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Short Communication

Topical Antibacterial Therapy from *Moringa oleifera* Extract Against *Staphylococcus epidermidis*

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ABSTRACT

Moringa oleifera is considered a versatile plant. It has been shown that antimicrobial properties exist in leaves, stem bark, root bark, cotyledon seeds, and seed coats of *Moringa oleifera*. These antimicrobial properties are believed to be due to secondary metabolites such as flavonoids, saponins, alkaloids, steroids, tannins and phenols in different plant parts. Acne (*Acne Vulgaris*) is a common skin condition believed to be caused by the build-up of dead skin cells, bacteria, and dried sebum that blocks the hair follicles in the skin. Natural products are known to possess phytoconstituents for treating many skin infections. This study aims to evaluate the antibacterial activity of ethanol extracts of *Moringa oleifera* leaves with concentrations of 5%, 10% and 15%, formulated into topical gel preparations against *Staphylococcus epidermidis*. The moringa leaves were macerated for three days in 70% ethanol. Carbopol 940 was used as the gelling agent. Three extract concentrations were prepared: 5%, 10%, and 15%. As a positive control, 1% clindamycin gel was used in an in vitro bacteriostatic activity test. The gel was physically evaluated for its organoleptic characteristics, homogeneity, pH, viscosity, and adhesion. The study showed that the extract concentrations affected the gel's viscosity, pH, and adhesion. Also, the bacteriostatic activity tests of the Moringa leaf extracts revealed that formulas 1, 2 and 3 had inhibition zones between 6.12 mm, 7.98 mm, and 8.35 mm, respectively. In conclusion, the formulations containing 5%, 10% and 15% moringa extract showed bacteriostatic activity against the test organism and normal range for organoleptic, homogeneity and viscosity tests, pH and adhesion tests.

Keywords: Moringa extract, Moringa gel, *Staphylococcus epidermidis*, antibacterial therapy

Introduction

Moringa oleifera is considered a versatile plant. It is an excellent natural source of nutrients; it acts as an energy booster and can be seen as a natural cure-all. A large number of essential compounds are found in this plant, including glucose, amino acids, vitamins, and minerals. According to various pharmaceutical research studies, extracts from *Moringa oleifera* have anti-microbial, anti-diabetic, and antioxidant properties and can fight cancer in the body, among other activities.[1-2] It has been shown that antimicrobial properties exist in the leaves, stem bark, root bark, cotyledon seeds, and seed coats of *Moringa oleifera*.[3-5] The aqueous extract of the pods' husks showed antibacterial activity against gram-positive and gram-negative pathogenic bacteria and yeast strains.[5-8]

Numerous studies have investigated the cosmeceutical properties of medicinal plants that grow in various climatic zones. Phenolic-rich plant extracts can be formulated into cosmetic cream products, significantly enhancing their commercial value. It is possible to prepare herbal extracts from various plant parts and integrate them into different skin care cosmetic creams, lotions and ointments.

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Herbal cosmetics can effectively prevent the skin from developing various skin conditions, allergic reactions, and diseases. Herbal cosmetics are preferred over synthetic cosmetics due to their superior safety profile, effectiveness, high quality, affordability, and fewer side effects.[9-11]

Skin health, especially facial skin, is very important for everyone today as it can be an important part of your appearance. One of the most common skin problems is acne. If the skin is oily, this will lead to clogged pores, producing a rough facial appearance, often referred to as acne. Cosmetic creams are mostly used in treating acne. Some bacteria (*Propionibacterium acne*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) have been implicated in acne. Acne caused by a bacterial infection can be treated with antibiotics. However, antibiotics have been associated with serious side effects and increased development of resistance strains, affecting the chemotherapy of serious infections and surgical procedures. *Moringa oleifera* leaves have antibacterial properties due to secondary metabolites such as flavonoids, saponins, alkaloids, steroids, tannins and phenols.[11-12] Moringa leaf extract was reported to have antibacterial properties against *Propionibacterium acnes* in a previous study of Moringa leaves ointment preparations. There has been significant inhibitory activity against *Staphylococcus aureus* using diluted leaf extracts at concentrations of 5%, 10%, and 15% in ointments.[12]

