**RESEARCH ARTICLE**

# Formulation and Antibacterial Test of *Moringa oleifera* Seed Extract Gel Preparations on *Staphylococcus aureus* Bacteria

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**ABSTRACT**

**Background:** Human skin can occur infection by viruses, bacteria, fungi, or parasites. One of the pathogenic bacteria that often infect the skin is *Staphylococcus aureus*. The incidence of bacterial infections on the skin continues to increase and pathogenic bacteria *S. aureus* easily experience antibiotic resistance. The development of herbal treatments from plants with antibacterial potential can be one of the alternative treatment options. One of the plants often used for alternative traditional medicine is moringa seeds (*Moringa oleifera*).

**Purpose:** Knowing and analyzing the gel formulation of moringa seed extract (*Moringa oleifera*), which has optimal physical characteristics and antibacterial activity against *S. aureus*.

**Methods:** The type of research conducted was true experimental with a post-test-only control group design. In this study, there was a treatment group that was given moringa seed extract gel with a concentration of 5%, 10%, 20%, 40%, and 80%, a positive control group of clindamycin gel, and a negative control group of sterile aquadest with *S. aureus* as test bacteria. The method of antibacterial testing uses the disc diffusion method. The results of the study were analyzed using the Kruskal-Wallis statistical test.

**Results:** All formulations of gel preparations have optimal physical characteristics. The diameter of moringa seed extract gel inhibition zone against *S. aureus* bacteria at concentrations of 5%, 10%, and 20% have a weak antibacterial potential. In comparison, a concentration of 40% (5.05 mm) is categorized as medium potential, and a concentration of 80% (11.70 mm) is classified as having strong potential. The analysis results found significant differences between treatment groups with a significance value of $p < 0.05$.

**Conclusion:** Formula 4 and 5 are optimal formulations of moringa seed extract gel preparations because they have optimal physical characteristics and antibacterial activity against *S. aureus* bacteria.

**Keywords**: *Moringa oleifera*, *Staphylococcus aureus*, Gel, Antibacterial

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## INTRODUCTION

The skin is one of the particular human organs because it is located on the outer side of humans. Viruses, bacteria, fungi, or parasites can infect human skin. One of the pathogenic bacteria that often infect the skin is *Staphylococcus aureus*.[1] Based on the Global Burden of Disease study in 2019, skin diseases, especially infections caused by bacteria (including pyoderma and cellulitis), are a significant global disease burden. The incidence of bacterial skin diseases by age increased from 1990 to 2019 by 7.38%.[2] *S. aureus* bacteria are bacteria that quickly develop antibiotic resistance. The increasing and widespread use of antibiotics with uncontrolled doses can trigger *S. aureus* resistance to several types of antibiotics. Research conducted by the Department of Dermatology and Venereology at Cipto Mangunkusumo Hospital (RSCM) in 2006 highlighted the growing issue of antibiotic resistance, particularly in the treatment of skin infections. The study revealed that a small proportion of *S. aureus* exhibited resistance to erythromycin (28.6%), doxycycline (14.3%), and clindamycin (14.3%). [3] Furthermore, research data from Southern Brazil in 2016 also reported resistance of *S. aureus* to topical antimicrobial agents.[4] The existence of antibiotic resistance causes problems in treating infectious diseases. The development of herbal medicine from plants with antibacterial potential can be an alternative treatment option. One of the plants that can be used and widely available in East Nusa Tenggara is the *Moringa oleifera* plant. Moringa seeds have antibacterial properties such as tannins, flavonoids, saponins, phenols, and alkaloids. Based on research in 2020 by Ali and Utari also proved that moringa seed extract has an average diameter of the inhibition zone on the growth of *S. aureus* at

