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Antibacterial Effectiveness Test of *Moringa oleifera* Leaf Extract on the Growth of *Staphylococcus epidermidis Bacteria* Causing *Acne Vulgaris*

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ABSTRACT

Acne vulgaris is a common skin condition in teenagers and young adults, often caused by bacterial infections, including *Staphylococcus epidermidis*. The use of antibiotics as a primary treatment carries the risk of bacterial resistance, highlighting the need for safer natural alternatives such as moringa leaf (*Moringa oleifera*) extract. This study aimed to evaluate the antibacterial effectiveness of ethanol-based moringa leaf extract against *Staphylococcus epidermidis*. A true experimental post-test only control group design was used, applying the disc diffusion (Kirby-Bauer) method. Extracts at concentrations of 25%, 50%, 75%, and 100% were tested, alongside positive (Doxycycline) and negative (distilled water) controls. Inhibition zones were measured in millimeters. Results showed all extract concentrations produced inhibition zones ranging from 0.25 mm to 2.3 mm (classified as resistant), while Doxycycline produced a 28.7 mm zone (classified as sensitive). An conclusion, phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, alkaloids, phenolics, and steroids. Although the antibacterial activity of moringa extract is relatively low, it shows potential for further development.
Keywords: *acne vulgaris*; *Moringa oleifera*; *Staphylococcus epidermidis*; natural antibacterial; inhibition zone

INTRODUCTION

Everyone, wants beautiful, clean, and flawless facial skin, free from skin problems such as acne or hyperpigmentation.[1] Acne, which often occurs at a young age, especially in teenagers and young adults, can affect appearance; studies show that around 83-85% of women and 65-80% of men aged 16-17 experience acne, with the possibility of acne cases in Southeast Asia reaching 40-80%.[2–4] In Indonesia, Acne vulgaris is the third most common disease in the Department of Skin and Sex Health Sciences, with the highest prevalence at the age of 14-17 years.[4] The main factors involved in acne formation include increased sebum production, keratinocyte decay, inflammation, cosmetics, food, genetic factors, excessive stress, and bacterial growth, including *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. *Propionibacterium acnes*, as a normal skin flora, plays a role in the pathogenesis of acne which can cause inflammation.[5] *Staphylococcus epidermidis*, although generally not pathogenic, can cause disease if the population is excessive.[6,7] Acne treatment caused by bacterial infections often uses antibiotics, but their use can cause side effects and bacterial resistance, so safer alternatives are needed, such as moringa leaf extract.[8,9] The moringa plant (*Moringa oleifera*) is known to contain various important nutrients and bioactive compounds, including cytokinins which have antioxidant, anti-aging, and anti-inflammatory properties, and can help treat acne.[10,11]

Acne vulgaris is one of the most common dermatological conditions, especially among teenagers and young adults. The disease is characterized by the appearance of blackheads, papules, pustules, and in more severe cases, it can cause scarring. The main cause of acne vulgaris is the blockage of hair follicles by sebum and keratin, which then becomes an ideal environment for the growth of bacteria, especially *Cutibacterium acnes* (formerly known as *Propionibacterium acnes*) and *Staphylococcus epidermidis*.[12,13] *Staphylococcus epidermidis*, which is part of the normal skin flora, can act as an opportunistic pathogen when there is an imbalance in the skin microbiota.[14,15] Infections caused by *Staphylococcus epidermidis* are often related to the use of cosmetic products containing preservatives that can affect the dynamics of skin microflora.[16] The use of antibiotics to treat bacterial infections, including those caused by *Staphylococcus epidermidis*, is often faced with the challenge of antibiotic resistance. This resistance has increased significantly, requiring alternative approaches in the treatment of bacterial infections.[17,18]

In this context, plant extracts, such as moringa leaves (*Moringa oleifera*), have gained attention as a potential source of natural antibacterial compounds. Moringa leaves are known to contain various bioactive compounds, including flavonoids, alkaloids, and phenolic compounds, which have significant antibacterial activity.[19,20] Previous research has shown that moringa leaf extract can inhibit the growth of various types of pathogenic bacteria, including *Staphylococcus aureus* and Escherichia coli.[21] One promising approach is the use of moringa leaf extract as an antibacterial agent to overcome the growth of *Staphylococcus epidermidis* which contributes to the development of acne vulgaris. Research shows that compounds in moringa leaves can disrupt the biofilm process produced by *Staphylococcus epidermidis*, which is one of the main pathogenicity mechanisms of this bacterium.[22,23] The biofilm that forms can protect the bacteria from attacks by the immune system and antibiotic treatment, making it easier for infections to develop.[24,25] Therefore, testing the effectiveness of moringa leaf extract on the growth of *Staphylococcus epidermidis* is important to explore the potential of alternative

therapies in the treatment of acne vulgaris. In addition, a better understanding of the interaction between *Staphylococcus epidermidis* and other skin microflora, as well as how moringa leaf extract can modulate this interaction, can provide new insights into the development of more effective treatment strategies. This study aims to evaluate the antibacterial effectiveness of moringa leaf extract against the growth of *Staphylococcus epidermidis* which plays a role in the development of acne vulgaris, as well as to understand the mechanism of action of the active compounds contained in the extract.[26,27]

