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Antibacterial Activity Test of Green Betel Leaf Extract Clay Mask (*Piper Betle* L) Against Acne-Causing Bacteria (*Propionibacterium Acnes*)

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ABSTRACT
Propionibacterium acnes is one of the bacteria that causes acne. Green betel leaf (*Piper betle* L.) is a plant that has been used for generations. Green betel leaf extract contains flavonoids, tannins, saponins, alkaloids, and phenols that have antibacterial potential. *Clay masks* are solid mask preparations formulated with clay minerals as the base ingredient. This study aims to determine the characteristics of an anti-acne *clay mask* made from green betel leaf extract and its antibacterial activity against *Propionibacterium acnes*. Green betel leaves were extracted through maceration method using 96% ethanol solvent then made 4 *clay mask preparations* which were tested for organoleptic, pH, homogeneity, spreadability, adhesive power and drying time and antibacterial test was carried out using well method, inhibition zone data were tested using *One-Way ANOVA* and Duncan test to determine whether there were significant differences between each formula. The results of this study aimed to determine the physical quality and antibacterial activity of green betel leaf extract *clay mask preparations against Propionibacterium acnes* . The *clay mask formula* used consisted of F0 (without extract), F1, F2, and F3 with concentration variations of 2.5%, 5% and 7.5% extract. Evaluation of physical quality included organoleptic, homogeneity, pH, spreadability, adhesive power, and drying time tests. The results of the physical preparation test of the green betel leaf ethanol extract *clay mask* showed a soft, homogeneous shape, green color, betel leaf aroma, Ph around 5-6, spreadability of 5.23-6.73 cm, adhesiveness of 15.5-24.7 seconds and drying time of 12.7-15 minutes. The antibacterial activity test showed that F3 produced the largest inhibition zone of 3 2.9 mm. Statistical analysis using One-Way ANOVA showed that variations in extract concentration had a significant effect on antibacterial activity. Thus, the green betel leaf extract *clay mask* , especially the F3 formula, has good physical quality and the highest effectiveness as an anti-acne preparation.

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INTRODUCTION

Acne is *a* common skin problem in adolescents and young adults. This condition is often found in adolescent girls aged 14–17 years with a prevalence of 83–85% and in adolescent boys

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aged 16–17 years reaching 95–100% (Zahrah *et al* ., 2018). *Acne vulgaris* remains the most common skin disorder, especially in the adolescent age group. Almost everyone has experienced this condition so it can be categorized as a skin disease. *Acne vulgaris* is skin inflammation due to overproduction of sebaceous glands that cause lesions such as pustules or cysts, generally appearing in areas with many oil glands such as the face and chest. Acne usually begins to appear during puberty until young adulthood (Setiyadi & Qonitah, 2020) . In addition to being influenced by excess sebum production, the emergence of acne is also closely related to the activity of the bacteria *Propionibacterium acnes* which triggers inflammation of the skin .

Propionibacterium acnes is a normal bacterial flora that dominates skin areas with numerous sebaceous glands, with populations reaching 20–70%. The growth of this bacteria is influenced by the amount of lipids, skin pH, sweat production, and sebum secretion. This bacteria produces proteins that can damage skin tissue, causing inflammation (Hikmah & Hasanah, 2023) . *Propionibacterium acnes* acts as an inflammatory trigger and is found in higher amounts on the skin of adolescents with acne than those without. This gram-positive bacterium is an important factor in the pathogenesis of acne (Putri & Muflihah, 2024) . With the presence of *Propionibacterium acnes* , the bacteria that causes inflammation in acne, requires an antibacterial agent that can suppress its growth. One natural ingredient with potential as an anti-acne agent is green betel leaf **.**

Green betel leaf (*Piper betle* L.) is a traditional medicinal plant used for generations, especially the leaves. Research shows that betel leaves contain flavonoids, tannins, saponins, alkaloids, and phenols (Futri et al., 2023). These compounds provide benefits as antibacterials, anti-inflammatory, antioxidants, antiseptics, and anti-inflammatory agents, and can stop bleeding (Hermanto *et al* ., 2023) . The antibacterial activity of betel leaf extract against *Propionibacterium acnes* is higher than that of tea leaf and breadfruit leaf extracts at the same concentration. Natural ingredients with antibacterial activity can be applied topically in the form of facial masks that can provide healing effects, refresh the skin, and provide short- and long-term protection. One type of mask that is in demand is *the clay mask* , which can absorb excess oil, soften the skin, and prevent acne. The addition of effective natural antibacterial ingredients is needed to help inhibit the growth of acne-causing bacteria such as *Propionibacterium acnes.* (Lucita & Tensiska, 2021) .

