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POTENTIAL OF MORINGA LEAF EXTRACT GEL (*Moringa oleifera* Lam.) AS AN ANTIBACTERIAL AGAINST *STAPHYLOCOCCUS AUREUS* CAUSES OF ACNE

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ABSTRACT

Moringa leaves (Moringa oleifera Lam.) can be utilized by the community to heal wounds, beriberi, and fever; have anti-inflammatory and analgesic properties; and treat skin disorders caused by bacteria. One of the skin diseases is acne, which is caused by the bacterium Staphylococcus aureus. The aim of this study is to determine the antibacterial activity of a gel made from moringa leaf extract against Staphylococcus aureus and to identify the secondary metabolite compounds of the extract through phytochemical screening. This research is a laboratory experiment that involves creating moringa leaf extract gel at concentrations of 2%, 4%, 6%, and 8% to inhibit the growth of Staphylococcus aureus. Antibacterial testing was conducted using the well diffusion and disc diffusion methods and analyzed using one-way ANOVA. Based on the results of the phytochemical screening, the extract contains flavonoid, alkaloid, saponin, tannin, and terpenoid compounds. The results of the study showed that the antibacterial activity of the gel with the well method at concentrations of 2%,

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4%, 6% and 8% obtained an average inhibition zone of 7.68 mm; 8.8 mm; 9.18 mm; 9.55 mm. While with the well method, the average inhibition zone at concentrations of 2%, 4%, 6% and 8% was 8.55 mm; 9.03 mm; 9.17 mm; 9.88 mm. The ANOVA test analysis obtained a result of p = 0.000 smaller than α = 0.05 which means there is a significant difference in the average diameter of the inhibition zone between the treatment groups.

INTRODUCTION

Moringa leaves *(Moringa oleifera* Lam.) are one type of plant that can be utilized as vegetables and medicinal plants to cure various diseases. According to research, moringa leaves contain alkaloids, flavonoids, phenolics, triterpenoids/steroids, and tannins. (Agung *et al*., 2016). Traditionally, the community uses moringa leaves to heal wounds, beriberi, fever, muscle aches, and anemia, as well as for their anti-inflammatory and analgesic properties, and to treat skin disorders caused by microorganisms (BPOM, 2016). One common skin disease is acne, which is caused by the bacterium *Staphylococcus aureus*.

This bacterium is an aerobic bacterium that belongs to the gram-positive type and is one of the normal flora of humans on the skin and mucous membranes. *Staphylococcus* is a spherical bacterium that lives in pairs or forms short and long chains, depending on the species and growth conditions. These bacteria are homofermentative, and some species produce lactic acid rapidly under anaerobic conditions. (Rifai, 2021).

In addressing acne on the skin, there are currently many medicinal preparations that can be used. One of them is gel preparations. Gel preparations with polar solubility are easier to clean from the skin surface after use and do not contain oils that can worsen acne severity. (Chairunnisa and Eni, 2017). In addition, gel preparations are non-sticky, provide a cooling sensation, and are relatively stable, thus having better potential for topical formulation. (Ramadan, 2020).

According to previous research conducted by Christy *et al,* (2022) Effectiveness of Antibacterial Gel Formulation of Moringa Oleifera Leaf Extract Against *Propionibacterium Acne*. The research results indicate that the gel formulation of moringa leaf extract consistently affects the growth of *Propionibacterium acne* bacteria, with an average inhibition zone diameter of 14.5 mm (strong effectiveness) using the well method and 13.0 mm (strong effectiveness) using the disc diffusion method. disc diffusion method. The findings indicate that the gel formulation consistently reduces the growth of Propionibacterium acnes bacteria. Whereas in the study Riswana *et al,* (2022). Antibacterial activity test of moringa leaf extract (*Moringa oleifera*) against the growth of acne-causing bacteria. The results of this study indicate that *Moringa oleifera* leaf extract has potential as an antibacterial agent against acne-causing bacteria *Staphylococcus aureus, Staphylococcus epidermidis, and Propionibacterium acnes.*

The effectiveness of using extracts topically on the skin as an anti-acne treatment needs to be improved by formulating moringa leaf extract into a gel preparation, which is expected to allow for the rapid penetration of active compounds through the skin. In this study, the activity of the moringa leaf extract gel preparation in inhibiting the growth of *Staphylococcus aureus* bacteria was tested, with variations in extract concentration in the gel being 2%, 4%, 6%, and 8%.