Acne vulgaris is one of the most common skin problems, particularly among teenagers and young adults, characterized by the appearance of blackheads, papules, pustules, and scarring that can affect the quality of life of those affected. In Indonesia, this condition ranks third highest in dermatology and venereology clinics, with the highest prevalence among individuals aged 14–17 years. The causes are complex, including increased sebum production, abnormal keratinization, inflammation, and colonization by bacteria such as *Cutibacterium acnes* and *Staphylococcus epidermidis*. *Staphylococcus epidermidis*, although a normal skin flora, can become an opportunistic pathogen when there is an imbalance in the skin microbiota and plays a role in the formation of biofilms that protect bacteria from the immune system and antibiotics. The use of antibiotics as a treatment for acne vulgaris often leads to bacterial resistance, necessitating safer and more effective alternatives. One potential candidate is moringa leaf extract (*Moringa oleifera*), which is known to contain bioactive compounds such as flavonoids, alkaloids, and phenolic compounds with antibacterial and anti-inflammatory activities. Previous studies have shown that moringa leaf extract can inhibit the growth of various pathogenic bacteria, including *Staphylococcus aureus* and *Escherichia coli*, and disrupt biofilm formation. Therefore, this study is important to evaluate the antibacterial efficacy of moringa leaf extract against *Staphylococcus epidermidis*, which contributes to the pathogenesis of acne vulgaris, to support the development of safer and more natural alternative therapies.

METHODS

The research was carried out at the Research Laboratory of the Faculty of Medicine, Universitas Muslim Indonesia, Jl. Urip Sumoharjo KM 5, Panaikang, Panakkukang District, Makassar City, South Sulawesi, from June to November 2024. This study used a true experimental post-test only control group design, which allows researchers to control all variable that affect the course of the experiment.[28–30] The method applied was disc diffusion (Kirby Bauer method)[31] to measure the sensitivity of moringa leaf extract (*Moringa oleifera*) to the growth of *Staphylococcus epidermidis* bacteria by measuring the zone of inhibition formed.

The population in this study was moringa leaves taken from a house yard on Jl. Poros Taman Sudiang Indah No. E5, Makassar, South Sulawesi. The samples used were moringa leaves extracted in concentrations of 25%, 50%, 75%, and 100%. Inclusion criteria include extracts made from moringa leaves that are not infected with fungi, pests, or chemicals, as well as undamaged Mueller Hilton Agar (MHA) medium.

The dependent variable in this study was *Moringa oleifera* leaf extract, while the independent variable was the zone of inhibition of *Staphylococcus epidermidis* bacterial growth. The objective criteria for the inhibition zone were divided into three categories: sensitive (≥21 mm), intermediate (15-20 mm), and resistant (≤14 mm). Data was collected by measuring the inhibition zone formed after treatment with moringa leaf extract on MHA medium inoculated with *Staphylococcus epidermidis*. The inhibition zone was measured using a ruler in millimeters (mm). The data obtained from the measurement of the inhibition were analyzed descriptively to determine the effectiveness of moringa leaf extract at various concentrations. The results of the inhibition zone from moringa leaf extract were compared with positive control (*clindamycin*) and negative control (*aquadest*) to evaluate antibacterial effectiveness.

This research had met ethical standards in accordance with applicable regulations and had undergone a review process by the Research Ethics Committee. Ethical approval for the conduct of this research had been granted by the Research Ethics Committee of the University of Muslim Indonesia with reference number: 629/A.1/KEP-UMI/XII/2024 dated December 10, 2024. With this approval, all research procedures were guaranteed to comply with research ethics principles, including the protection of research subjects, the use of test materials, and safety and security aspects throughout the research process.