Clay mask is a solid mask preparation made from clay minerals such as bentonite and kaolin to cleanse, soften and brighten the skin (Maharani et al., 2024). Physical qualities include organoleptic, homogeneity, pH, spreadability, adhesiveness, and drying time. This study aims to design a formula and evaluate *a clay mask* containing green betel leaf extract as an active ingredient in acne treatment. It is hoped that this research can contribute to the development of natural-based skin care formulations and become a new alternative in acne prevention through the utilization of the potential of green betel leaves in the form of *a clay mask.* (Yovitasari et al., 2025).

Research on the use of green betel leaf extract (*Piper betle* L) as an antibacterial agent has been carried out in various dosage forms such as solutions, gel and creams (Julianti et al., 2023). Betel leaf extract contains active compounds such as flavonoids, tannins, phenols, and

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essential oils, which are known to inhibit the growth of Propionibacterium acnes, the primary bacteria that causes acne (Hidayati *et al*., 2020). However, research on the use of green betel leaf extract in clay mask preparations as an antibacterial is still very limited. *Clay masks* offer advantages over other topical formulations because they absorb excess sebum, cleanse pores, softens and brightens the skin and increase the contact time of active ingredients with the skin, thus increasing antibacterial effectiveness (Maharani *et al*., 2024). Furthermore, clay masks provide a cooling sensation and provide comfort during use, making them a preferred choice for acne-prone skin care products (Pamungkas *et al*., 2024).

METHOD

Tools and materials

The tools used are mortar and stamfer, stirring rod (*Pyrex* ®) , digital scales (Ohaus pioneer *Pyrex* ®), petri dish , loop needle, magnetic stirrer, autoclave, oven, refrigerator, 20/60 mesh sieve, blender, spatula, measuring cup (*Pyrex* ®), glass beaker (Pyrex ®) , test tube (*Pyrex* ®) filter paper, dropper pipette, parchment paper , porcelain cup, pH paper , glass object, water bath, *laminar air flow* (LAF), adhesive power tester, spread power tester, viscometer, handscoon, mask, spiritus, tripod, crucible, desiccator , tweezers, micropipette, caliper , test tube rack, well punching tool. The materials used are green betel leaves, 96% ethanol, kaolin, bentonite, xanthan gum, glycerin, oleum rose, aquadest, FeCl3, meyer reagent, dragendoof.

Production of Green Betel Leaf Ethanol Extract

The green betel leaves used are fresh leaves characterized by green leaves, old leaves are chosen because they produce high antibacterial activity. The green betel leaves are then washed with running water to remove any dirt attached. Then they are dried by oven at 40°C. After that, the sample is ground using a blender and sieved to become a 40 mesh size powder and weighed as the weight of the simplicia. 1 kg of green betel leaf simplicia is then macerated with 96% ethanol solvent for 3x24 hours in a closed container and protected from light that has been ground and stored in a clean container protected from light. The results of all macerates are filtered using a Buchner funnel and evaporated with *a vacuum rotary evaporator* at 50 °C until a constant weight of the thick extract is obtained.

Yield Calculation

The calculation of extract yield is done by weighing the obtained extract against the weight of the dry powder before the extraction process. The calculation formula is as follows:

$$\text{Rendemen} = \frac{\text{Berat hasil ekstraksi}}{\text{Berat awal simplisia}} \text{ x } 100\%$$

After obtaining the yield extract, standardization of the extract is carried out consisting of two parameters, namely specific organoleptic parameters, screening (phytochemical identity) and non-specific parameters (determination of drying loss).

Phytochemical Screening

a. Identification of flavonoid compounds
   Extract 0.5 grams of green betel leaf extract and drip it with concentrated Mg and HCl . The results are indicated by the appearance of orange to red colors (Putri et al., 2023).

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b. Identification of tannin compounds
0.5 grams of extract was added to hot aguadest, filtered and $FeCl_{3\ was\ added}$. The result was marked by a color change to brownish green or blackish blue indicating the presence of tannins (Sukbakti et al., 2025).

c. Identification of saponin compounds
A 0.5 gram extract was added to 10 mL of hot distilled water, then shaken for 30 seconds. The result was indicated by the presence of foam in the shaken solution (Afifah et al., 2020).

d. Identification of alkaloid compounds
The extract was first treated with 2N HCl, then divided into two test tubes. Afterward, Mayer's reagent formed a white precipitate, and Dragendorff's reagent produced an orange precipitate. These results positively indicated the presence of alkaloids. (Sukbakti et al., 2025).

e. Identification of phenol compounds
0.5 grams of extract was dissolved in 96% ethanol and added with iron (lll) chloride solution. If a greenish black color appears, it indicates the presence of polyphenol compounds (Maharani et al., 2024).

