testing. This is done by looking at the physical appearance of plants by observing which includes smell, color, shape, and taste. The results of organoleptic tests can be seen in the following table:

Organoleptic Test	Result
Smell	Strong aromatic
Colour	Purplish red
Form	Flat like a heart with a tapered tip
Flavour	Bitter and spicy

C. Plant Yield

The yield of this research extract was obtained through the extract process using the maceration method. Yield is the ratio of the amount of extract produced from plant extraction, the resulting yield indicates the value of the extract produced is increasing, the yield can be expressed from the comparison of the weight of the extract with the weight of the simplicia powder. The yield of plants can be seen in the following table:

Sample	Powder Weight (g)	Extract Weight (g)	Extract Yield Value (%)
Red Betle Leaf	100 g	13,5 g	13,5%

D. Phytochemical Screening

Phytochemical screening is a preliminary stage in a phytochemical research with the aim of providing an overview of the class of compounds contained in the plant to be studied. The

phytochemical screening method that will be carried out is by looking at the color test reaction using a color reagent. The results of phytochemical screening can be seen in the following table:

Secondary Metabolic Compound	Extract Test Results	Reagen	Information
Flavonoid	+	*Wagner*	Chocolate precipitate
Alkaloid	+	FeCl3 1%	Blue or greenish black color
Tannin	+	Concentrated HCl + Mg	Dark red color
Saponin	+	Aquadest	Forms stable foam
Esential Oil	+	Ethanol 96%	Presence of residue characteristic odor

E. Inhibitory Zone Diameter Test

The antibacterial bioactivity test was carried out using samples of red betel leaf with three replications of treatment. The antibacterial bioactivity test of the red betel leaf ethanol extract used four concentration variations, namely 70%, 80%, 90%, and 100%. The results of the diameter of the inhibition zone can be seen in the following table:

Treatment	Average Inhibition Zone Diameter (mm)	Information
70%	16 mm	Intermediate
80%	18 mm	Intermediate
90%	18,5 mm	Intermediate
100%	21 mm	Sensitive
Control (+)	26 mm	Sensitive
Control (-)	0	Resistant

Information:
Positive control = Clindamycin
Negative control = Aquadest

DISCUSSION

In this study, the sample used was red betel plant. The plant parts used in this study were leaves that had been collected in February 2022. The red betel plants that have been collected are then determined at the Research Center for Biology-Indonesian Institute of Sciences, Cibinong, West Java. Determination of plants is done to ensure the correctness of the plants to be used, by matching the morphological characteristics of red betel leaf and green betel leaf to the literature. Based on the results of the determination that has been made, it can be seen that the red betel leaf plant is included in the type of *Piper crocatum Ruiz and Pav*. with the *Piperaceae* family.

Furthermore, after determining the plants, organoleptic tests were carried out on red betel leaf plants. The organoleptic test aims to see the physical appearance of red betel leaf and green betel leaf which includes smell, color, shape, and taste. Based on the results of organoleptic tests, it is known that red betel leaf has a purplish red color, while for smell, shape, and taste, it has a strong aromatic odor, flat leaf

shape resembling a heart with a tapered leaf tip, and has a bitter and spicy taste. These results are the same as research conducted by Nani and Tri (2016) that red betel leaves have red leaves with a purple underside, have an elliptical shape with a tapered top, and have a bitter taste.

In this research, the red betel leaf which has been determined and organoleptic test is then extracted using 70% ethanol solvent by maceration method. According to Gede et al. (2019) Ethanol solvent is a polar solvent so it is often used to identify bioactive compounds. Ethanol can dissolve compounds in plants because ethanol is able to degrade cell walls so that bioactive compounds are easier to get out of plant cells. Then from the results of the maceration extraction, the results of the viscous extract were obtained, where the viscous extract was calculated to obtain the yield value of the extract. Extract yield is the ratio of the weight of the resulting extract to the weight of the sample simplicia. High extract yields indicate that the amount of juice that can be extracted or removed from the plant tissue is quite optimal. The yield of red betel leaf extract obtained in this study is higher when compared to the yield of red betel leaf extract obtained in Septiani's research (2017) using 70% ethanol solvent, which is only 10.10% with the maceration method.

The next research data obtained are the results of phytochemical screening. Phytochemical screening is a test carried out to determine the content of the secondary metabolite compounds contained in plants. Based on the results obtained in this phytochemical screening, red betel leaf positive contains alkaloids, flavonoids, tannins, saponins and essential oils. The results of this phytochemical screening are the same as those of the study conducted by Vianey et al. (2022), which in his research compared the antioxidant activity of red betel leaf using a maceration extraction process with 70% ethanol solvent, both of which were positive for alkaloids, flavonoids, tannins, and saponins, while the essential oil content was in accordance with Ayu's research (2018) where in his research it was stated that the positive red betel leaf contains essential oil and qualitative analysis was carried out.