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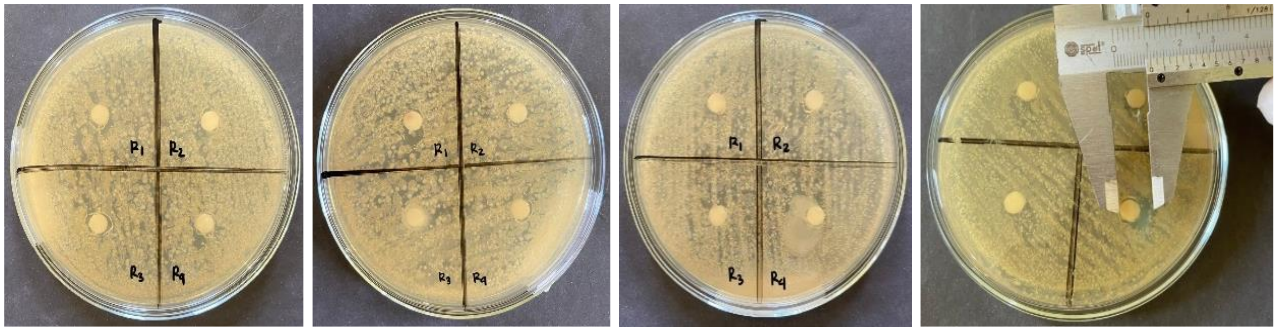


Concentration 5% Concentration 10% Concentration 20% Concentration 40%

Concentration 80% Positive Control Negative Control

Figure 1. Antibacterial Test Results of Moringa Seed Extract Gel Against *S. aureus* bacteria

concentrations of 5%, 10%, 20%, and 40%, respectively 6.7 mm, 7, 21 mm, 7.23 mm, and 7.6 mm.[5] However, in the treatment of skin infections, drug preparations are needed that can provide optimal effects on the skin, such as gel preparations. Gel preparations can create a moist, calm, and well-absorbed environment on the skin and are easily washed off with water.[6]

## METHODS

### Sample Determination

Moringa plant determination was carried out to confirm the type and species of plant samples used in the study. The determination was carried out at the Indonesian Biology Generation Laboratory.

### Extract Preparation

Moringa seed extract was prepared by drying the seeds in an oven at 100°C for one hour, followed by grinding with a blender and filtering. The resulting Moringa seed powder was then macerated in 70% ethanol at a 1:10 ratio for three days. After maceration, the mixture was filtered and concentrated using a rotary evaporator. The extract yield was calculated as the ratio of the final extract weight to the initial seed weight, multiplied by 100%. To ensure the absence of ethanol, the extract was tested using $H_2SO_4$ and $K_2Cr_2O_7$. Furthermore, phytochemical screening was carried out with reagents to determine the content of natural antibacterial secondary metabolites of the ethanol extract of Moringa seeds. Phytochemical screening involves testing for flavonoids, alkaloids, tannins, and saponins.

### *Moringa oleifera* Seed Extract Gel Preparations

The formulation of Moringa seed extract gel containing *M. oleifera* extract is presented in Table 1. The gel preparation began with dissolving the gelling agent, carbopol 940, in 10 mL of water at 70°C, followed by the addition of the extract to form the first mixture. Separately, methylparaben was dissolved in a small amount of water, then combined with glycerin, triethanolamine, and propylene glycol, forming the second mixture. The first and second mixtures were then combined, stirred thoroughly, and water was added until the total weight reached 20 grams. Finally, the mixture was stirred until a homogeneous gel was obtained.[7]

### Evaluation of the physical characteristics of the gel preparation

The Moringa seed extract gel was evaluated for the physical characteristics of the gel preparations. The physical characteristics of the optimum gel were organoleptic (semi-solid consistency, clear yellowish colour, and the characteristic odour of Moringa gel or

Table 1. Formulation of Moringa Seed Extract Gel

| Composition | Formulation | | | | | Function |
|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | |
| Moringa seed extract | 5% | 10% | 20% | 40% | 80% | Active ingredient |
| Karbopol 940 | 5% | 5% | 5% | 5% | 5% | Gelling agent |
| Triethanolamine (TEA) | 3% | 3% | 3% | 3% | 3% | Alkalizing agent |
| Propylene glycol | 15% | 15% | 15% | 15% | 15% | Stabilizer |
| Methylparaben | 0,02% | 0,02% | 0,02% | 0,02% | 0,02% | Preservative |
| Glycerin | 8% | 8% | 8% | 8% | 8% | Humectant |
| Sterile water | 100 ml | 100 ml | 100 ml | 100 ml | 100 ml | Solvent |