Drying Loss

The drying shrinkage test was carried out by weighing 2 g of the simplicia carefully into a shallow, capped weighing bottle that had previously been heated to a certain temperature and tared. The simplicia powder used was then leveled by shaking the bottle to form a layer with a thickness of ± 5–10 mm. Next, the weighing bottle was inserted into the drying chamber with the lid open and dried at a predetermined temperature until a constant weight was obtained. Before each subsequent drying stage, the weighing bottle was tightly closed and cooled in a desiccator until it reached room temperature (Depkes RI, 2017).

Making *Clay Mask* from Green Betel Leaf Ethanol Extract

clay mask is made by weighing all the ingredients according to the formula. Bentonite is dissolved in hot water and allowed to expand, then mixed with xanthan gum to form a gel base. Next, glycerin is added as a moisturizer and green betel leaf extract as an active ingredient with antibacterial, antioxidant, and anti-inflammatory properties. Kaolin is added little by little until evenly mixed, followed by oleum rosae for fragrance. The mixture is then ground until homogeneous to obtain a smooth *clay mask preparation* that is ready to use (Sari *et al*., 2024)

Table 1. *Clay Mask* Formula Green Betel Leaf Ethanol Extract

Material	F0	F1	F2	F3	Function	Material
Green Betel Extract	-	5%	7.5%	10%	Main Active Ingredients	Green Betel Extract
Kaolin	25%	25%	25%	25%	*Clay mask* base	Kaolin
Bentonite	1.5 %	1.5 %	1.5 %	1.5 %	*Clay mask* base	Bentonite
Glycerin	8%	8%	8%	8%	Moisturizer	Glycerin
Xanthan Gum	0.5 %	0.5 %	0.5 %	0.5 %	Thickener	Xanthan Gum
Oleum rosae	1 %	1 %	1 %	1 %	Fragrance	Oleum rosae

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Material	F0	F1	F2	F3	Function	Material
Aquadest	ad 100	ad 100	ad 100	ad 100	Solvent	Aquadest

Physical Preparation Test of *Clay Mask* Ethanol Extract of Green Betel Leaves

a. Organoleptic test
Testing of green betel leaf extract *clay mask preparations* , organoleptic tests included odor, color, texture and form of the preparation (Ningsih *et al* ., 2023) .

b. pH test
The pH test was performed using universal indicator pH paper. The pH paper was dipped into the preparation and left for approximately 1 minute. The color change that occurred on the pH paper was observed and compared with the universal pH indicator standard, which indicates the pH value of *the clay mask preparation.* (Julianti *et al* ., 2023) .

c. Homogeneity test
The test was conducted by spreading the green betel leaf *clay mask* on a transparent glass. The preparation was considered homogeneous, indicated by the even distribution of *the clay mask color* and the absence of any clumps (Ningsih *et al* ., 2023) .

d. Spreadability test
The clay mask preparation was weighed at 500 mg, then placed between two transparent glass plates with a block of millimeter paper underneath, and waited for 60 seconds. The measurement of the distribution diameter was repeated for each 150-gram load. The distribution diameter was measured using a vernier caliper. (Ningsih *et al* ., 2023)

e. Adhesion test
The preparation was weighed as much as 0.25 grams and then placed on *a glass object* . A 50 gram load was added and left for 1 minute. The load was released until *the glass object* separated. The release time from *the glass object* was observed and recorded . (Hidayati *et al* ., 2020) .

f. Dry time test
The preparation was weighed as much as 0.5 grams of mask, then applied to a glass plate with an area of 5.0 x 2.5 cm and a thickness of 1 mm to form a thin, even layer. The observation time started from the mask was applied until the layer was completely dry using a stopwatch (Zainal *et al* ., 2023) .

Antibacterial Activity Test

Antibacterial activity testing was carried out by first sterilizing all equipment wrapped in brown paper using an autoclave at 121°C for 20 minutes, and all procedures were carried out aseptically to prevent contamination (Rosyadi et al., 2022). One loop of pure *Propionibacterium acnes culture* was inoculated into 10 mL of *Nutrient Broth* (NB) media with turbidity adjusted to the *McFarland standard* of 0.5 (≈1×10^8 CFU/mL) and then incubated for 24 hours. The melted *Nutrient Agar* (NA) was poured into a petri dish as a base layer and allowed to solidify; then 1 mL of bacterial suspension was mixed onto the NA surface until homogeneous and solidified again. Six wells with a diameter of 4 mm were made on the surface of

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the media to administer the treatment in the form of a *clay mask preparation* containing ethanol extract of green betel leaves at concentrations of 2.5%, 5%, and 7.5%, as well as positive and negative controls. All plates were treated and then incubated at 37°C for 24 hours and the inhibition zone was observed to assess the antibacterial activity of the *clay mask preparation* (Indarto et al., 2019) .

