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ETANOL-ACTIVATED ANTIBACTERY EXTRACT OF KELOR LEAVES (*Moringa oleifera* L.) AGAINST *Staphylococcus aureus* ATCC 43300

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ABSTRACT

Kelor leaves (*Moringa oleifera* L.) have active compounds such as flavonoid, alkaloid, phenol, tanin, and saponin. The content of these compounds is stated to have antibacterial activity. the development of an alternative treatment of natural ingredients is needed to treat infections caused by *Staphylococcus aureus* bacteria, one of which is Moringa leaves *(Moringa oleifera* L.). To analyze the antibacterial activity from ethanol extract of moringa leaf in inhibiting of *Staphylococcus aureus* growth at concentrations of 25%, 50%, and 100%. The method used is a true experimental posttest-only control group in vitro. The samples of this study were divided into five groups: the positive control group (vancomycin) and the negative control group (96% ethanol solvent), with concentrations of 25%, 50%, and 100%. The results proved that the extract of ethanol from moringa leaves without dilution (100% of concentration) showed about zone of inhibition against *Staphylococcus aureus* ATCC 43300 bacteria with an inhibition diameter of 8.4 mm and the positive control vancomycin has an inhibition zone diameter of 17.4 mm. Moringa leaf ethanol extract showed inhibition against bacterial growth in *vitro* at a concentration of 100%.

Keywords : Antibacterial activity., *Moringa oleifera* L., *Staphylococcus aureus*

INTRODUCTION

Infectious disease is a disease caused by the presence of a pathogenic microorganism, one of which is the bacterium *Staphylococcus aureus*. This bacterium can be widespread and has become a worldwide health threat and has recently been classified as a high-level II pathogen by the World Health Organization (WHO).[1]

Staphylococcus aureus bacteria are known to attack all body tissues on the skin, joints, respiratory tract, and others. Most *Staphylococcus aureus* bacterial infections occur in patients who have multiple risk factors. It is also known that if this bacteria cannot be handled properly, it triggers bacteremia which can cause around >80% of deaths in hospitals.[2] Most of these *Staphylococcus aureus* bacteria can be found on the surface of the objects we hold and are very easy to identify if someone is infected with these bacteria.[3]

It is known that the use of antibiotics sometimes triggers resistance, so some antibiotics are resistant to *Staphylococcus aureus* bacteria such as ampicillin, amoxicillin, clavulanic acid, penicillin G, sulbenicillin, chloramphenicol, and ciprofloxacin.[3] In addition, this bacterium is also known to be resistant to the semisynthetic beta-lactam class known as *Methicillin Resistant Staphylococcus aureus* (MRSA).[4]

Based on the existing weaknesses, new modalities from natural materials are needed. Moringa plants are easy to find anywhere, especially in tropical environments.[5] One part of the Moringa plant, namely Moringa leaves *(Moringa oleifera* L.), is known to have flavonoid, alkaloid, phenol, saponin, and tannin compounds that have antibacterial properties.

Flavonoid compound work by inhibiting nucleic acid synthesis, cell membrane function, and energy metabolism resulting in cytoplasmic membrane damage.[6] The mechanism of action of alkaloid is by interfering with the components of the peptidoglycan peptidoglycan, causing the cell wall layer to not form intact and the death of cells. intact and the occurrence of cell death.[7] Saponin work by increasing membrane permeability resulting in hemolysis of bacterial cells.[8] Tannin work as antibacterials by interfering with bacterial surface receptors by binding to adhesin proteins in bacteria which will cause inhibition of protein synthesis for cell wall formation and a decrease in bacterial attachment.[9] polyphenols are known to damage bacterial cells by inhibiting the synthesis and cell membrane.[10]

based on this, research on the antibacterial activity test of moringa leaf ethanol extract against staphylococcus aureus ATCC 43300 in vitro needs to be carried out to

determine the inhibitory activity of moringa leaf extract against staphylococcus aureus bacteria. it is hoped that it will be able to become a scientific basis in clinical application for the following.