Table 2. Results of Evaluation of Physical Characteristics of Gel Preparations

| Formula | Organoleptic Test | | | Homogeneity | pH | Spreadability |
|---|---|---|---|---|---|---|
| | Consistency | Colour | Odour | | | |
| F1 | Semi-solid | Transparent | Typical gel | Homogenous | 6,19 | 5,36 |
| F2 | Semi-solid | Transparent | Typical gel | Homogenous | 6,03 | 5,29 |
| F3 | Semi-solid | Transparent | Typical gel | Homogenous | 5,94 | 5,58 |
| F4 | Semi-solid | Clear yellowish | Typical Moringa | Homogenous | 6,32 | 5,59 |
| F5 | Semi-solid | Yellow | Typical Moringa | Homogenous | 6,14 | 5,33 |

Table 3. Results of Measurement of Inhibition Zone Diameter

| Formula | Inhibition Zone Diameter (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Repetition 1 | Repetition 2 | Repetition 3 | Repetition 4 | Mean | Potential |
| F1 | 0 | 0 | 0 | 0 | 0 | Weak |
| F2 | 0 | 0 | 0 | 0 | 0 | Weak |
| F3 | 0 | 0 | 0 | 0 | 0 | Weak |
| F4 | 10,20 | 10,00 | 0 | 0 | 5,05 | Medium |
| F5 | 12,44 | 13,50 | 11,76 | 9,10 | 11,70 | Strong |
| Positive Control | 33,40 | 36,20 | 29,00 | 31,68 | 32,57 | Very strong |
| Negative Control | 0 | 0 | 0 | 0 | 0 | Weak |

seeds), homogeneous gel, pH value of 4.5–6.5, and spreadability value of 5–7 cm.[8]

**Antibacterial Test Against *S. aureus***

Bacterial confirmation test by gram stain and catalase test. Nutrient Agar medium was prepared by heating and dissolving it in 1000 mL of sterile distilled water, followed by sterilization, homogenization, and pouring into petri dishes. Once solidified, the media was inoculated with *S. aureus* and incubated at 37°C for 16–18 hours. The bacterial suspension was prepared by suspending *S. aureus* in a 0.9% NaCl solution to achieve a turbidity standard of 0.5 McFarland. The antibacterial activity test was performed by dipping a sterile cotton swab into 1 mL of the *S. aureus* suspension, spreading it evenly on the Nutrient Agar surface, and allowing it to stand for 10 minutes to ensure absorption. Sterile tweezers were used to immerse 6 mm diameter paper discs in Moringa seed extract gel, clindamycin gel, and sterile distilled water for 30 minutes. The discs were then placed onto the agar plates containing the bacterial suspension. The plates were incubated at 37°C for 24 hours, after which observations were made. The diameter of the inhibition zone was measured using calipers.