RESULT

Maceration using 96% ethanol solvent was the extraction method used. Moringa leaves were washed thoroughly and dried. Maceration was carried out with 96% ethanol solvent at a ratio of 500 g of material in 4500 ml of solvent and left to stand for 72 hours (changed every 24 hours for 3 days). Filtering was carried out using filter paper. Then the extraction results were concentrated and a thick moringa leaf extract was obtained. Mueller Hilton Agar (MHA) powder was weighed at 5.7 grams and then dissolved in 150 ml of distilled water using an Erlenmeyer flask. After that, it was homogenized and sterilized in an autoclave at 121ºC for 15 minutes, then poured into a petri dish.

The bacterial culture was taken with a sterile inoculation needle and then suspended in a tube containing 2 ml of 0.9% NaCl solution until the same turbidity as the McFarland solution turbidity standard is obtained. After that, a cotton swab was scratched on MHA medium using the streak plate method. The disc was immersed for 15 minutes in negative control, positive control, and the 4th extract concentration, then placed in MHA medium which had been given bacterial suspension. Incubate the medium in the incubator for 1 x 24 hours. Measure the inhibition zone with a vernier caliper (mm).

Table 1 presents the results of phytochemical screening of moringa leaf extract, which showed the presence of several bioactive compounds with potential as antibacterial agents. The positive result for alkaloids indicates that the extract contains this compound, which is known to have various biological activities, including antibacterial effects that can inhibit the growth of pathogenic bacteria. In addition, the confirmed presence of flavonoids indicates that the extract is rich in this compound, which has antioxidant and anti-inflammatory properties, and has the potential to disrupt bacterial cell membranes, increasing antibacterial effectiveness. The positive result for steroid compounds also indicates that the extract contains these components, which can play a

role in biological processes, including anti-inflammatory effects that help reduce acne symptoms. The presence of positively detected phenolic compounds indicates that moringa leaf extract is rich in these compounds, which are known to have antibacterial and antioxidant activity and can protect the skin from free radical damage. However, negative results for saponins indicate that the extract does not contain this compound, which usually has antibacterial properties. However, the absence of saponins does not reduce the antibacterial potential of other compounds in the extract. Overall, the results of this phytochemical screening support further research on the use of moringa leaf extract in the treatment of acne vulgaris.

Table 2 shows the results of inhibition zone testing formed at various concentrations of Moringa leaf extract against the growth of *Staphylococcus epidermidis.* The results showed that at a concentration of 25%, the zone of inhibition formed was 0.25 mm, which is interpreted as resistance. At a concentration of 50%, the zone of inhibition increased to 1.05 mm, but is still categorized as resistance. A concentration of 75% results in a zone of inhibition of 0.8 mm, which also indicates resistance. Although the concentration of 100% shows an increase in the inhibition zone to 2.3 mm, this result is still categorized as resistance. For comparison, the positive control using Doxycycline showed a significant inhibition zone of 28.7 mm, which is interpreted as sensitive, while the negative control with distilled water shows no inhibition zone at all, with a result of 0 mm, which shows that distilled water has no antibacterial activity. These results indicate that although moringa leaf extract has antibacterial potential, its effectiveness against *Staphylococcus epidermidis* is still limited, and all concentrations tested are not effective enough to overcome the growth of this bacterium.

Table 1. Phytochemical screening results

No.	Examination	Result	Note
1	Alkoloid	(+)	(+) Positive: Contains a class of compounds (-) Negative: Does not contain a class of compounds
2	Flavonid	(+)	
3	Steroid	(+)	
4	Phenolic	(+)	
5	Saponin	(-)	

Table 2. Inhibitory zones formed in various Moringa leaves constrictions

Concentration	Zone of inhibition on *Staphylococcus epidermidis*	Interpretation
25%	0,25 mm	Resistance
50%	1,05 mm	Resistance
75%	0,8 mm	Resistance
100%	2,3 mm	Resistance
Control (+) doxycycline	28,7 mm	Sensitive
Control (-) aquades	0 mm	Resistance