The combination of Carbopol 940 and an ethanol extract of Moringa leaves extract was successfully used in developing an antiacne gel for acne treatment. Since gel absorbs less water than ointments and has a lower absorption rate, gels have a significantly higher potential for use as topical drugs. Gels are more attractive than ointments, are less sticky, require less energy to form, and are more stable. Also, gel preparations are more effective because they are rapidly absorbed, allowing active ingredients to be more easily absorbed into acne-prone areas.[14-15]

In this study, ethanol extracts of *Moringa oleifera* leaves at different concentrations of 5%, 10% and 15% were formulated into a topical gel preparation. The physical properties of the Moringa leaves gel, including organoleptic properties, pH, homogeneity, adhesion, and antibacterial activity, were evaluated.

Research Methods

Extraction Method
Moringa leaf used for this study was collected from Kupang City, East Nusa Tenggara, Indonesia, in June 2022. It was identified, and voucher no: Co2021 was assigned. The leaves were dried and ground. Subsequently, 500 g of the powdered sample was macerated for 24 hr with 2.5 L of ethanol (70%) with intermittent shaking. The macerate was filtered with Whatman No. 1 filter paper, and the filtrate was concentrated *in vacuo* in a rotary evaporator at 40℃. The crude extract obtained was kept in a refrigerator at 4℃ until further use.

Phytochemical Qualitative screening
In this research, the ethanolic leaf extract of *Moringa oleifera* underwent a screening process to detect the presence of phytochemical compounds, specifically tannins, saponins, flavonoids, and alkaloids. The qualitative findings from this screening were analyzed and interpreted based on the outcomes observed in each individual assay.[8]

Gel Preparation and Formulation
The topical gel of Moringa leaf extract was prepared based on the formula in Table 1. Firstly, Carbopol 940 was dispersed in hot distilled water until the carbomer base expanded and was ready for use. To achieve the gel consistency, the methylparaben was dissolved in propylene glycol, which was added to the base and stirred continuously to achieve complete dissolution. The base was stirred with sterile water for a smooth, homogeneous mixture. The mixture was fortified with the Moringa leaf extract to produce 5%, 10%, and 15% gel concentrations.

Evaluation of the Moringa Topical Gel Preparation Properties
The Moringa leaf extract antiacne gel's physical properties were evaluated using different physical parameters: organoleptic properties, homogeneity, viscosity, pH, adhesiveness and stability tests. The tests were done in triplicates.

Organoleptic test
The main characteristics of the gel were observed and recorded, including its consistency, colour, odour, and texture.

Homogeneity test
The gel sample (500 mg) was dispersed on a microscopic and examined under a microscope to determine if the gel preparation was homogeneous.

Table 1. Moringa Extract Gel Formula

Components	**Formula (%)**		
	F1	F2	F3
Moringa ethanolic Extract	5	10	15
Carbopol 940	1	1	1
Propylene Glycol	10	10	10
Glycerin	5	5	5
Methyl paraben	0.5	0.5	0.5
Ethanol	2	2	2
Sterile water	Ad 50 g	Ad 50 g	Ad 50 g

Note:
F1: Moringa Gel formulation with 5% ethanolic Moringa extract
F2:Moringa Gel formulation with 10% ethanolic Moringa extract
F3:Moringa Gel formulation with 15% ethanolic Moringa extract

Viscosity test
The viscosity test was conducted with a Rion VT-04 viscometer using an in-house gel sample, and a sample tube filled with the rotor was placed until the rotor spindle was engulfed in the gel sample. After turning on the viscometer, the rotor was rotated until the spindle was completely submerged in the liquid, and then the viscometer was turned off. When the rotor pointing needle is moved, the viscosity is measured by reading the second rotor scale.

pH test
A pH meter was used to measure the pH of the gel. In this test, 1 g of gel was weighed and dissolved in 10 mL of sterile water. The pH test was performed at room temperature with an electrode dipped into the solution of interest. The pH meter reading was noted as a constant pH value was achieved.