## RESULT AND DISCUSSIONS

The determination results confirmed that the Moringa seeds used belonged to the *Moringa oleifera* L. species. A total of 4 kilograms of Moringa seeds were macerated, yielding 3 liters of Moringa seed extract. The liquid extract was then evaporated, resulting in 150 grams of thick Moringa seed extract. The extraction yield was calculated at 37.5%, which is considered optimal as it exceeds the standard value of 10%. Additionally, the ethanol-free test confirmed the absence of ethanol in the extract.[9] Phytochemical screening revealed that Moringa seed extract contained secondary metabolites, including flavonoids, alkaloids, tannins, and saponins. Flavonoids exhibit antibacterial activity by inhibiting nucleic acid synthesis, disrupting cytoplasmic membrane function, and interfering with bacterial energy metabolism.[10] The following secondary metabolites, alkaloids, act by inhibiting bacterial cell wall synthesis, altering membrane permeability, disrupting bacterial metabolism, and preventing nucleic acid and protein synthesis.[11] Tannins function as antibacterial agents by preventing bacterial adhesion to surfaces, leading to bacterial cell death, and restricting bacterial growth by inhibiting sugar and amino acid absorption.[12] Lastly, saponins exert antibacterial effects by damaging cell wall permeability, ultimately causing bacterial cell death.[13] Moringa seed extract containing secondary metabolites is formulated with a gel base and gelling agent. The formulation consists of 5 gel formulations with different concentrations of Moringa seed extract, namely 5%, 10%, 20%, 40%, and 80%. The results of the evaluation of the physical characteristics of the gel preparation are in Table 2. Organoleptic tests showed that all formulations had optimal shape or consistency, colour, and odour. Its normal consistency is semi-solid, clear yellowish, or yellow because it is mixed with Moringa seed extract, which is yellow and has a distinctive gel and Moringa seed aroma. The homogeneity test on all formulations showed that it was homogeneous, meaning that mixing the gel bacilli was evenly distributed and positively affected the homogeneity of the gel preparations. The pH assessment of the Moringa seed extract gel preparations for all formulations was good according to standards, meaning that the gel is safe for the skin and has the potential not to irritate. The gel spreadability test showed that all formulations were optimal and ensured even distribution of the gel when applied to the skin. The last test was the antibacterial activity test of moringa seed gel preparations from various formulations. Antibacterial test results on Moringa seed extract gel against *S. aureus* in Figure 1 and Table 3. The

results of measuring the average inhibition zone diameter of Moringa seed gel formulations against *S. aureus* showed that formulation 5, with an 80% concentration (11.70 mm), was categorized as strong, while formulation 4, with a 40% concentration (5.05 mm), was categorized as medium. Formulations 1, 2, and 3, with concentrations of 5%, 10%, and 20%, respectively, as well as the negative control (-), were classified as weak, as they exhibited no inhibition zone (0 mm). In contrast, the positive control (+), which used clindamycin gel, was categorized as very strong, producing an inhibition zone of 32.57 mm.[14] These findings align with research by Anggia, Sri, and Agung (2020), which reported that *Moringa oleifera* L. seed extract demonstrated the highest antibacterial activity at a 75% concentration, with an inhibition zone diameter of 14.75 mm against *S. aureus*.[15] However, when compared with previous studies, this research yielded differing results. A study by Ali and Utari (2020) found that Moringa seed extract at concentrations of 5%, 10%, 20%, and 40% produced inhibition zone diameters of 6.7 mm, 7.21 mm, 7.23 mm, and 7.6 mm, respectively. In contrast, the present study showed no antibacterial activity at 5%, 10%, and 20% concentrations. One factor influencing the inhibition zone diameter is the concentration of the gel base, carbopol 940. Higher carbopol 940 concentrations increase gel viscosity, making it more difficult for *S. aureus* to diffuse, ultimately reducing the antibacterial inhibition zone diameter.[16] This study used clindamycin gel as a positive control and sterile distilled water as a negative control. Clindamycin was chosen as a positive control because it effectively treats infections caused by anaerobic bacteria and is active against Gram-positive bacteria. It functions by inhibiting bacterial protein synthesis at the 50S ribosome level.[17] Meanwhile, the negative control exhibited no inhibition zone since sterile distilled water has no antibacterial properties. For data analysis, a homogeneity test was performed using Levene's test, which indicated non-homogeneous data (*p-value* > 0.05). Consequently, the Post Hoc test used is the Dunnett T3 test because the data is not homogeneous. This test determines which treatment groups have a significant difference in the mean diameter of the inhibition zone. On the results of the Dunnett T3 test, the treatment group had a significant difference in the mean or p value < 0.05, namely formulation 1 with formulation 5 and control (+), formulation 2 with formulation 5 and control (+), formulation 3 with formulation 5 and control (+), formulation 4 with control (+), formulation 5 with formulation 1, formulation 2, formulation 3, control (+), and control (-), control (+) with the five formulations and control (-), and control (-) with formulation 5 and control (+).

## CONCLUSION

Formulations 4 and 5 are optimal formulations of moringa seed extract gel preparations because they have optimal physical characteristics and antibacterial activity against *S. aureus*.

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