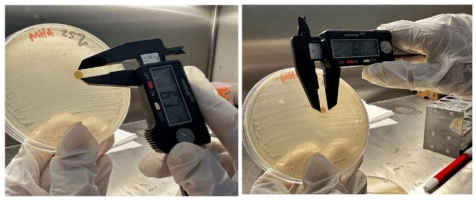

Figure 1. Sensitivity test results of moringa leaf extract 25% concentration

Figure 2. Sensitivity test results of moringa leaf 50% concentration

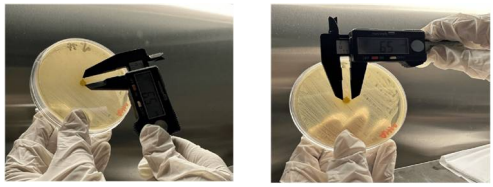

Figure 3. Sensitivity test results of moringa leaf extract concentration 75%

Figure 4. Sensitivity results of moringa leaf extract concentration 100%

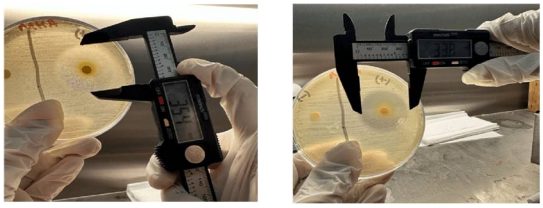

Figure 5. Sensitivity results of moringa leaf positive control (doxycycline)

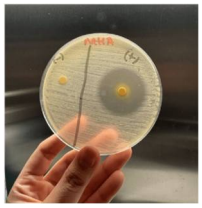

Figure 6. Sensitivity test results of moringa leaf negative control (aqua des)

DISCUSSION

Acne vulgaris is a common dermatological problem, especially among teenagers and young adults. This disease is characterized by inflammation of the sebaceous glands caused by several factors, including increased sebum production, follicular keratinization, and colonization of pathogenic bacteria such as Cutibacterium acnes (formerly known as *Propionibacterium acnes*) and *Staphylococcus epidermidis*.[16,32,33] In this context, the use of plant extracts as an alternative to antibacterial therapy is attracting increasing attention, especially moringa leaf extract, which is known to have various therapeutic properties. Moringa leaf extract has been shown to contain various bioactive compounds, including flavonoids, tannins, and saponins, which have potential as antibacterial agents.[34,35] Previous research has shown that ethanol extracts from various plants, including moringa leaves, can inhibit the growth of acne-causing bacteria such as *Staphylococcus epidermidis* and *Cutibacterium acnes*.[36–38]

This antibacterial activity can be measured by the agar diffusion method or the microdilution method, which allows researchers to determine the minimum inhibitory concentration (MIC) of the extract being tested.[39,40]

In this study, we aim to evaluate the antibacterial effectiveness of moringa leaf extract against the growth of acne-causing *Staphylococcus epidermidis.* Before testing, the moringa leaves will first be extracted using the maceration method until a thick extract is obtained. Maceration is the process of immersing samples in an organic solvent used at room temperature, making it very easy to perform and has many advantages. In addition, the medium is prepared as a medium for bacterial growth, namely MHA medium and bacterial culture suspension. The results of phytochemical screening show that the herbal powder and moringa leaf extract extracted using the maceration method contain several qualitative compounds, such as alkaloids, flavonoids, steroids, and phenolics, while for saponin compounds showed negative results, indicating the absence of saponin content in the extracted moringa leaves.[41,42] Previous research also explains that moringa leaves contain several secondary metabolite compounds that have antibacterial activity, but this depends on the concentration of the compound.

Tests of antibacterial effectiveness using the zone inhibition test method showed varying results at different extract concentrations. At a 25% extract concentration, the inhibition zone formed was 0.25 mm, at 50% it was 1.05 mm, at 75% it was 0.8 mm, and at 100% it was 2.3 mm. The increase in the inhibition zone as the concentration of the extract increases shows that the higher the concentration of moringa leaf extract used, the higher the antibacterial activity against *Staphylococcus epidermidis.* In comparison, the positive control used, the synthetic antibacterial Doxycycline, showed an inhibition zone of 28.7 mm, while the negative control (*aquadest*) showed no inhibition zone. According to the literature, moringa leaves can have a healing effect on acne vulgaris both through their antibacterial effect and through other effects of the compounds they contain, such as quercetin, chlorophyll, vitamin C, beta-sitosterol, oleic acid, and tannins, which can have an anti-inflammatory effect, reducing swelling and redness of acne, as well as antioxidant effects that can regenerate the skin.[43]

Although the results of the study show that moringa leaves have antibacterial potential against *Staphylococcus epidermidis*, the inhibition zone formed in the 25%, 50%, 75%, and 100% extract concentration groups is relatively smaller than the Doxycycline positive control. This may be due to the potential for bacterial resistance to moringa leaf extract, as well as other factors such as the amount of ingredients or simplisia used in the extraction process that can affect the antibacterial compound content in moringa leaves. Doxycycline, as a second-generation tetracycline antibacterial, has a broad spectrum and is bacteriostatic, so the resulting inhibition is greater than in moringa leaf extract.[44] Another study showed different results, where moringa leaf extract with concentrations of 5%, 10%, and 15% showed a larger inhibition zone, namely 23.01 mm, 23.34 mm, and 23.68 mm, with a comparative standard of 2% *clindamycin* of 31.69 mm.[45] The difference in results may be due to variations in the process of preparing the extract and the inhibitory zone test, as well as differences in the content of the compounds in the moringa leaf extract used.

