Antibacterial Activity Test of Noni Leaves (*Morinda citrifolia* L.) Ethanol Extract Ointment Against *Staphylococcus aureus* Bacteria

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**ABSTRACT**

Noni leaves (*Morinda citrifolia* L.) are known to provide antibacterial properties and can be used in ointment formulations to prevent or eliminate germs that might cause infections, such as *Staphylococcus aureus*. This research aimed to formulate an ointment containing noni leaf ethanol extract that fulfills the physical quality test standards and to determine the antibacterial activity of various concentrations of ethanol extract of noni leaves in ointment preparations against *Staphylococcus aureus* bacteria. The extraction technique employed was maceration with 96% ethanol. The extracted substance was formulated into ointments with concentrations of 2.5%, 5%, and 10%. The antibacterial activity of the ointment was evaluated using the agar diffusion method with wells. Organoleptic, homogeneity, pH, spreadability, and adhesion tests demonstrated that the ointment at the three concentrations of 2.5%, 5%, and 10% satisfied the physical quality test standards. The antibacterial activity test revealed that a 96% ethanol extract of noni leaves inhibited the growth of *Staphylococcus aureus* bacteria with the maximum diameter of the inhibition zone at a concentration of 10% (15.26 mm) and was classified as strong.

**INTRODUCTION**

Antibacterials are substances that can inhibit or eliminate bacteria that cause infection. Bacteria or pathogenic microorganisms cause infections, and these microbes can enter the body's tissues and multiply in the tissues. Among the bacteria that can cause infection is *Staphylococcus aureus*, which is a gram-positive bacterium that belongs to the normal flora of the skin. *Staphylococcus aureus* can cause infectious diseases in hair follicles and sweat glands, boils, and wound infections (Astriani *et al.*, 2021).

Plant extracts contain secondary metabolites that have antibacterial properties. Several phytopharmaceutical studies have employed plants to treat *Staphylococcus aureus* infections. Setyani *et al.* (2016) used Som Jawa (*Talinum paniculatum* (Jacq.) Gaertn) leaves to make antibacterial creams to treat *Staphylococcus aureus*. Furthermore, Naibaho *et al*. (2013) reported using basil leaf extract (*Ocimum sanctum* L.) to make an ointment on the skin of rabbits infected with *Staphylococcus aureus*. Considering the benefits of these plant medicines, it is essential to explore other plants that could potentially be utilized for treating *Staphylococcus aureus* infection.

Noni leaves are used as compresses to heal injured skin parts and reduce pain. In some areas, noni leaf infusion extract is consumed for treatment and analgesics. Noni leaves contain active compounds that function as antibacterial, and are bactericidal. The ethanol extract of noni leaf (*Morinda citrifolia* L.) can also inhibit *Staphylococcus aureus* growth. The inhibition zone of noni leaf extract on *Staphylococcus aureus* at 25% concentration was 6.35 mm, at 50% concentration was 6.73 mm, and at 75% concentration was 6.86 mm indicating that noni leaves have a moderate inhibition zone against
the growth of *Staphylococcus aureus* according to the Clinical and Laboratory Standards Institute (CLSI) (Erina *et al*., 2019). Noni plants contain several active compounds, namely anthraquinones, alkaloids, flavonoids, acubin, alizarin, tannins, and triterpenes (Baroroh *et al*., 2014; Djauhariya *et al*., 2016). Noni leaves also contain antibacterial chemical components such as anthraquinones, alkaloids, saponins, flavonoids, and terpenoids (Kameswari *et al*., 2013). One of the pharmaceutical preparations that is easily applied to the skin for the treatment of *Staphylococcus aureus* infection is an ointment. Therefore, the primary objective of this research was to create a noni leaf ointment with 96% ethanol extract that was antibacterial against *Staphylococcus aureus*. The other purposes of this study were to formulate an ointment containing noni leaf ethanol extract that fulfills the physical quality test standards and to determine the antibacterial activity of various concentrations of ethanol extract of noni leaves in ointment preparations against *Staphylococcus aureus* bacteria.