Adhesive test
An adhesive test was performed, firstly the object glass was coated with 500 mg of gel while it dried, followed by another piece of glass in the adhesive test. A timer recorded time intervals between when the object glass broke after 80 grams of the load were inserted into a test tool that contained an adhesive test tool containing the object glass.

Bacteriostatic activity of Moringa leaf extract against Staphylococcus epidermidis
Kirby-Bauer disc diffusion was used in this antibacterial activity test. As a first step, sterile cotton swabs were inserted into a *Staphylococcus epidermidis* suspension and rotated several times before being pressed against the tube wall to remove excess inoculum. Different agar media were used to inoculate *Staphylococcus epidermidis*. The formulations F1, F2, and F3 gels contain 5%, 10% and 15% of Moringa leaves' ethanol extracts. A paper disc was dipped in each sample and placed on the surface of the media. The discs were positioned approximately two centimetres from the edge of the Petri dish. Clindamycin gel (1%) was used as a positive control, while the preparation without Moringa extract was used as a negative control. The Petri dish was then incubated at 37°C for 24 hours, after which the diameter was measured to determine whether an inhibition zone had developed.

Statistical analysis
Before analyzing the variance of the data, normality and homogeneity were tested in SPSS, and the data were analysed using analysis of variance (ANOVA). An analysis of the data's normality was conducted to determine if the data followed a normal distribution. Significant results are considered normal ($\alpha = 0.05$). On the other hand, if the significance value was < 1.0, the data were not normally distributed. Also, a homogeneity test was conducted to determine whether the variances of several populations were the same. In the case of a non-significant homogeneity test, the variances of two or more populations were considered not to be the same. *P*-values >0.05 were considered not statistically significant.

Results and Discussion

Phytochemical screening
This study aimed to identify the secondary metabolites contained in the ethanol extract of Moringa leaves using qualitative phytochemical screening. Table 2 shows the phytochemical identification of Moringa's ethanol extract, which responded positively to the test for tannins, saponins, flavonoids and alkaloids. Indeed, much research has suggested that *Moringa oleifera* extract may have antibacterial properties due to its secondary metabolites. For instance, much evidence has suggested that tannins can cause protein denaturation in bacteria with the eventual of the organism. Moreover, saponins are compounds that act as antiseptics by causing damage to the protein permeability of the bacteria. *Moringa oleifera* extract also contains flavonoids that may inhibit bacteria's energy metabolism, thus inhibiting bacteria's growth and development. It is also believed that the alkaloids in *Moringa oleifera* extract are effective antibacterials because they interfere with the constituents of bacteria, causing cell death.[10]

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Moringa leaf ethanolic gel extract evaluation

A physical evaluation was conducted to check for differences in the formula and ensure that the standard and observation criteria were appropriate. The gel was subjected to physical stability tests at room temperature four weeks post-formulation. A summary of the organoleptic properties, pH, viscosity and adhesive properties is shown in Table 3.

Organoleptic test

Formula 1 and 2 are brown, but a dark brown colour was observed in Formula 3, possibly due to a higher extract concentration. Due to the higher concentration of the extract in Formula 3, the gel formulated with this extract also produced a higher level of gel consistency. The results of this study indicate that the organoleptic properties of the Moringa leaf ethanol extract are the same in all the formulations. It has been shown that the concentration of the extract can influence its odour.[15]

Homogeneity

We carried out a homogeneity test on the gel preparation to determine if it was homogeneous. All three gels with different formulations were homogeneous during dispersion and had no coarse grains. This indicates that all the formulated ingredients were well mixed without forming coarse grains.

Viscosity Test

An examination of the viscosity of the three formula gels was performed. It is recommended that gels range in viscosity between 50 and 1000 dPa for easy removal from tubes and containers.[14] The viscosity of gel Formulas 1 to 3 was 800 dPa.s, 900 dPa.s, and 1000 dPa.s respectively, as determined by the viscosity test of Moringa oleifera extract. In other words, they are within the normal range expected.

pH test

A pH test was carried out to ensure the preparation would be safe to apply to the skin. Topical preparations should have a pH of approximately 4.5-6.5, which is the normal pH of the skin. As shown in Table 3, Formulas 1, 2 and 3 had pH values of 4.55, 4.98 and 5.88, respectively. The pH values of all formulations were within the normal range for skin pH criteria and can, therefore, be considered safe for use. When the gel preparation was stored, the pH of the preparation decreased, indicating that it became more acidic than when freshly prepared.[16] This may be related to the temperature and storage conditions.