Observation and measurement of the diameter of the inhibition zone, namely the clear area around the well hole, using a vernier caliper. Statistical analysis using Analysis of Variance (ANOVA) with the SPSS program. Tests were carried out Normality using Shapiro Wilk is because the sample used in the research is less than 50. Normality is met if the test results are significant with a significant level (p>0.05). The homogeneity test is carried out to ensure equality of variance in several populations. Homogeneity is met if the significant value (p>0.05) is equal to or greater (≥) than 0.05, then the variance of two or more groups of measured data is homogeneous. One Way Test (Annova) is carried out if the data has been proven to be normally distributed and the hypothesis in this study is more than 2 groups and is not paired, with a 95% confidence level or p<0.05 followed by the Duncan test. The alternative test is Kruskal Wallis.

RESULTS AND DISCUSSION

The plants used for testing in this study were obtained from Jl. Tilaman, Imogiri District, Bantul Regency, Yogyakarta Special Region . Before being used, the plants had undergone an identification process at the Plant Systematics Laboratory, Faculty of Biology, Gajah Mada University. Number: 01014/S.Tb./XI/2025. This identification process aims to ensure the validity of the identity of the plants studied, avoid errors in sampling, and prevent possible contamination with other species (Wahyukurnia *et al* ., 2023) .

Table 2. Results of calculating the yield of green betel leaf simples

Sample weight (grams)	Extract weight (grams)	Yield (%)
500	46.2326	9.24

green betel leaf extract can be seen in Table 3. A thick extract yield of 46.2326 grams was obtained, while the yield of the thick extract obtained was 9.24 %. Yield is the ratio between the amount of extract obtained and the amount of extracted simplicia . This value indicates an efficient extraction process because the yield is within the achievable range (Manarisip *et al* ., 2020) .

Table 4. Phytochemical Screening Results

No	Compound name	Literature	Observation result	Information
1	Flavonoids	Red or Orange	Red	+
2	Tannin	Brownish green or blackish blue	Blackish blue	+
3	Saponin	Stable foam	Stable foam	+
4	Alkaloid	White sediment	No white sediment	–
5	Phenol	Blackish green	Blackish green	+

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green betel leaf extract . The results obtained for identity and organoleptic can be presented in table 4. The ethanol extract of green betel leaves was screened for phytochemistry with results as in table 5. From the results obtained that the ethanol extract of green betel leaves in flavonoid, tannin, saponin and phenol compounds is positive while alkaloids are negative.

Table 5. Drying Shrinkage Results

Parameter	Results
Drying shrinkage	10.11%±2.14

The results of the drying loss showed that the drying loss in green betel leaf powder was 10.11 %. The requirement for drying loss in green betel leaf powder to be <11% is then the results of the drying shrinkage test declared to meet the requirements. Water content of more than 1 % can affect the stability and purity of the powder, and can cause microbial growth. (Manarisip *et al* ., 2020) .

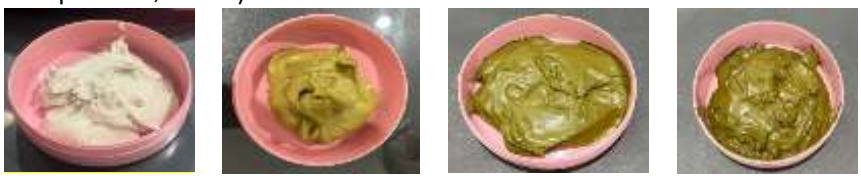

Figure 1. Results of Preparation

Table 6. Organoleptic Test Results

Preparation	Form	Color	Aroma
F0	Gentle	Milky white	Typical
F1	Gentle	Light green	Typical
F2	Gentle	Dark green	Typical
F3	Gentle	Brownish green	Typical

The preparation was made according to the procedure and a physical evaluation test *of the clay mask was carried out* . The organoleptic test aims to ensure that *the clay mask* has been made according to the specified parameters. This test involves evaluating the physical characteristics of the preparation, such as color, texture or shape and aroma of the preparation (table 7) F0, F1, F2 and F3 produced the same form, namely a soft texture and had a distinctive aroma. While the color produced in F1 was milky white, F2 was light green, F3 was dark green and for F4 was brownish green. Based on the test results that have been obtained, it shows that the more extract added, the more intense the color of the resulting *clay mask* . This indicates that the concentration of green betel leaf ethanol extract affects the results obtained.

