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Antibacterial Activity Test of Moringa Leaf Ethanol Extract Ointment of *Moringa oleifera Lamk*. on *Staphylococcus aureus* Bacteria

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ABSTRACT

Staphylococcus aureus is gram-positive bacteria and can cause infections and disorders of the skin. This study aims to determine the physical stability of ointment preparations containing ethanol extract from Moringa leaves based on variations in concentration and antibacterial activity against *Staphylococcus aureus*. This research is a laboratory experiment with the stages of making an ointment from the ethanol extract of Moringa leaves and testing its antibacterial activity against *Staphylococcus aureus*. Data analysis using One-Way ANOVA. The results of the research on moringa leaf ethanol extract ointment preparations were physically and chemically stable through organoleptic testing, homogeneity, dispersion, viscosity, and pH by including a cycling test. Antibacterial activity test showed that moringa leaf ointment had antibacterial activity against *Staphylococcus aureus* with the diameter of the inhibition zone each extract concentration of 15% (16.1mm), 20% extract concentration (17.3mm), 25% extract concentration (18.1mm) in inhibiting bacteria staphylococcus *aureus.* Ethanol extract ointment preparations with each concentration have a strong category of inhibition against the *Staphylococcus aureus* bacteria.

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Introduction

Traditional medicine has long been known and used by people in Indonesia for the purpose of treatment and health care. The use of medicinal plants as medicine in the community continues to grow and be passed on to the next generation. Traditional medicine develops starting from several traditional ingredients that have been used in the community and then develops into an herb that is believed to have certain properties for the human body [1].

Skin is a layer of tissue that covers all parts of the body from dangers that come from outside. The skin usually gets injured, whether it's a scratch, an infection, or a burn. A wound is a condition where body tissue is damaged caused by several factors, such as trauma, animal bites, scratches from sharp objects, and others [2].

Wounds are a problem that is often experienced by every human being, but they are often considered light, even though wounds can cause infection. Therefore wound management is necessary to prevent the risk of infection, which can make the wound worse and difficult to heal [3].

Boils (furuncles) are infections that often occur on the skin, characterized by a reddish lump on the skin that enlarges to reveal pus spots or are called pus eyes caused by the bacteria *Staphylococcus aureus,* and because of that, boils can also be interpreted as a local infection of the deep skin. This ulcer disease can affect anyone, from infants to children, considering that the body's immune system is still susceptible to disease, but that does not mean that adults are free from boils. Boils can attack almost all parts of the body, especially the parts where there are folds that allow frequent friction, such as the armpits and buttocks [4].

Infectious disease is a disease caused by pathogenic microbes and is very dynamic. The process of the occurrence of infectious diseases generally involves three factors that often interact, namely: disease-causing factors (agents), human factors or hosts (hosts), and environmental factors. One of the infectious diseases that can be caused by the bacteria *Staphylococcus aureus, namely* skin infections and open wounds such as ulcers, burns, and surgical scars, which are likely to be infected by bacteria[5].

In humans, the bacteria *Staphylococcus aureus* could cause various kinds of infectious diseases, namely infections of the skin, ulcers, and furunculosis. Besides that, *Staphylococcus aureus* can also cause infection of postoperative wounds in hospitals. Bacteria *Staphylococcus aureus* is a spherical gram-positive bacterium and is the main pathogenic bacteria in humans. This bacterium can cause various infections, such as acne and boils, and can also cause serious and fatal infections, which generally occur in contaminated post-surgical patient wounds [6].

Plants are a source of various types of chemical compounds that are efficacious as drugs [7]. The utilization of plants as medicine is an alternative way to prevent and treat disease

because they are considered not to cause many side effects. Besides that, it can reduce the level of resistance to antibiotics. Increased bacterial resistance to antibiotics can provide a great opportunity to obtain antibacterial compounds by utilizing bioactive compounds as a result of secondary metabolism from the richness of biodiversity.

Extracts from various plants have shown an important role in inhibiting pathogens; even the use of plant extracts with antibacterial activity can prevent infection. Moringa is a vegetable tree that is highly nutritious and has various benefits [8]. Moringa leaves contain a variety of useful chemical substances, while the phytochemical content contained in moringa leaves includes tannins, steroids and triterpenoids, flavonoids, saponins, anthraquinones, and alkaloids and contains minerals, essential amino acids, antioxidants and vitamins [9].