MATERIAL AND METHODS

This study was designed to determine the anti-bacterial effect of Moringa leaf extract on *Staphylococcus aureus*. Post-test-Only Control Group Design is used in this study. The samples of this study were divided into five groups namely control +, control -, concentration 25%, concentration 50%, concentration 100% and tested using the disc diffusion method *(Kirby-Bauer*). This study was conducted in October-November 2023 at the Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University, and has received approval from the Medical Research Ethics Commission of Udayana University with Ethical Clearance Number*:* 2022.01.1.0405. Following equipments were used in this study: buckets, ovens, analytical scales, blenders, beakers, stir bars, measuring cups, filter paper, rotary evaporators, macerators, test tubes and their shelves, steam baths, electric stoves, sterile cotton cloth, tweezers, Petri dishes, Erlenmeyer tubes, micropipettes, blue tips, yellow tips, autoclaves, paper discs, incubators, and calipers. The materials that used were Moringa leaves *(Moringa oleifera* L.), 96% ethanol, *Staphylococcus aureus* culture, Mueller-Hinton Agar (MHA), Mc Farland 0.5 solution, antibiotic discs, and blank discs. The obtained Moringa leaves were cleaned with running water then dried for 24 hours at room temperature. After that, the leaves were cut into small pieces and mashed using a blender. Then the weight of powder was measured using an analytical balance (g). The powder was dissolved into solvent for 24 hours. The resulting extract was filtered and evaporated using a rotary evaporator with a maximum temperature of 70 ℃ to obtain a thick extract. The obtained extract was collected in a sterile bottle and then stored in the refrigerator.[11] Furthermore, the Moringa leaf ethanol extract was divided into 3 groups, namely 25%, 50%, and 100%. All concentration of extract were used to prove their anti-bacterial property against the growth of *S. aureus* bacteria.

Inhibition testing against *S. aureus* was performed using disc diffusion (Kirby-Bauer) method on a Petri dish containing agar media. The first step of this method was preparing the paper discs on an empty Petri dish. Then, the Moringa leaf ethanol extract with concentrations of 25%, 50%, and 100% were added into each paper disc and they were left there for about 1 hour. After that, the suspended bacteria were applied evenly on the surface of the agar media. Paper discs that filled with ethanol extract were placed in Petri dishes according to the predetermined concentration and control group and incubated in an incubator at 37℃ for 24 hours. After the incubation period was completed, the diameter of the inhibition zone was measured using a caliper. The capability of Moringa leaf ethanol extract as anti-bacterial agent is shown by the diameter of bacterial growth inhibiton sone around the paper disc. Acquired diameter data were analysed using Statistical Package for the Social Sciences (SPSS) software The obtained diameter of the inhibition zones was analysed using normality and homogeneity tests to determine whether the data were normally distributed and homogeneous data or not. For normally distributed and homogenous data, One-Way Anova would be used to assess the difference of inhibition diameter of each class. Meanwhile, if the data were not normally distributed and/or un-homogenous, Specific differences of each class were assessed with Mann-Whitney test.

RESULT

The inhibition diameter of moringa leaf extract in three concentrations (25%, 50%, and 100%), control +, control – against *S. aureus* ATCC 43300 by disc diffusion method is shown in **Figure 1.** Repetititon I to V showed no inhibition zone in moringa leaf extract in concentrations of 25%, 50%, and control -. Meanwhile in control + abd moringa leaf ethanol extract with 100% concentration. There was an inhibition zone with different result in each repetition. Inhibiton zone of moringa leaf ethanol extract can be seen in **Table 1**.

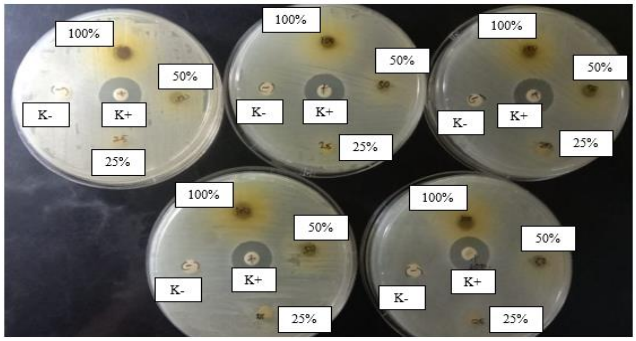

Figure 1. Inhibition zone diameter result data

Table 1. Measurement Result of Zone of Inhibition of Moringa Leaf Ethanol Extract (*Moringa oleifera* L.) Against *Staphylococcus aureus* ATCC 43300

Treatment	Inhibition Zone Diameter (mm)					Mean+-SD
	I	II	III	IV	V	
Control +	17	18	19	16	17	17.4±1.14
Control -	0	0	0	0	0	0
Concentration 25%	0	0	0	0	0	0
Concentration 50%	0	0	0	0	0	0
Concentration 100%	9	7	8	8	10	8.4±1.14

According to normality and, the data were normally distributed in the control + and concentration 100% is 0.814 (p>0.05). However, the homogenity test results showed that the research data is not homogeneous. Thus, non-parametric test was used, namely Mann-Whitney test to evaluate whether there is a significant difference between the group. The results of the Mann-Whitney test can be seen in **Table 2**.