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RESEARCH METHODS

Tools and Materials

The tools used are stirring rods, porcelain dishes, beakers, measuring cylinders, watch glasses, parchment paper, mortars and pestles, dropper pipettes, water baths, test tube racks, test tubes, rotary evaporators, spatulas, analytical balances, ovens, incubators, Petri dishes, and micropipettes. The material used is 96% ethanol extract of moringa leaves. Using other additives: carbopol 940, triethanolamine, glycerin, sodium metabisulfite, aquadestillata, Mueller Hinton agar medium, H_2SO_4,$BaCl_2$, BaCl2, NaCl, magnesium powder, HCl (p), Dragendorff, Mayer, Wagner, FeCl3, n-hexane, and Liebermann-Burchard.

Sample

The sample used in this study is fresh Moringa leaves (Moringa oleifera Lam.) taken from Pasir Pengaraian, Rokan Hulu Regency, Riau.

The Process of Making Moringa Leaf Simplisia

The process of making moringa leaf simplicia involves taking fresh moringa leaves (*Moringa oleifera* Lam.), performing wet sorting by cleaning off any dirt attached to the leaves and separating the stems, then washing them with running water. Next, the leaves are chopped into small pieces and dried at room temperature (±25°C) for about 1 week until dry. After that, dry sorting is done to separate dirt, foreign materials, and some damaged simplicia, then blended until fine, weighed, and stored in a tightly closed container. (Mardhiyani and Afriani, 2021).

Preparation of Moringa Leaf Extract

The extraction process uses 96% ethanol as a solvent with the maceration method. The moringa leaf simplicia was weighed at 500 grams and placed into 96% ethanol in a dark glass container until fully submerged, then macerated for 3 days. It was kept in a cool place protected from light and stirred several times a day for 3 days. The maceration result was filtered and collected in a glass bottle, then the residue was macerated again for 3 days, and this process was repeated for a total of 3 macerations. Then the results of the maceration are combined into one or evaporated using a rotary evaporator until a thick extract is obtained (Ginara *et al*, 2022). The formulation percentage of the extract can be calculated using the formula as follows :

$$\%\text{Extraction yield} = \frac{\text{the weight of the obtained thick extract (g)}}{\text{extracted simplicia weight (g)}} \times 100\%$$

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Phytochemical Screening

1. Flavonoid Test:

   Weigh 1 gram of moringa leaf extract and add 50 mL of distilled water; stir, then divide into 2 parts: one for the alkaloid test and one for the flavonoid, saponin, and tannin tests; heat for 5 minutes using a hot plate, then filter. Then, 1 mL of the filtrate is placed into a test tube, adding ½ spatula of Mg powder, followed by 1 mL of amyl alcohol and 1 mL of HCl (p). If a yellowish and orange color forms in the upper layer, it indicates a positive result for the presence of flavonoids.

2. Saponin Test:

   25 mL of the heated extract is then placed into a test tube, and 10 mL is added along with 1 mL of HCl (p) and shaken for 10 seconds. If foam forms that lasts for 10 minutes and does not disappear, it indicates the presence of saponins.

3. Tannin Test

   1 mL of the heated extract was added with 3 drops of $FeCl_3$. If a blue or dark green color change occurs, the result indicates the presence of tannins.

4. Terpenoids/Steroids Test

   One spatula of the extract sample was added to 10 ml of n-hexane and allowed to sit for 15 minutes. Then it is filtered, the filtrate is evaporated with a hot plate in a dish until dry, and 5 drops of the Liebermann-Burchard reagent are added to each side through the wall of the dish. If a green color forms, it indicates the presence of steroids, and a purple color indicates the presence of terpenoids. (Yulia, 2022).