Table 7. pH Test Results

Replication	F0	F1	F2	F3
1	6	5	5	5
2	6	5	5	5
3	6	5	5	5
Average	6	5	5	5

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The pH evaluation was conducted to determine the pH of *the clay mask formulation* . *The clay mask* preparation should have a skin pH of 4.5-6.5 (Setiawan *et al. al* ., 2023) . From the results obtained, it shows that F0 produces a pH of 6 and for formulas F1, F2 and F3 produces a pH of 5, and meets the test requirements at pH, this preparation is very important because if the preparation is too acidic it can cause skin irritation and cause temporary stinging, preparations that are too alkaline have the potential to make the skin dry and cause itching (Tatambihe *et al* ., 2025) .

Table 8. Results of the Homogeneity Test

Replication	F0	F1	F2	F3
1	Homogeneous	Homogeneous	Homogeneous	Homogeneous
2	Homogeneous	Homogeneous	Homogeneous	Homogeneous
3	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Average	Homogeneous	Homogeneous	Homogeneous	Homogeneous

The homogeneity test was conducted on all formulations, with the aim of determining the homogeneity level of the *clay mask* . This test was conducted by observing the particles present in the formulation. Homogeneity affects the distribution of the active ingredient in the green betel leaf in *the clay mask* . The results obtained from the four formulations showed that the formulation did not show any coarse grains when applied to a transparent glass object. The results obtained indicated that the green betel leaf extract *clay mask* had good homogeneity. Differences in extract concentrations did not affect the homogeneity of the formulation. This good homogeneity indicates that the green betel leaf extract was optimally dispersed in the formulation base. In addition, differences in extract concentrations in each formulation did not affect the level of homogeneity, so all formulations met the criteria for good homogeneity. (Asthyananda & Bakri, 2024) .

Table 9. Results of Spreadability Test

	Spread diameter (cm)			
Replication	F0	F1	F2	F3
1	6.6	6.2	5.6	5.2
2	6.7	6.1	5.63	5.3
3	6.9	6.2	5.7	5.2
Average	6.73	6.16	5.64	5.23

The spreadability test aims to measure the ability of *the clay mask* to spread on the surface of the skin. The higher the spreadability test value, the wider the contact area of the active substance with the skin. The results of the spreadability test are as shown in Table 10. Based on the results of the spreadability test, the average spreadability diameter is as follows: F0 of 6.73 cm, F1 of 6.16 cm, F2 of 5.64 cm and F3 of 5.23. From this data, it shows that F0, F1, F2, F3 have a spreadability that is still within the ideal range (5-7 cm), thus meeting the requirements for comfortable application on the skin. F0 and F1 are close to the ideal range, this indicates the texture or shape *of the clay mask.* which is lighter and easier to spread . Overall, all formulas still meet the spreadability standards for good topical preparations. (Ningsih *et al* ., 2023) .

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Table 10. Results of adhesion test

Adhesion time (seconds)				
Replication	F0	F1	F2	F3
1	24	21	19	15
2	24.6	21.6	20	15.6
3	25.5	22	20.1	16
Average	24.7	21.5	19.7	15.5

Based on the results, it shows that all *clay mask formulas* have met the requirements for good adhesion for topical preparations, which is more than 4 seconds (Apriyanti *et al* ., 2022) . This shows that increasing the concentration of green betel leaf extract in *Clay masks* tend to have lower adhesive power. This means that a higher concentration of extract makes *Clay masks* are more easily removed from the skin, which may reduce the long-term effectiveness of the formula . F0 , F1, F2 and F3 have optimal adhesion for topical preparations .

Table 11. Drying Time Test Results

Adhesion time (minutes)				
Replication	F0	F1	F2	F3
1	15	15.20	14.45	12.33
2	15	15.26	14.27	12.21
3	15	15.17	14.16	12.17
Average	15	14.26	13.91	13.83

From the results of the speed time test The drying results obtained on clay masks with 4 variations of formulas and bases provided good and stable drying times during storage with an average F1 of 14.26, F2 of 13.91, F3 of 12.83, and F0 of 15 minutes. The specifications for a good drying time for clay masks are in the range of 10-20 minutes, as can be seen in Table 12 (Syamsidi *et al* ., 2021) .

Table 12. Antibacterial Activity Results of *Clay Mask Preparations Made* from Ethanol Extract of Green Betel Leaves (*Piper belte* L.)