Acne vulgaris is one of the most common skin conditions caused by inflammation of the sebaceous glands, triggered by various factors such as excessive sebum production, follicular keratinization, and colonization by pathogenic bacteria such as *Cutibacterium acnes* and *Staphylococcus epidermidis*. This study shows that moringa leaf extract has antibacterial activity against *Staphylococcus epidermidis*, one of the bacteria that causes acne. The zone inhibition test results indicate that an increase in extract concentration is directly proportional to an increase in the inhibitory effect on bacterial growth, although it is still lower than the positive control, Doxycycline. This supports the theory that bioactive compounds in plants, such as flavonoids, tannins, and phenolics, play a role in inhibiting bacterial activity through cell membrane damage or protein synthesis inhibition. However, the inhibitory activity at concentrations ranging from 25% to 100% was relatively low compared to Doxycycline, which may be attributed to several factors such as the extraction process, varying content of active compounds, or the possibility of bacterial resistance to natural compounds. Nevertheless, the antibacterial effects of moringa extract still show potential, especially if further developed using extraction methods that can optimize the content of active compounds, such as Soxhlet extraction or ultrasonic extraction. Additionally, previous studies have noted that compounds such as quercetin, vitamin C, and oleic acid in moringa leaves possess anti-inflammatory and antioxidant activities that can accelerate acne healing by reducing inflammation and promoting skin regeneration.

Research using extract concentrations of 5%, 10%, and 15% yielded significantly larger inhibition zones, exceeding 23 mm, which may be attributed to differences in extraction techniques, the type of solvent used, and environmental conditions during laboratory testing. These differences emphasize the importance of standardization in research methods to ensure valid results and high consistency in replication. Thus, although the effectiveness of moringa leaf extract has not yet matched that of synthetic antibiotics, the results of this study support the use of moringa extract as a candidate for natural antibacterial agents that can be further developed. Further studies on the mechanism of action of the active compounds and their interactions with bacteria, as well as in vivo tests, are needed to strengthen the scientific basis for its use as a safer and more natural alternative therapy for acne. This study provides a clear picture of the antibacterial effectiveness of moringa leaf extract against *Staphylococcus epidermidis*, the cause of acne vulgaris. The results obtained show that although moringa leaf extract has antibacterial potential, its effectiveness still needs to be improved to compete with synthetic antibacterials. Further research is needed to explore more deeply the potential of moringa leaf extract, including purification of the extract and further testing of different concentrations and extraction methods. This research is expected to contribute to the development of alternative therapies for acne, as well as to add insight into the potential of medicinal plants in dermatological treatment.

This study has several limitations that should be acknowledged. First, the sample size of 20 rats, while sufficient for preliminary analysis, may limit the generalizability of the findings. A larger sample size could provide more robust data and enhance the statistical power of the results. Second, the study was conducted over a relatively short treatment period of 14 days, which may not fully capture the long-term effects of VCO and EVOO on pancreatic health. Future studies should consider longer treatment durations to assess the sustained impact of these oils. Additionally, the research focused solely on male Wistar rats, which may introduce gender

bias; including female rats in future studies could provide a more comprehensive understanding of the effects of VCO and EVOO. Furthermore, while histopathological analysis was performed, the study did not explore the underlying molecular mechanisms by which these oils exert their effects on pancreatic tissue, which could provide valuable insights into their therapeutic potential. Lastly, the study's reliance on animal models may limit the applicability of the findings to human subjects, necessitating further clinical trials to validate the results in a human population. Addressing these limitations in future research will enhance the understanding of the therapeutic roles of VCO and EVOO in managing obesity and diabetes.

CONCLUSION

Based on the results of the study, moringa leaf extract showed antibacterial activity against *Staphylococcus epidermidis* with increasing effectiveness as the concentration of the extract increased. A concentration of 100% was the most effective compared to other concentrations, with an inhibition zone of 2.3 mm, although it was still classified as resistant. This indicates that while moringa leaf extract has potential as a natural antibacterial agent, its effectiveness against *Staphylococcus epidermidis* is still lower than that of synthetic antibiotics such as Doxycycline. Therefore, further research is needed using more optimal extraction methods, testing of active fractions, and *in vivo* testing to support the development of moringa leaf extract as a safer and more natural alternative therapy for acne.

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