Leaf extracts and seeds from the moringa plant contain compounds that have antibacterial properties, so they can be used as an infection medicine. Based on research conducted by Ref. [10], by testing the antibacterial activity of Moringa leaf extract on bacteria *Escherichia coli* and *Staphylococcus aureus*, using a concentration of 5% has the smallest antibacterial inhibition results. Ref. [11] also said that Moringa leaf extract could be used as an antibacterial against *Staphylococcus aureus* with an inhibition zone of 20.50 mm with a concentration of 80%.

Based on this background, researchers will conduct research on the formulation of Moringa leaf ethanol extract *Moringa oleifera Lamk.* in the form of an ointment against bacteria *Staphylococcus aureus* with concentrations of 15%, 20%, and 25%. Looking at the content contained in moringa leaves which are so large, and the plants are easy to obtain and easy to use, it attracts the interest of researchers to be able to explore the active ingredients contained in these moringa leaves.

Methods

The instrument used to test the antibacterial activity was sterilized in an autoclave at 121°C for 15 minutes [12]. Na media (Nutrient agar) is prepared by weighing 2 grams of NA. Then put it in an Erlenmeyer flask, dissolve it with 100 ml of distilled water, and heat it on a hot plate until the NA dissolves and is clear in color. Then put it in the autoclave for 15 minutes at 121°C.

Preparation of MHA media *(mueller hinton agar)* was carried out by weighing as much as 38 grams according to the composition on the package (2g beef extract, 17.5 g *casein hydrolysate*, 1.5 g starch, and 17 g of jelly), then put into an Erlenmeyer flask and dissolve it in 1 L of aqua dest with the help of heating using a hot plate for ± 10 minutes until the media is dissolved and clear. Then the media was put into the autoclave for 15 minutes at 121^0C. The media that has cooled is then put into a petri dish of as much as 5 ml and allowed to solidify.

Prepare a test tube, then put 3 mL of NA medium into the test tube. Then sterilized in the autoclave for 15 minutes at 121^{0}C. Then lay it horizontally. After the medium thickens, the test tube is straightened, and the medium is tilted.

The bacteria used are bacteria *Staphylococcus aureus*. Bacterial inoculation was carried out by instilling 1 ose of bacteria into 3 ml of slanted NA medium. Done by scraping, then incubated for 24 hours at 37^{0}C. Then the bacterial colonies that have formed are taken with an ose needle and suspended by placing them in a test tube containing 10 ml of 0.9% NaCl.

After that, 0.2 ml of the suspension was taken and poured into 10 ml of NA media in a test tube and homogenized, then the mixture of bacterial suspension with NA was put into a petri dish containing ± 5 ml of NA media. The antibacterial test of moringa leaf extract ointment was carried out using MHA media which aims to grow the bacteria *Staphylococcus aureus.* Antibacterial testing of Moringa leaf extract ointment is done by using the agar diffusion method with the *well* technique. This is done by making wells on MHA media (so that the diameter of the wells is ±6.7 mm). In the MHA media which had been planted with the test bacteria, 5 wells were made in each plate using an iron scraper, then in each well, a negative control (ointment base), positive control (gentamicin sulfate ointment) was added, 15% concentration of Moringa leaf extract ointment. , 20%, and 25%, after which it was incubated at 37oC for 1 time 24 hours. After that, the inhibition zone was measured using a vernier caliper.

Data were analyzed and processed using *Analysis Of Variance* (ANOVA) using the SPSS program. Data is tested first by testing normality and then homogeneity as a requirement of data analysis before conducting *an Analysis Of Variance* (ANOVA). The normality test aims to show whether the data that is carried out has a normal distribution or not. Normality is fulfilled if the results are significant (α = 0.05). If the significant value is greater than α, then the data is normally distributed. Conversely, if the significance value is less than α, it means that the data is not normally distributed [13].