Test treatment	Inhibition zone diameter (mm)			Mean ± SD
	Replication 1	Replication 2	Replication 3	
F1	22.3	25.8	27.52	25.20 ± 2.17
F2	30.69	32.32	32.12	31.71 ± 0.72
F3	32.42	33.04	33.24	32.9 ± 0.34
F0	33.34	34.74	37.14	35.07 ± 1.56
Control (-)	0	0	0	0

The results of the antibacterial activity test of the green betel leaf ethanol extract clay mask showed the formation of an inhibition zone against *Propionibacterium acnes* . Formulas F1, F2, and F3 produced inhibition zone diameters of 25.20 ± 2.17 mm; 31.71 ± 0.72 mm; and 32.90 ± 0.34 mm, respectively. The positive control (clindamycin) showed the highest inhibition of 35.07 ± 1.56 mm, while the negative control did not produce an inhibition zone. Based on the category of Sakramentia *et al*. (2019), all test formulas were classified as having

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strong antibacterial activity (>20 mm). The absence of inhibition in the negative control confirmed that the antibacterial effect came from the betel leaf extract in the preparation. In addition, the higher the extract concentration, the larger the inhibition zone formed (Dewi *et al*., 2019).

Table 13. Statistical Test Results

Treatment	P Shapiro Wilk	P Levene Test	P One Way Annova
F1	0.712		
F2	0.307		
F3	0.173	0.188 > 0.05	0.234 > 0.05
F0	0.355		
K (-)	0		

The effectiveness test of the green betel leaf ethanol extract *clay mask preparation* was conducted to determine the extent to which the potential or concentration of a compound is able to provide an effect on microorganisms. The antibacterial effectiveness test of the green betel leaf ethanol extract *clay mask* with varying extract concentrations of 2.5%, 5%, and 7.5% showed that the green betel leaf ethanol extract was able to inhibit the growth of *Propionibacterium acnes bacteria* because there was a clear area around the well. The higher the concentration of the green betel leaf ethanol extract , the wider the inhibition zone formed. This indicates that a higher concentration of the green betel leaf ethanol extract has a greater effect in inhibiting the growth of *Propionibacterium acnes bacteria* which can be seen by the increasing width of the inhibition zone.

Figure 2. Antibacterial Test Results F0

Figure 3. Antibacterial test results F1, F2, F3

(Figure 3). The resulting inhibition zone diameter was then followed by a statistical analysis test using SPSS (Table 13). The statistical data of the inhibition zone of the green betel leaf ethanol extract *clay mask* were processed. A normality test was conducted to ensure that the data had a normal distribution. A normal distribution is considered fulfilled if p>0.05. In this study, the results of the normality significance test (p>0.05) indicated that the data were normally distributed. Furthermore, a homogeneity test was conducted to ensure that one or more samples had uniform variance. The homogeneity test using the Levene Test produced

a significance value of 0.062 which met the requirements of p>0.05. After the data was confirmed normal and homogeneous, the analysis was continued with the *One-Way ANOVA* test . The results of the *One-Way ANOVA test* showed a significance value of 0.234 (p>0.05), so it can be concluded that there was no significant difference between the groups. (Hasanah, 2022) . Therefore, further tests (*post hoc*) such as Duncan's are not necessary . The results of the study showed a clear difference between the positive control and the three test formulas (F1, F2, F3) using green betel leaf extract as the active ingredient. The positive control using clindamycin powder produced the largest inhibition zone of 35.07 mm, making it the main standard of comparison in the antibacterial effectiveness test. Meanwhile, the formula with green betel leaf extract showed lower inhibition than the positive control, but still provided strong antibacterial activity. Formula III (7.5%) produced an inhibition zone of 32.09 mm, which is close to the effectiveness of clindamycin, while formula II (5%) provided an inhibition zone of 31.71 mm. Formula I (2.5%) showed the lowest inhibition, namely 25.20 mm. This difference in results is caused by differences in the main variables, namely the type and concentration of antibacterial ingredients used.

CONCLUSION

Clay mask preparation formulation Ethanol extract of green betel leaves (*Piper betle L*) meets the characteristics A suitable *clay mask* with potential as an anti-acne agent. *Propionibacterium acnes* at a concentration of 7.5 % with an average diameter of 36.91 mm is considered strong. The higher the concentration, the larger the inhibition zone. In this study, the *clay mask* formulation of green betel leaf ethanol extract has good physical characteristics and shows potential as an antibacterial agent against an *Propionibacterium acnes*. However, this study still has limitations, such as the scope of the test which only focused on laboratory testing without direct evaluation on users or skin safety testing. Therefore, further research is needed that includes irritation testing, long-term stability, and in vivo effectiveness testing to support the use of green betel leaf extract clay masks as acne skin care products in the future.