Table 2. Analysis Result Mann Whitney

Treatment	N	Mean+-SD	P-Value
Control +	5	17.4±1.14	0,008
Concentration 100%		8.4±1.14	

The Mann-Whitney analysis test data in Table 2 above shows that the comparison of control + with concentration 100% is <0.05, which means there is a significant difference.

DISCUSSION

Based on the results of the study, it is known that moringa leaf ethanol extract *(Moringa oleifera* L.) can inhibit the growth of *Staphylococcus aureus* bacteria only at 100% concentration. The average diameter of the inhibition zone of each concentration varies and it can be concluded that the inhibitory effect of moringa leaf ethanol extract against *Staphylococcus aureus* depends on the dose or is dose-dependent. To assess the strength of the inhibitory effect, the inhibition zone category is used as a guideline. The inhibition zone is considered weak if its diameter is ≤ 5mm, moderate if its diameter is 5-10mm, strong if its diameter is 10-20mm, and very strong if its diameter is ≥ 20mm.[12] The results of the study in **Table 1** show that moringa leaf extract with concentrations of 25% and 50% did not show significant inhibitory activity. However, at a concentration of 100% moringa leaf extract showed moderate inhibitory activity against *S. aureus* bacteria.

The result of this study is slightly different with previous similar study which is previous study showed anti-bacterial effect in lower concentration. This difference could be caused by difference of extraction process for example the maceration of moringa without stirring and uncontrolled collection time. Furthermore, collection location could affect the content of secondary metabolites that are thought to be active as antibacterials. The decrease in the inhibition zone area occurred due to the high solubility of ethanol solution so that it can attract polar and polar and non-polar compounds in plants.[13,14]

Previous research showed that the concentration with the best effect of Moringa leaf extract in inhibiting the growth of *S. aureus* was 40% compared to concentrations of 30% and 50%.[14] ethanol and aqueous extracts of Moringa leaves have antibacterial activity against the bacteria Staphylococcus aureus hasmaximum inhibitory level compared to water extract which is 9.67 mm.[15] From the description above, compared to previous research with the current study, it is known that the diameter of the inhibition zone produced can vary due to factors affecting extraction, differences in the type of antibacterial test method, and differences in concentration levels.

Vancomycin is an antibiotic produced by *Streptococcus orientalis* and *Mycolatopsis orientalis*. Vancomycin works by interfering with cell wall synthesis and only affects gram-positive organisms with cells.[16]

Staphylococcus aureus is one of the gram-positive bacteria that has a cell wall consisting of a thick peptidoglycan layer and a cell membrane composed of proteins, lipids, and teichoic acids. These bacteria contain polysaccharides and proteins that act as antigens and cell wall structures.[17] The peptidoglycan structure is one of the

reasons that makes the cell wall of gram-positive bacteria easily damaged by antibiotics and certain active compounds contained in plant extracts such as moringa leaves.

Leaves from moringa can contain various compounds that active such as flavonoids, alkaloids, phenols, terpenoids, and tannins. Alkaloids and phenols work by disrupting the peptidoglycan structure found in gram-positive bacterial cell walls. Flavonoids work by inhibiting nucleic acid synthesis, cell membrane function, and energy metabolism. Terpenoids can form strong polymer bonds when interacting with porins, which are transmembrane proteins, causing damage to porins and reducing bacterial cell wall permeability.[18] Tanin compounds inhibit the activity of reverse transcriptase and DNA topoisomerase enzymes and inhibit bacterial cell growth.[19] Steroids can damage lipid membranes causing leakage in liposomes which ultimately results in bacterial death.[20]

CONCLUSION AND ADVICE

Moringa leaf ethanol extract showed inhibition against bacterial growth in *vitro* at a concentration of 100%. The suggestions in this study are the need to consider the location of taking natural ingredients, maceration treatment, and evaporation which risks disrupting the stability of active substances before conducting research, and further examine the modality of natural ingredients that have the potential to inhibit *S. aureus* bacteria.

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