Moringa Leaf Extract Gel Manufacturing

Table 1. Formulation of the preparation

Ingredients	**Concentration (% b/v)**			
	F1(2%)	**F2 (4%)**	**F3 (6%)**	**F4 (8%)**
Moringa Leaf Extract	2 g	4 g	6 g	8 g
Carbopol 940	1 g	1 g	1 g	1 g
TEA	0,5 mL	0,5 mL	0,5 mL	0,5 mL
Gliserin	1 mL	1 mL	1 mL	1 mL
Natrium Metabisulfit	0,2 g	0,2 g	0,2 g	0,2 g
Aquadest ad	100 mL	100 mL	100 mL	100 mL

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Weigh all the ingredients according to the formulation first. Making moringa leaf gel from moringa leaf extract. Prepare a mortar, and then develop 1 g of Carbopol 940 with hot water and grind it vigorously until a gel base is formed. Then add 0.5 mL of TEA. Next, add the other ingredients. Sodium metabisulfite was weighed at 0.2 grams and added to 1 mL of glycerin, then 2% ethanol extract from moringa leaves was added to the mortar and stirred slowly until homogeneous. For the preparation of gels with concentrations of 4%, 6%, and 8%, the same method was used as for the preparation of the moringa leaf extract gel. (Ginara *et al*, 2022).

Organoleptic testing

The organoleptic testing of the gel preparation is conducted by visually observing the prepared gel, including its shape, color, and odor. (Yusuf *et al.*, 2017).

Mueller Hinton Agar Media (MHA)

Add 3.8 grams of MHA media into a 500 mL volumetric flask, then add 350 mL of distilled water. Next, stir and heat the MHA media using a hot plate. Then, autoclave the MHA media for 15 minutes at 121℃ (Mardhiyani and Afriani, 2021). After that, the MHA media are removed from the autoclave and poured evenly into each petri dish, then left to solidify.

Mc. Farland Standard

A 0.36 N H_2SO_4 solution of 99.5 mL is mixed with a 0.5 mL 1% $BaCl_2 \cdot 2H_2O$ solution in an Erlenmeyer flask, then shaken until a cloudy solution forms. This turbidity serves as the standard for the bacterial test suspension.

Antibacterial Activity Test

Welling Method

A 0.36 N H_2SO_4 solution of 99.5 mL is mixed with a 0.5 mL 1% $BaCl_2 \cdot 2H_2O$ solution in an Erlenmeyer flask and then shaken until a cloudy solution forms. This turbidity serves as the standard for the bacterial test suspension. (Ginara *et al*, 2022).

Disc Diffusion Method

Sterile cotton swabs are dipped into a test suspension in a test tube, then the cotton swabs are squeezed against the walls of the test tube while being rotated. After that, the test suspension is applied to the surface of the medium in a zigzag manner until the entire medium is evenly coated. Then the chloramphenicol disc is taken and placed on the surface of the medium, which is used as a positive control, (+) and the gel base with a negative control (-). Next, an empty disc is placed on the surface of the medium, and the gel is introduced with a concentration test of 2%, 4%, 6%, and 8% using a 2 µL micropipette. Repetitions were carried out three times. Next, the petri dishes were incubated upside down in an incubator for 48 hours at 37°C. The zones of bacterial growth inhibition from each disc were measured as research data. (Mardhiyani and Afriani, 2021).

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RESULTS AND DISCUSSION

The moringa leaves used in the research were obtained from Pasir Pengaraian, Rokan Hulu Regency, Riau. Next, a wet sorting is carried out by cleaning the dirt attached to the moringa leaves and separating the stems, then washing them with running water. Next, it is chopped into small pieces to increase the surface area of the simplicia, thereby accelerating the extraction and drying processes. The moringa leaves are then dried at room temperature (± 25°C) for approximately 1 week (Ramadan, 2020). The purpose of drying is to obtain simplicia that is not easily damaged, allowing it to be stored for a long time. Thereafter, a dry sorting is performed to separate dirt, foreign materials, and some damaged simplicia. Next, it is blended until smooth and stored in a tightly closed container (Slamet *et al*., 2020).