**METHODS**

**Extraction**

Noni leaf samples were taken from the noni plants growing in Makassar City, with the coordinates of 5° 07'36.6"S 119° 25'37.3"E. Extraction used the maceration method, namely 600 grams of noni leaves with a ratio of 1:10 (600 grams: 6000 ml), so the ethanol needed was 6 liters. The method was as follows: Place leaves into the maceration container, after moistening first with the solvent for 15 minutes, then adding 96% ethanol solvent up to 3.2 liters, stir evenly, then cover and allow to stand for three days while stirring occasionally. After three days, the samples from the maceration were filtered and concentrated using a rotary evaporator to obtain a dark green thick extract.

**Ointment Making**

This ointment was made with two fat bases: adeps lanae and vaseline album, both of which can be melted without previously melting the base. The ointment was made by weighing all of the ingredients according to the design formula. Then, the ethanol extract of noni leaves was put into a heated mortar. First, propylene glycol was gradually added while crushing until the mixture was homogeneous and smooth. Then, the process was repeated with the addition of adeps lanae, methylparaben, and vaseline album sequentially and alternately one by one to create a homogeneous and soft ointment. Furthermore, the ointment was placed in a container labeled according to its concentration. The design formula is shown in the Table 1.

![Figure 1. The Results of the Formulation of 96% Ethanol Extract Noni Leaf Ointment](image)

<table>
<thead>
<tr>
<th>Table 1. Noni leaf ethanol extract ointment formula design</th>
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<tbody>
<tr>
<td>Materials</td>
</tr>
<tr>
<td>Noni Leaf Ethanol Extract</td>
</tr>
<tr>
<td>Adeps lanae</td>
</tr>
<tr>
<td>Propylene glycol</td>
</tr>
<tr>
<td>Methylparaben</td>
</tr>
<tr>
<td>Vaseline album ad</td>
</tr>
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<tr>
<th>Table 2. Organoleptic test results of noni leaf 96% ethanol extract ointment</th>
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<tr>
<td>Physical Properties of Preparations</td>
</tr>
<tr>
<td>Color</td>
</tr>
<tr>
<td>Smell</td>
</tr>
<tr>
<td>Materialize</td>
</tr>
</tbody>
</table>

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Evaluation of Ointment Preparations

Organoleptic tests were conducted by observing the ointment preparations' shape, color, and smell. A good ointment preparation is a semi-solid preparation with a color-like extract and a characteristic odor from the sample or does not have a stinky smell (Naibaho et al., 2013).

The homogeneity test was conducted by observing the results of applying the ointment to the glass plate. Homogeneous ointments are characterized by the absence of lumps in the smearing results, an even structure, and a uniform color from the start to the end of the application (Elmitra, 2017).

The pH test was conducted using a pH meter dipped in the ointment preparation. The pH value of a good ointment is 4.5-6.5 (Soemarie et al., 2016).

Test the spreadability of the ointment; namely, as much as 0.5 g is placed on a glass plate with a diameter of 15 cm, another glass is placed on it and left for 1 minute, and then the diameter of the ointment spread is measured. After that, add a load of 100 g and then 150 g alternately every 1 minute and then measure the diameter of the constant power spread. A good range of ointment spreadability is 5-7 cm (modification from Soemarie et al., 2016).

Adhesion test: as much as 0.1 g of the ointment preparation was weighed and placed on a glass object, and another glass object was placed on it. Then pressed with a load of 100 g for 1 minute; after that, it was released then the time was recorded until both were released (modification from Putri et al., 2020).

Preparation of NA (Nutrient Agar) Medium
Composition:
- Meat extract: 3 g
- Agar: 15 g
- Peptone: 5 g
- Distilled water ad: 1000 mL
- pH: 7

Ways of making:

All ingredients were put into the Erlenmeyer and dissolved with distilled water up to 1000 mL, then heated until the ingredients were entirely dissolved then the pH was measured until it reached pH 7. They were sterilized in an autoclave at 121°C with a pressure of 2 atm for 15 minutes for sterilization.