Based on the results table, it is known that the ethanolic extract of red betel leaf has antibacterial activity against the growth of P. acnes which is indicated by the formation of an inhibition zone around the disc. From the results of the data that has been obtained based on the measurement of the diameter of the inhibition zone of the red betel leaf ethanol extract at concentrations of 70%, 80%, 90%, and 100%, which is 16 mm; 18mm; 18.5mm; and 21 mm. From the measurement results, it can be seen that the red betel leaf ethanol extract test with a concentration of 70%, 80%, 90% showed intermediate results, while the red betel leaf ethanol extract with a concentration of 100% showed sensitive results. Based on the results, red betel leaf extract was proven to inhibit the growth of *P. acnes* bacteria. The results of this study are in accordance with the research conducted by Wahyuningsih & Sumaryono (2021) which also proved that the ethanolic extract of red betel leaf can inhibit the growth of *P. acnes* bacteria.

As for the positive control, the antibiotic clindamycin was used, with an average inhibition zone of 26 mm in the sensitive category. Clindamycin has a greater lipophilic effect because it has the element of chlorine it has, its penetration into bacterial cells is better than lincomycin. It is known that clindamycin is effective against gram-positive anaerobic bacteria, one of which *is P. acnes*. It has an antibacterial effect mechanism by binding to the 50S ribosomal subunit of bacteria and inhibiting bacterial protein synthesis due to its activity against S. aureus, streptococci, and anaerobes (Akramullah, 2021). The results of the positive control of clindamycin in this study were higher than those conducted by Mayefis et al. (2020) where the positive control results of clindamycin on P. acnes showed an inhibition zone diameter of 9.03 mm, while the negative control used aquadest, where the results did not show an inhibition zone. This indicates that the control used has no effect on the antibacterial test.

Determination of the antibacterial activity of red betel leaf extract was carried out by the agar diffusion method. The principle of this method is to use solid media and paper discs, then the inhibition of bacterial growth is determined by measuring the diameter of the zone of inhibition of bacterial growth. The inhibition zone for bacterial growth is a clear area around the paper disc (Susi, 2018).

This diffusion bioactivity test was started by rubbing the bacterial suspension on the surface of the *Mueller Hinton agar* media, then attaching a paper disk containing red betel extract with varying concentrations, a positive control paper disk for the antibiotic clindamycin, and a paper disk containing a negative control of distilled water. Then incubated at 37°C for 24 hours. After 24 hours, the inhibition zone was measured using the Kirby-bauer method, namely measuring the inhibition zone around the paper disk vertically and horizontally using a caliper or ruler with units of millimeters (mm). Then record the diameter and compare

the measurement results according to the 2015 CLSI (*Clinical Laboratory Standards Institute*) table to determine the bacteria in the category of resistant, intermediate, or sensitive. A bacterium is said to be sensitive to antibiotics if the bacteria can be inhibited properly which is characterized by the formation of a clear zone around the disc during the test, the intermediate category if the bacteria can be inhibited but with weaker inhibition, and the resistant category if the bacteria can be inhibited but show inhibition. very weak or no inhibition is formed at all (Susi, 2018).

The limitation of this research is the possibility of confounding variables such as contamination from plant raw materials and bioactivity tests. Because plant raw materials are natural products, plants can be degraded by various microorganisms, both bacteria and fungi. Contaminants of plant raw materials can come from soil, post-harvest processing, transportation, and storage. The effect that can arise due to the presence of microorganisms in plant raw materials is a decrease in active compounds, therefore prevention can be done by drying plants properly which can reduce microorganism contamination in raw materials, where the condition for growing microorganisms in a material is the presence of water (Ningsih, 2014).

Furthermore, the main causes of contamination include unhygienic procedures carried out and airborne microorganisms during the test process. Prevention that can be done to reduce the occurrence of contaminants is to store test materials to be used according to a predetermined temperature, maintain body and environmental hygiene, and carry out aseptic procedures (John *et al*., 2019).

CONCLUSION

The average diameter of the inhibition zone of red betel leaf at concentrations of 70%, 80, 90% indicates the intermediate category, and at a concentration of 100% indicates the sensitive category. So it can be concluded that the sensitive category of red betel leaf extract was obtained at the highest concentration of 100%.

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Article Error You may need to use an article before this word.

Article Error You may need to use an article before this word. Consider using the article **the**.

Article Error You may need to remove this article.

P/V You have used the passive voice in this sentence. Depending upon what you wish to emphasize in the sentence, you may want to revise it using the active voice.

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Frag. This sentence may be a fragment or may have incorrect punctuation. Proofread the sentence to be sure that it has correct punctuation and that it has an independent clause with a complete subject and predicate.

Sentence Cap. Remember to capitalize the first word of each sentence.

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S/V This subject and verb may not agree. Proofread the sentence to make sure the subject agrees with the verb.

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Wrong Form You may have used the wrong form of this word.

Article Error You may need to remove this article.

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Prep. You may be using the wrong preposition.

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Missing "," You may need to place a comma after this word.

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