REFERENCE

Afifah, R., Utomo, D. H., & Laily, A. N. (2020). Skrining Fitokimia Familia Piperaceae. *Jurnal Biologi Dan Pembelajarannya (JB&P)*, *7*(1), 28–32. https://doi.org/10.29407/jbp.v7i1.14805

Apriyanti, R., Rahmat, N., Melia, I., & Cania, L. (2022). Formulasi dan Uji Sifat Fisik Masker Pasta Clay yang Mengandung Jojoba Oil (Simmondsia chinensis) Untuk Kulit Wajah. *Jurnal Pelita Sains Kesehatan*, *2*(1), 1–10.

Asthyananda, M., & Bakri, F. F. D. (2024). Formulasi Clay Mask Daun Binahong (Anredera cordifolia (Ten.) Steenis) Dan Uji Inhibisi Staphylococcus aureus. *Kartika : Jurnal Ilmiah Farmasi*, *9*(2), 105–114. https://doi.org/10.26874/kjif.v9i2.680

Depkes RI. (2017). Farmakope Herbal Indonesia Edisi II 2017. *Departemen Kesehatan Republik Indonesia*, 1–561. https://doi.org/10.1201/b12934-13

Futri, C. L., Siregar, I., Yanti, S., & Natunnah, S. (2023). Formulasi Ekstrak Daun Sirih Hijau

(Piper Betle L) Sebagai Anti Jerawat. *Jurnal Kesehatan Ilmiah Indonesia (Indonesian Health Scientific Journal)*, *8*(2), 175–185. https://doi.org/10.51933/health.v8i2.1239

Hasanah, R. (2022). *Uji Efektifitas Daya Hambat Ekstrak Umbi Bawang Dayak (Eleutherine palmifolia) Terhadap Bakteri Staphylococcus aureus*.

Hermanto, L. O., Nibenia, J., Sharon, K., & Rosa, D. (2023). Review artikel: Pemanfaatan tanaman sirih (Piper betle L) sebagai obat tradisional. *Pharmaceutical Science Journal*, *3*(1), 33–42. http://openjournal.wdh.ac.id/index.php/Phrase/index%0APHRASE

Hidayati, N., Styawan, A. A., & Muslimah, I. N. (2020). Uji Stabilitas Formula Optimum Masker Gel Peel Off Ekstrak Buah Mahkota Dewa (Phaleria macrocarpa (Scheff.) Boerl). *Jurnal Ilmu Farmasi*, *11*(2), 32–39. https://doi.org/10.61902/cerata.v11i2.145

Hikmah, F., & Hasanah, N. (2023). Uji Hambat Aktivitas Bakteri Propionibacterium acnes Terhadap Ekstrak Etanol Rimpang Lengkuas Merah (Alpinia purpurata (K.) Schum). *E-Jurnal Medika Udayana*, *12*(1), 74–78. https://doi.org/10.24843/mu.2023.v12.i01.p13

Indarto, Narulita, W., Anggoro, B. S., & Novitasari, A. (2019). Aktivitas antibakteri ekstrak daun binahong terhadap propionibacterium acnes. *Jurnal Tardis Biologi*, *10*(1), 67–78. http://ejournal.radenintan.ac.id/index.php/biosfer/index

Julianti, P. A., Hutahaen, T. A., & Februyani, N. (2023). Formulasi Sediaan Gel Antiacne Ekstrak Daun Sirih. *Indonesian Journal of Health Science*, *3*(2), 308–319.

Lucita, A., & Tensiska. (2021). Aktivitas Antibakteri Ekstrak Daun Teh, Daun sukun, dan Daun Sirih Terhadap Propionbacterium acne s serta aplikasinya Pada Masker Antijerawat Antibacterial Activity of Tea, Breadfruit, and Piper Betle Leaves Extracts Against Propionibacterium acne Staphyl. *Penelitian Pascapanen Pertanian*, *18*(2), 105–112.

Maharani, T. A., Norhabibah, Ira Wati, N. R., Putri, L. R., & Malahayati, S. (2024). Pengembangan Formulasi Clay Mask Stick Ekstrak Rumput Gandum (Triticum Aestivum L) Komoditas Lokal yang Berpotensi Sebagai Antioksidan. *Jurnal Surya Medika*, *10*(2), 6–14. https://doi.org/10.33084/jsm.v10i2.7718

Manarisip, G. E., Fatmawali, & Rotinsulu, H. (2020). *Standarisasi Ekstrak Daun Sirih Hijau (Piper betle L) dan Uji Antibakteri Terhadap Bakteri Pseudomonas Aeruginosa*. *9*(4), 533–541.