Preparation of Test Bacteria
The test bacteria used were Staphylococcus aureus obtained from stocks of the Laboratory of Microbiology, Makassar High School of Pharmacy.

Rejuvenation of Staphylococcus aureus bacteria: Staphylococcus aureus bacteria were rejuvenated by inoculating one loop of NA slanted medium in a test tube by scraping aseptically and inoculating at 37 °C for 24 hours.

McFarland Solvent Making: This step was done by taking 99.5 ml of 1% H₂SO₄ solvent mixed with 0.5 ml of 1.175% BaCl₂ solution in a 100 mL Pyrex® Erlenmeyer, shaking until a cloudy solvent was formed. This turbidity is used as a standard for the turbidity of the bacterial test suspension.

Preparation of Bacterial Suspension: Rejuvenation tests of bacteria 24 hours old were taken using a loop, then suspended with sterile 0.9% NaCl as much as 10 ml, put into a clean test tube, and homogenized until entirely mixed. Then observed and compared turbidity with McFarland solvent.

Sample Testing: Antibacterial activity test of noni leaf 96% ethanol extract ointment formulation against Staphylococcus aureus was conducted by the agar diffusion method using a 6 mm diameter reservoir. The preparation of the test medium begins by making the base layer Based layer by pipetting 10 ml of NA (Nutrient Agar) medium and then pouring it into a cup and allowing it to solidify; then, on the surface of the medium placed five scavengers then, adding 5 ml of NA medium which has been added to the bacterial test suspension. Allowed to solidify after solidification, the buffer is removed. The three formulated ointments without diluting, the negative control (additives without active substance) and the positive control (tetracycline) were put into the wells using a micropipette of as much as 0.1 µl. The Petri dishes were labeled to differentiate the tested samples and then incubated at 37 °C for 1x24 hours in an incubator, and then the diameter of the inhibition zone that occurs was observed, measured and recorded.

RESULTS AND DISCUSSION

The sample used in this study was noni leaf extracted using 96% ethanol because it has less water content to avoid damage to the extract due to microbial growth and is not toxic. The extraction of 600 grams of noni leaves obtained a 10.03% yield (60.21 grams thick section). Pictures of noni leaf 96% ethanol extract ointment can be seen in Figure 1.
The physical quality tests are conducted to determine that the preparations meet the requirements of an excellent physical quality test following the provisions of topical preparations. The bases used in the formulation of ointment preparations are hydrocarbon bases, adeps lanae, and vaseline album. This base is chosen because adeps lanae has a sticky nature and is easier to wash with water than a fatty base. Vaseline album can provide optimum stability for several active substances and as an emollient that can maintain skin moisture. Adeps lanae and vaseline albums can retain water and reduce the ointment’s consistency when combined (Alfilaili et al., 2022). Methylparaben cannot dissolve in a fatty base. However, adding other ingredients, namely propylene glycol, can increase the solubility and the antibacterial activity of methylparaben so that the components in noni leaf ointment are compatible. (Parrott, 1970; Rowe et al., 2006).

The organoleptic test was conducted by observing the preparation's physical properties in the ointment’s shape, color, and smell. The criteria for a good ointment preparation are preparations that are semi-solid in condition, have a color extract, have a characteristic odor of sections, or do not have a stinky smell. Judging from the results of the organoleptic testing, Table 2 shows that the ointment base formulation and the three ointment concentrations meet the criteria for a good ointment (Lasut et al., 2019).

The homogeneity test determines which ointment is homogeneous or evenly mixed between the active substance and the ointment base. The homogeneity test was done by observing the results of applying the cream to the glass plate. No lumps characterize a homogeneous cream at the time of application, an even structure, and a uniform color from the application’s start to the end. The ointment base and noni leaf ethanol extract ointment with concentrations of 2.5%, 5%, and 10% in this test had good homogeneity (Table 3), and it can be concluded that this ointment met the homogeneity requirements (Elmitra, 2017).