After that, a homogeneity test is carried out. The aim of this test is to find out whether the variances of the several populations show the same or not. If the significant value in the homogeneity test is less than α, then the variances of two or more population data groups are not the same. Conversely, if the significant value is greater than α, then the variance of two or more data population groups is the same [13].

Results and Discussion

Antibacterial activity testing was carried out using moringa leaves 96% ethanol extract ointment moringa *oleifera Lamk.*) in inhibiting bacterial growth with concentrations of 15%, 20%, and 25%. The selected bacteria are *Staphylococcus aureus*, where this bacterium is a bacterium that is widely found on the skin.

The method used to see the activity of bacteria is using the *well* method, according to Ref. [14]. The advantages of the *well* method are that it is easier to measure the area of the inhibition zone formed because it can move not only outside the surface but to the subsurface, while the drawback of this method is that the media is very susceptible to contamination when making holes and when entering samples because it often opens the cup. The principle of this method is to make holes in the agar that has been inoculated with bacteria, then each concentration of the formula is dripped into each well that has been made. The inhibition of the growth of microorganisms can be seen by the presence of an inhibition zone (clear area) formed around the wellbore.

Table 1. The diameter of the inhibition zone

Formula	Replication			Diameter	Category
	I	II	III		
Basis	0	0	0	0	No
BE	15	17	16.5	16.1	Strong
BE	18	17	17	17.3	Strong
SONS	18.3	17	19	18.1	Strong
K+	19.7	19.3	22.3	20.4	Very strong

Remark:
Basis : Ointment without extract
BE : Ointment of ethanol extract of moringa leaves with a concentration of 15%
BE : Ointment of ethanol extract of moringa leaves with a concentration of 20%
SONS : Ointment of ethanol extract of moringa leaves with a concentration of 25%
K(+) : positive control
K(-) : negative control
>20mm : very strong
10-20 : strong
<5mm : weak

In this test, the positive control used was 0.1% gentamicin sulfate ointment. The reason for selecting the positive control was because the mechanism of action of gentamicin sulfate ointment was to inhibit protein synthesis and can cause errors in translocation of the genetic code to inappropriate codon-anticodon interactions and can cause the breakdown of polysomes into nonfunctional monosomes, which can result in cell death.

Based on the microbiological evaluation results for each formula and the average diameter of inhibition varied, we found out that the higher the concentration of Moringa leaf extract, the greater the inhibition obtained. In the formulation of 96% ethanol extract of moringa leaves with a concentration of 15% obtained an inhibition zone of 16.3 mm in the strong category, formula II 96% ethanol extract of moringa leaves with a concentration of 20% obtained an inhibition zone of 17 mm in the strong category, and formula III extract ethanol 96% Moringa leaves with a concentration of 25% obtained an inhibition zone of 18.3 mm in the strong category. It was stated that the 96% ethanol extract ointment of moringa leaves with these 3 formulations had an inhibitory effect on the bacteria *Staphylococcus aureus.* This is due

to the presence of active compounds that act as antibacterials, such as flavonoids, alkaloids, tannins, saponins, and vitamins. This is indicated by phytochemical screening by Ref. [15]. As for formulas I, II, and III, they have the same inhibitory potential, which falls within the *strong* category. However, still, the strongest formula is formulation 3. This is because the greater the concentration of the extract contained in the preparation, the greater the inhibition zone formed, and also the smaller the zone. The inhibition formed can be influenced by biological factors and chemical factors. Biological factors include plant species, harvesting time, plant extraction chemical factors, and extraction methods. The concentration of 5% has the smallest inhibition, namely <5 mm (weak category).

Conclusion

Preparation of moringa leaf ethanol extract ointment *Moringa oleifera Lamk.* has antibacterial activity against *Staphylococcus aureus,* and formulations of moringa leaf ethanol extract ointment preparations with concentrations of 15%, 20%, and 25% can provide antibacterial effects against *Staphylococcus aureus*. Further research is needed regarding whether antibacterial can be used with other ingredients. Nevertheless, this study has demonstrated that *Moringa oleifera Lamk.* has antibacterial properties, and the formula can be made into other forms, such as lotions, creams, and pastes.

Conflict of Interest

The authors declare that there is no conflict of interest.

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