Ningsih, W. P., Widiastuti, R., & Eltivitasari, A. (2023). Formulasi dan Uji Karakteristik Fisik Sediaan Masker Clay Serbuk Biji Kopi Robusta (Coffea robusta. *Sinteza*, *3*(1), 1–8. https://doi.org/10.29408/sinteza.v3i1.7427

Pamungkas, A. S., Fauziah, & Samodra, G. (2024). Evaluasi Sifat Fisik Masker Clay Ekstrak Etanol Kulit Pisang Ambon (Musa paradisiaca (L.) Kunt. var. sapientum) Dengan Kaolin Dan Bentonit Sebagai Basis Masker. *Pharmacy Genius*, *03*(03), 149–160.

Putri, A. T., Suproborini, A., & Kusumawati, D. (2023). Kandungan metabolit sekunder ekstrak etanol daun sirih hijau (Piper betle L.). *Seminar Nasional Prodi Farmasi*, 226–229.

Putri, & Muflihah, C. H. (2024). Uji Aktivitas Antibakteri Penyebab Jerawat Propionibacterium acnes Dan Staphylococcus aureus Menggunakan Ekstrak Dan Fraksi Jintan Hitam (Nigella sativa) Serta Bioautografinya. *Usadha Journal of Pharmacy*, *3*(3), 272–287. https://doi.org/10.23917/ujp.v3i3.404

Setiawan, R., Masrijal, C. D. P., Hermansyah, O., Rahmawati, S., Sari, R. I. P., & Cahyani, A. N.

(2023). Formulasi, Evaluasi Dan Uji Stabilitas Fisik Sediaan Gel Antioksidan Ekstrak Tali Putri (Cassytha filiformis L). *Bencoolen Journal of Pharmacy*, *3*(1), 27–33. https://doi.org/10.33369/bjp.v3i1.27649

Setiyadi, G., & Qonitah, A. (2020). Optimasi Masker Gel Peel-Off Ekstrak Etanolik Daun Sirih (Piper Betle L.) dengan Kombinasi Carbomer dan Polivinil Alkohol. *Pharmacon: Jurnal Farmasi Indonesia*, *17*(2), 174–183. https://doi.org/10.23917/pharmacon.v17i2.11976

Sukbakti, I. A. P., Wardani, T. S., & Fitriawati, A. (2025). Formulasi Sediaan Masker Gel Peel-Off Kombinasi Ekstrak Daun Sirih (Piper Betle L.) dan Madu (Mel Depuratum) Serta Uji Efektivitas Antibakteri Terhadap Bakteri Staphylococcus aureus ATCC 25923 dan Propionibacterium acnes ATCC 6919. *Jurnal Farmasindo: Jurnal Penelitian Dan Pengabdian Masyarakat*, *9*(1), 124–135. https://farmasindo.poltekindonusa.ac.id

Syamsidi, A., Syamsuddin, A. M., & Sulastri, E. (2021). Formulation and Antioxidant Activity of Clay Mask of Tomato (Solanum lycopersicum L .) Lycopene Extract with Variation of Concentration of Kaoline and Bentonite Bases). *Jurnal Farmasi Galenika*, *7*(1), 77–90. https://doi.org/10.22487/j24428744.2021.v7.i1.15462

Tatambihe, N. A., Yuhara, N. A., Rawar, E. A., Farmasi, F., Kristen, U., Yogyakarta, I., & Istimewa, D. (2025). *Jurnal Kesehatan Perintis*. *11*(2), 136–148. https://jurnal.upertis.ac.id/index.php/JKP%0AAktivitas

Wahyukurnia, P. T., Yuhara, N. A., & Atmaja, S. P. (2023). Aktivitas Antibakteri Fraksi N-Heksan, Etil Asetat, N-Butanol Daun Jambu Mete (Annacardium occidentale) terhadap Bakteri Penyebab Ulkus Diabetik (Staphylococcus aureus dan Escherichia coli). *Jurnal Kesehatan Perintis*, *10*(2), 114–123. https://jurnal.upertis.ac.id/index.php/JKP%0AAktivitas

Yovitasari, T. A., Rachmawati, P., & Notario, D. (2025). Formulasi Dan Evaluasi Sediaan Clay Mask Yang Mengandung Kopi Robusta (Coffea canephora). *Damianus Journal of Medicine*, *24*(1), 29–38.

Zainal, T. H., Ulfa, M., Nisa, M., & Pawarrangan, T. J. (2023). Formulasi Masker Clay Mask Kulit Buah Pisang Muli cuminata L.). *Jurnal Penelitian Farmasi Indonesia*, *12*(1), 7–12. https://doi.org/10.51887/jpfi.v12i1.1760