### Table 3. Homogeneity, spreadability, pH, and adhesion test results of noni leaf 96% ethanol extract ointment

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Homogeneity Test</th>
<th>Spreadability Test (cm)</th>
<th>pH Test</th>
<th>Adhesion Test (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before Load Added</td>
<td>Load 100g</td>
<td>Load 150g</td>
</tr>
<tr>
<td>Ointment Base</td>
<td>Homogeneous</td>
<td>5.32</td>
<td>6.22</td>
<td>6.02</td>
</tr>
<tr>
<td>Concentration Ointment 2.5%</td>
<td>Homogeneous</td>
<td>5.02</td>
<td>5.75</td>
<td>4.96</td>
</tr>
<tr>
<td>Concentration Ointment 5%</td>
<td>Homogeneous</td>
<td>5.12</td>
<td>6.15</td>
<td>5.14</td>
</tr>
<tr>
<td>Concentration Ointment 10%</td>
<td>Homogeneous</td>
<td>5.17</td>
<td>6.17</td>
<td>5.26</td>
</tr>
</tbody>
</table>

### Table 4. Results of antibacterial activity test measurement of inhibitory power zone of noni leaf 96% ethanol extract ointment

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Ointment Concentration</th>
<th>(+)</th>
<th>(-)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5%</td>
<td>5%</td>
<td>10%</td>
</tr>
<tr>
<td>Replication</td>
<td>Barrier Diameter (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>7.74</td>
<td>9.9</td>
<td>12.08</td>
</tr>
<tr>
<td>II</td>
<td>8.04</td>
<td>10.54</td>
<td>16.72</td>
</tr>
<tr>
<td>III</td>
<td>11.32</td>
<td>15.21</td>
<td>16.98</td>
</tr>
<tr>
<td>Amount</td>
<td>27.1</td>
<td>35.65</td>
<td>45.78</td>
</tr>
<tr>
<td>Average</td>
<td>9.03</td>
<td>11.88</td>
<td>15.26</td>
</tr>
</tbody>
</table>

**Figure 2.** Activity Test of Noni Leaf Extract Ointment (*Morinda citrifolia* L).

Information: a) Concentration 2.5%; b) Concentration 5%; c) Concentration 10%; d) Negative control
The spreadability test aims to see the ability to spread ointment preparations on the skin, where the ointment base must have good spreadability to ensure maximum drug absorption. The results of the spreadability test in Table 3 show that there are differences in the area of spreadability of noni leaf ointment before and after adding the load, namely for a 2.5% concentration of 5.75 cm, a 5% concentration of 6.15 cm, a 10% concentration of 6.17 cm and an ointment base of 6.22 cm from the results obtained. The spreading power of the four ointment preparations decreased, whereas the ointment base has the most excellent spreading power. The result shows that the consistency of the ointment is softer because the use of adeps lanae and Vaseline album as a base will affect the preparations’ spreadability. Adeps lanae and albumin vaseline can hold water. They can also reduce the consistency of the ointment preparation, causing the spreading power to increase with each additional load for the three ointment preparations with a concentration of 2.5%, 5%, and 10% (Sandi and Yaumi, 2018). The results follow the literature, which indicated that a good range of spreadability of ointment preparations is 5-7 cm (Lasut et al., 2019).

pH testing is conducted to determine the nature of the ointment in irritating the skin. The pH test is done by dipping the pH meter into the ointment preparation. The results in Table 3 of noni leaf extract ointment at a concentration of 2.5% have a pH of 4.96, a concentration of 5% has a pH of 5.14, and a concentration of 10% has a pH of 5.26. The results show that noni leaf extract ointment with concentrations of 2.5%, 5%, and 10% have met the pH value requirements for topical preparations, namely 4.5-6.5, will not irritate when applied to the skin and have met the requirements pH value for ointment preparations that are good and safe for human skin, while is 4.0-6.5 (Novasella et al., 2022; Sari and Maulidya, 2016; Tranggono and Latifah, 2007).