The maceration process is carried out by soaking moringa leaf simplicia powder in a solvent at a ratio of 1:10.(Farmakope Herbal, 2017). The solvent used is 96% ethanol because ethanol has advantages such as being inert, non-toxic, and having excellent and universal absorbance. 96% ethanol is a semi-polar solvent and can extract most of the chemical content from simplicia; 96% ethanol also dissolves polar compounds. (Fathonah, 2019). The obtained extract is filtered and then re-soaked using the same solvent for 3 repetitions. The results of the maceration are filtered, and the filtrate is evaporated using a vacuum rotary evaporator until a thick extract is obtained. The vacuum rotary evaporator is used so that the solvent can be separated from the solute without high heating, preventing the compounds contained in the solvent from being damaged by high temperatures.

Based on the results obtained, the yield value of the moringa leaf extract is 24.61%. This result indicates that the extract yield meets the requirements of the Indonesian Herbal Pharmacopoeia, which states that the yield of moringa leaves should not be less than 9.2% (Farmakope Herbal, 2017). The value is influenced by several factors, including the type of solvent, solvent concentration, size of the simplicia particles, and the duration of the extraction time. (Chairunnisa *et al.*, 2019). The yield value of this moringa leaf extract shows that the higher the yield value, the more extract value is produced.

Phytochemical screening of moringa leaf extract aims to determine the content of secondary metabolite compounds contained in moringa leaves. The moringa leaf extract contains secondary metabolites such as flavonoids, alkaloids, saponins, tannins, and terpenoids (Table II). The results obtained are consistent with Yunita *et al*., (2020) which states that moringa leaf extract contains flavonoid, alkaloid, saponin, tannin, and terpenoid compounds. Flavonoid compounds are the main focus as antibacterial agents in this study because the mechanism of action of flavonoids is similar to the antibiotic compounds used in Medi-klin® gel to inhibit bacterial activity. The antibacterial mechanism of flavonoids inhibiting nucleic acid synthesis involves rings A and B, which play a crucial role in the intercalation process or hydrogen bonding by stacking nucleic acid bases that inhibit DNA and RNA. As a result, flavonoid compounds can cause damage to bacterial cell wall permeability, microsomes, and lysosomes due to the interaction between flavonoids and bacterial DNA. (Mahmiah *et al*., 2020).

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Table 2. Phytochemical screening of moringa leaf extract

Secondary Metabolites	Reagents	Test Results			Color Change	Conclusion
		I	II	III		
Flavonoid	Mg + HCl Powder (P)	+	+	+	Formed top layer yellowish color	Positive
Alkaloid	Dragendorff	+	+	+	Deposits are formed brownish in color	Positive
	Mayer	-	-	-	No white deposits formed	Negatives
	Wagner	+	+	+	Deposits are formed brownish in color	Positive
Saponin	HCl (P)	+	+	+	Stable foam formed	Positive
Tanins	$FeCl_3$	+	+	+	Formed a blackish-green color	Positive
Terpenoid	N-Heksan + Liebermen-burchard	+	+	+	Purple color formed	Positive
Steroid	N-Heksan + Liebermen-burchard	-	-	-	No green color formed	Negatives

Organoleptic tests are carried out by visually observing the smell, color and shape of the preparation (Yusuf *et al.*, 2017). In all the gel preparations produced, the form and smell are the same, which is a semi-solid form with a characteristic moringa leaf scent. However, they have different colors: the gel base is clear, the 2% gel preparation has a greenish color, the 4% preparation has a greenish color, the 6% preparation has a dark greenish color, and the 8% preparation has a dark greenish color. The higher the concentration of moringa leaf extract, the greener or darker it becomes because the amount increases, as shown in Table III.