Adhesive Test

The longer the interaction of the gel with the skin, the greater the absorption of the active ingredient. Therefore, the gel base will release more active ingredients if the adhesion is greater. Generally, the adhesion value criteria for topical preparations should not be less than four seconds.[15] The adhesion test results of the Moringa leaf ethanol extracts of formula 1 to 3 showed that it would adhere to skin for 4.09, 4.98 and 5.37 seconds, respectively. Therefore, all the formulas fulfil the requirements of the criteria for good adhesion.

Table 2: Moringa extract phytochemical analysis

Phytochemicals	Inference
Tannins	+
Saponins	+
Flavonoids	+
Alkaloids	+

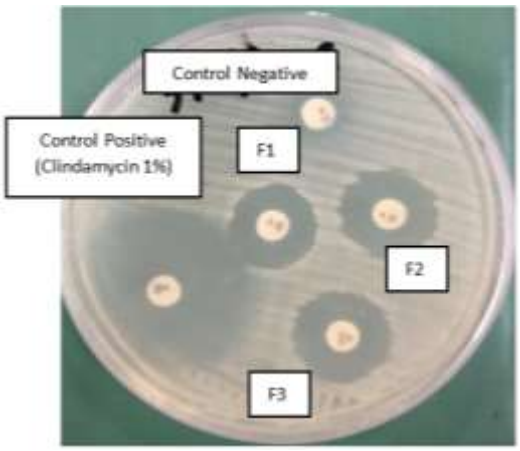

Figure 1: Inhibition zone of Control Positive (A), Control Negative, Formula 1 (F1), Formula 2 (F2), and Formula 3 (F3) against *Staphylococcus epidermidis*

Table 3: Moringa Leaves Gel Ethanol Extract Physical Analysis

Criteria	F1	F2	F3
Colour	Brown	Brown	Dark Brown
Smell	Leaf-like	Leaf-like	Leaf-like
Consistency	Viscous	Viscous	Very Viscous
Homogeneity	Homogenous	Homogenous	Homogenous
Viscosity (dPa.s)	800 ± 0	900 ± 0	1000 ± 0
pH	4.55 ± 0.01	4.98 ± 0.01	5.88 ± 0.01
Adhesive (sec)	4.09 ± 0.09	4.98 ± 0.12	5.37 ± 0.62

Table 4: Inhibition zone of gel preparation against *Staphylococcus epidermidis*

	Inhibition Zone Diameter (mm)	Antibacterial Activity
Control Positive (Clindamycin 1%)	28.10 ± 1.05	Very Strong
Control Negative	-	-
Formula 1	6.12 ± 0.2	Moderate
Formula 2	7.98 ± 1.56	Moderate
Formula 3	8.35 ± 0.27	Moderate

Antibacterial activity

Formulas 1, 2 and 3 in this study show inhibition zones measuring 6.12 mm, 7.98 mm and 8.35 mm, respectively (Figure 1). An inhibition zone diameter of 28.10 mm was observed in the positive control, indicating significant inhibitory activity, while no inhibition zone was observed in the negative control. At concentrations of 5%, 10%, and 15%, Moringa ethanol extract was tested for its ability to inhibit *Staphylococcus epidermidis* growth. Comparatively, formula 3 produced the highest inhibition zone with a 15% concentration of Moringa extract compared to the other formulas.

Conclusion

There has been evidence that moringa leaves ethanolic extract is effective against *Staphylococcus epidermidis* when applied as a topical gel. Furthermore, the formulations containing 5%, 10%, and 15% moringa extract also showed moderate antibacterial activity, homogeneity, viscosity, pH, and adhesion tests were within normal limits.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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