The adhesion test was conducted to determine the ability of the ointment to adhere to the skin. The longer the cream is attached to the skin, the greater the absorption of the drug because the bond that occurs between the ointment preparation and the skin is longer, conversely if the ointment is easily separated from the skin, the absorption of the active substance/drug substance is less than optimal (Ismarani et al., 2014; Yusuf et al., 2017). The adhesion test results in Table 3 show that the noni leaf extract ointment time increased from 35-95 seconds to adhere to the skin. For an ointment base, the adherence time is 35 seconds; an ointment with a concentration of 2.5% adheres to 41 seconds; a cream with a 5% concentration adheres to 75 seconds; and a balm with a 10% concentration adheres to 95 seconds. The results showed that the ointment with a concentration of 10% adhered longer than the ointment with a concentration of 2.5% and 5%, the results obtained from the three formulations fulfilled the adhesion test requirements, namely not less than 4 seconds (Prasetya et al., 2016). That is due to the influence of the base of the ointment used, which is a fatty hydrocarbon base. As a result, its bond with the active substance of the ethanol extract of noni leaves becomes strong, which allows the preparation time to contact the skin longer so that the penetration of the ointment can maximize better drug absorption (Ulaen et al., 2012).

The effect of the concentration of noni leaf extract in the form of an ointment formulation was tested on inhibition of the growth of *Staphylococcus aureus* bacteria using the agar diffusion method using wells. The choice of the method using wells is due to the view of the ointment preparation, which uses a heavy base, so it is possible to test the antibacterial activity using this method. The advantages of the sound method are that it makes it easier to calculate and measure the area of the clear zone formed around the wells because the bacterial isolates can move to the bottom of the NA media (Misna and Diana, 2016). Testing using a suitable method can produce a more expansive inhibition zone (Haryati et al., 2017). The agar diffusion method was used to determine the diameter of the inhibition zone formed. The results of measuring the diameter of the inhibition zone showed that noni leaf extract in ointment preparations with concentrations of 2.5%, 5%, and 10% could inhibit the growth of *Staphylococcus aureus* bacteria during the 24-hour incubation period, which was indicated by the formation of clear zones around the reservoir holes. The result shows that noni leaf extract ointment contains antibacterial compounds in noni leaves, namely flavonoids, saponins, and tannins, which can inhibit the growth of *Staphylococcus aureus* bacteria (Afiff and Amilah, 2017; Simatupang et al., 2017).

The result further showed that noni leaf extract in ointment preparations produced an average inhibition zone diameter at a
concentration of 10%, 15.26 mm. The lowest average diameter of the inhibition zone at a concentration of 2.5% was 9.03 mm (Table 4 and Figure 2). This finding follows the literature, which indicated that the higher the concentration, the larger the diameter of the inhibition zone (Pelczar and Chan, 1988). Other literature stated that antibacterial activity is based on the diameter of the inhibition zone, which is <5 mm, which is categorized as weak, while the inhibition zone of 5-10 mm is classified as moderate, and the inhibition zone of 11-20 mm is categorized as strong. The inhibition zone of >20 mm is categorized as very strong (Davis and Stout, 1971; Simatupang et al., 2017).

The negative control did not form an inhibition zone. Based on the study results, it can be concluded that noni leaf extract ointment additives do not have antibacterial properties against Staphylococcus aureus bacteria. The positive control (antibiotic sensitivity disks tetracycline 30 µg) had a more effective inhibition zone diameter of 26.11 mm. This is because tetracycline is included in a broad-spectrum and bactericidal class of antibiotics that work by inhibiting protein synthesis in bacterial cells, thereby slowing bacterial growth and having the ability to fight pathogens such as gram-positive bacteria (Staphylococcus aureus) and gram-negative bacteria (Escherichia coli) (Djide and Sartini, 2008; Sanu et al., 2015).

CONCLUSIONS

The results of the physical quality tests of the ointment preparations at concentrations of 2.5%, 5%, and 10% and the ointment base have met the requirements for organoleptic, homogeneity, pH, spreadability, and adhesion tests for ointment preparations.

The results of the antibacterial activity test of the 96% ethanol extract of noni leaves in the form of an ointment preparation showed that the 96% ethanol extract of noni leaves could inhibit the growth of Staphylococcus aureus bacteria with the largest diameter of the inhibition zone at a concentration of 10% (15.26 mm) and categorized as strong.

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