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Table 3. Organoleptic test of moringa leaf extract gel preparation

Concentration	Features		
	Color	Construction	Shape
2%	Greenery	Typical moringa leaves	Semi-solid
4%	Greenery	Typical moringa leaves	Semi-solid
6%	Blackish green	Typical moringa leaves	Semi-solid
8%	Blackish green	Typical moringa leaves	Semi-solid
K (-)	Clear	Odorless	Semi-solid

Next, the antibacterial activity of the gel preparation of moringa leaf extract against S. aureus bacteria was tested using the well and disc methods. Antibacterial activity can be observed by the presence of the inhibition zone diameter. Then compared with the negative control and the positive control. The negative control used is a gel base or gel preparation that does not contain active substances (moringa leaf extract). The negative control serves as a comparison and to determine whether the gel base used has antibacterial activity against *S. aureus* or not. (Ramadan, 2020). Meanwhile, the positive control used was Medi-klin® gel with a concentration of 1%. Medi-klin® gel has the same mechanism as the compounds found in moringa leaves, which are flavonoids that are effective as bacteriostatic agents against both Gram-positive and Gram-negative bacteria.

In the well method, gel preparations made with moringa leaf extract at concentrations of 2%, 4%, 6%, 8%, negative control, and positive control are placed into wells created in agar media that have been inoculated with *S. aureus* bacteria and then incubated for 24 hours. (Ginara *et al*, 2022). Then the diameter of the inhibition zone was measured with a calliper. The diameter of the inhibition zone at concentrations of 2%, 4%, 6%, 8%, negative control, and positive control were 7.68 mm, 8.8 mm, 9.18 mm, 9.55 mm, 6 mm, and 24.77 mm, as shown in Table IV.

Table IV. Diameter of the buffer zone with the grouting method

Concentration	Clear Zone Diameter (mm)				Category
	P1	P2	P3	Average	
2%	7.1	7.75	8.2	7.68	Medium
4%	8.25	8.45	9.7	8.8	Medium
6%	8.65	9.05	9.85	9.18	Medium
8%	8.85	9.25	10.55	9.55	Medium
K (+)	24.8	24.8	24.7	24.77	Very Powerful
K (-)	6	6	6	6	Weak

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Then, test the antibacterial activity using the disc method by soaking a blank disc in the gel preparation at concentrations of 2%, 4%, 6%, 8%, negative control, and positive control. Then, place the disc that has been soaked for 15 minutes on agar media that has been inoculated with *S. aureus* bacteria and incubate for 24 hours. (Ginara *et al*, 2022). The average zone diameters at concentrations of 2%, 4%, 6%, 8%, negative control, and positive control were 8.55 mm, 9.03 mm, 9.17 mm, 9.88 mm, 6 mm, and 25.06 mm, respectively, as shown in Table V.

Table 5. Diameter of the inhibition zone by disc method

Concentration	Clear Zone Diameter (mm)				Category
	P1	P2	P3	Rata-rata	
2%	7.45	8.65	9.55	8.55	Medium
4%	8.6	8.8	9.7	9.03	Medium
6%	8.85	8.85	9.8	9.17	Medium
8%	9.75	9.85	10.05	9.88	Medium
K (+)	25.2	27.4	22.6	25.07	Very Powerful
K (-)	6	6	6	6	Weak

Based on the antibacterial activity results of the gel against *S. aureus*, using both the well method and the disc method, the best concentration is 8%. Thus, the higher the concentration of the extract in the gel preparation, the higher the inhibition of the Staphylococcus aureus bacteria. The inhibition produced falls into the moderate category. (Datta *et al*., 2019). According to Datta *et al* (2019) Antimicrobial inhibition zone activity is grouped into four categories, namely: weak (<5 mm), medium (5-10 mm), strong (>10-20 mm), very strong (>20-30 mm).

The use of the well method and the disc method has both advantages and disadvantages. The well method is advantageous because it allows for easier measurement of the inhibition zone area, as the isolate is active both on the surface of the agar medium and below it; in contrast, the disc method is beneficial due to its simplicity and minimal time requirement. (Kirtanayasa, 2022). The results of the inhibition zone on the disc medium are larger than on the well. This difference is because the disc method uses filter paper that is soaked in the medium for 15 minutes, so the amount of secondary metabolites absorbed by the filter paper is likely greater than in the well method, which uses a micropipette to take 50 µL. The comparison graph between the disc method and the well method can be seen in Figure 1.

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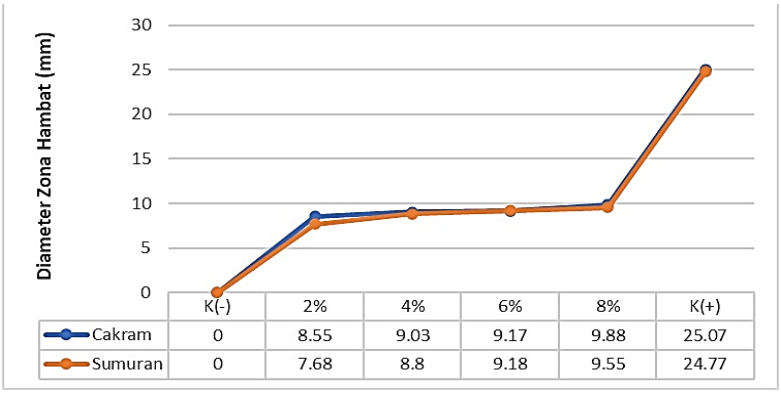

Figure 1. Graph of the diameter of the buffer zone by the ridge and disc method

Next, the results were statistically evaluated using ANOVA to see if there were significant differences in the diameter of the inhibition zone between the positive control and each formula. The results obtained showed a significant value of 0.000, which means $p < 0.05$, so it can be concluded that there is an effect of the concentration of the moringa leaf extract gel on the growth of Staphylococcus aureus, with differences in the diameter of the inhibition zone between the positive control and various concentrations of the moringa leaf extract gel. Based on the Pearson correlation test results table with a significance value of <0.01, it means there is a relationship between the concentration of the gel extract and the inhibition zone on the agar media using the well method and the disc method. The Pearson correlation test shows a value of $r = 0.858$ for the well method. The results indicate that in the well method, 85.8% of the observed bacterial growth inhibition is due to the application of the moringa leaf extract gel. Meanwhile, for the disc method, the Pearson correlation test shows a correlation value of $r = 0.845$. The results indicate that in the disc method, 84.5% of the observed bacterial growth inhibition is due to the application of the moringa leaf extract gel or other external factors.

Thus, the gel preparation of moringa leaf extract has antibacterial activity, based on phytochemical screening, which showed that the moringa leaf extract contains flavonoid secondary metabolites. Flavonoid compounds that are effective as bacteriostatic agents against both Gram-positive and Gram-negative bacteria. The antibacterial mechanism of flavonoids inhibiting nucleic acid synthesis involves rings A and B, which play an important role in the intercalation process or hydrogen bonding by stacking nucleic acid bases that inhibit DNA and RNA. As a result, flavonoid compounds can cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes due to the interaction between flavonoids and bacterial DNA. (Mahmiah *et al*., 2020). This result is also in line with the antibacterial mechanism of medi-clin (clindamycin phosphate), which has an antimicrobial mechanism where clindamycin binds to the 50S ribosomal subunit of bacteria and inhibits protein synthesis. Clindamycin shows three mechanisms of action, namely reducing the percentage of free fatty acids, having an anti-inflammatory effect, and decreasing the number of propionibacteria.

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CONCLUSION

Based on the results and discussion in the study, it can be concluded that the preparation of Moringa oleifera Lam. leaf extract gel has antibacterial activity against *Staphylococcus aureus*, which is indicated by the diameter of the inhibition zone (clear zone). In the well method with concentrations of 2%, 4%, 6% and 8%, the average inhibition zone was 7.68 mm; 8.8 mm; 9.18 mm; 9.55 mm. Meanwhile, with the well method, the average inhibition zone at concentrations of 2%, 4%, 6% and 8% was 8.55 mm; 9.03 mm; 9.17 mm; 9.88 mm.

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