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Original Research Paper

The potential of bilimbi leaf *(Averrhoa bilimbi L.)* extract as an inhibitor of *Staphylococcus epidermidis* growth

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Abstract
Coagulase-negative staphylococci (CoNS) are part of the normal skin flora and are often considered to be minimally pathogenic or even non-pathogenic. However, in certain cases, CoNS bacteria can lead to increased morbidity and mortality, particularly in high-risk patients. This study aims to examine the inhibition zones of *Staphylococcus epidermidis* following treatment with bilimbi *(Averrhoa bilimbi L.)* leaf extract at concentrations of 50%, 75%, and 100%, and to compare the inhibition zones across these three concentrations. This research employed a laboratory experimental design using the Kirby-Bauer disc diffusion method. The results showed that the inhibition zones for *Staphylococcus epidermidis* treated with 50% extract concentration were 12.78 mm, 13.01 mm, and 13.10 mm, classified as intermediate. At a 75% concentration, the inhibition zones were 13.07 mm, 13.40 mm, and 15.12 mm, ranging from intermediate to sensitive. Meanwhile, the 100% concentration produced inhibition zones of 15.64 mm, 16.89 mm, and 17.1 mm, classified as sensitive. A comparative analysis of the inhibition zones indicated that the 100% concentration produced the largest zone of inhibition among the three concentrations tested.

Keywords: *Averrhoa bilimbi L.; inhibition zone; Staphylococcus epidermidis*

1. Introduction

Staphylococcus is a Gram-positive coccal bacterium that is facultatively anaerobic, non-motile, non-flagellated, non-spore-forming, and catalase-positive. Species within the *Staphylococcus* genus are classified based on their ability to produce the enzyme coagulase, resulting in two main groups: coagulase-positive *Staphylococcus* (CoPS) and coagulase-negative *Staphylococcus* (CoNS). Unlike CoPS, which are well-known pathogens, CoNS are part of the normal skin flora and are often regarded as minimally pathogenic or non-pathogenic. However, in certain cases, CoNS can contribute to increased morbidity and mortality, particularly among patients with specific risk factors (Sudirman, 2022; Yazan et al., 2022). As of 2019, approximately 50 CoNS species have been identified. Among them, six species are commonly associated with clinical infections: *S. epidermidis*, *S. saprophyticus*, *S. haemolyticus*, *S. capitis*, *S. hominis*, and *S. lugdunensis*. Certain CoNS species, such as *Staphylococcus haemolyticus* and *Staphylococcus epidermidis*, are often linked to infections resulting from the insertion of foreign medical devices, such as intravenous catheters (Argemi et al., 2019; Smith & Andam, 2021).

The emergence of antibiotic resistance in CoNS has limited treatment options. Notably, *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*, which are significant contributors to nosocomial infections, have shown resistance to methicillin, commonly referred to as methicillin-

resistant coagulase-negative *Staphylococcus* (MR-CoNS), with resistance rates reaching up to 80% (Sudirman, 2022). According to the there are around 20,000 plant species with potential medicinal uses. Plants play an important role in traditional medicine, with various parts such as roots, wood, leaves, flowers, bark, and seeds used in herbal remedies. However, the use of traditional medicine requires scientific validation, including toxicological, pharmacological, and phytochemical studies to isolate and identify active compounds. Medicinal plants are considered promising sources of antibacterial agents, especially in tropical countries like Indonesia, where bacterial infections are prevalent. An advantage of using medicinal plants is their generally lower risk of side effects compared to synthetic drugs.

One such tropical plant widely found in Indonesia is the bilimbi (*Averrhoa bilimbi*), locally known as *belimbing wuluh*. It is known to contain active compounds such as flavonoids, triterpenoids, alkaloids, and tannins, all of which have demonstrated antibacterial properties.(Fadel et al., n.d.; Firmansyah et al., 2022; Hasanah & Novian, 2020; Idrus et al., 2018; Riset et al., 2022). Antibacterial agents are natural chemical compounds that, even at low concentrations, can inhibit or kill bacteria. Determining the minimum inhibitory concentration (MIC) is essential to enhance the efficacy of these compounds and to prevent bacterial resistance, which may occur due to the continuous or excessive use of antibacterial agents. The antibacterial activity of a plant extract is influenced by several factors, including extract concentration, the presence of antibacterial compounds, diffusion capacity, and the type of bacteria being inhibited. (Kuspradini et al., 2016; Marselia et al., 2015; Menon & Satria, 2016).

Based on this background, the author initiated a study entitled **"**Inhibition Zone Test of Bilimbi Leaf (*Averrhoa bilimbi L.*) Extract Against *Staphylococcus epidermidis*," using different extract concentrations: 50%, 75%, and 100%.

2. Research Method

This research was conducted in 2024 at the Research, Publication, and Community Service Unit *(UP3M),* Faculty of Medicine of Universitas Muslim Indonesia. This study employed a true experimental laboratory design using a post-test-only approach with the Kirby-Bauer disc diffusion method to observe the inhibition zone of bilimbi leaf (*Averrhoa bilimbi* L.) extract against *Staphylococcus epidermidis*. The sample used was fresh green leaves, measuring 7–10 cm in length and 1–3 cm in width. The leaves were sterilized using clean water, chopped into small pieces, and immersed in a reagent containing 96% ethanol as the solvent. The mixture was left to stand for 72 hours (3×24 hours), after which it was evaporated using a rotary evaporator until a thick extract was obtained. The concentrations of the extract tested were 50%, 75%, and 100%, prepared using sterile distilled water and DMSO. The resulting inhibition zones were categorized as resistant, intermediate, or sensitive. Gentamicin was used as the positive control, while sterile distilled water served as the negative control. The antibacterial test was carried out by rejuvenating *Staphylococcus epidermidis* on nutrient agar (NA) medium, preparing a bacterial suspension in 0.9% NaCl solution, and spreading paper discs soaked in the extract onto the medium. The Petri dishes were incubated at 37°C for 24 hours, after which the diameter of the inhibition zones was measured to evaluate the antibacterial effectiveness of the extract in comparison with the antibiotic gentamicin. The findings of this study demonstrate the antibacterial potential of bilimbi leaf extract against *Staphylococcus epidermidis*.

3. Results and Discussion

3.1.Result

The inhibition zones formed at various concentrations of bilimbi leaf (*Averrhoa bilimbi* L.) extract against *Staphylococcus epidermidis* are presented in Table 1.

Table 1. Inhibition Zones Formed at Various Concentrations of Bilimbi Leaf (*Averrhoa bilimbi* L.) Extract

Concentration	Inhibition Zone on *Staphylococcus epidermidis*	Interpretation of Inhibitory Response	Conclusion
50%	12.78 mm; 13.01 mm; 13.10 mm	Intermediate	100% Intermediate
75%	13.07 mm;13.40 mm;15.12 mm	Intermediate; Intermediate; Sensitif	33%; Sensitive
100%	15.64 mm; 16.89 mm; 17.11 mm	Sensitive	100%; Sensitive
Control (+) Gentamicin	31.96 mm	Sensitive	100%; Sensitive

Source: Primary Data, 2024

Table 1 shows that an increase in the concentration of the plant extract corresponds to a greater diameter of the inhibition zone. Specifically, the 50% concentration of bilimbi leaf (*Averrhoa bilimbi* L.) extract produced inhibition zones interpreted as intermediate. At the 75% concentration, the results showed both intermediate and sensitive responses. Meanwhile, the 100% concentration resulted in inhibition zones classified as sensitive against *Staphylococcus epidermidis*.

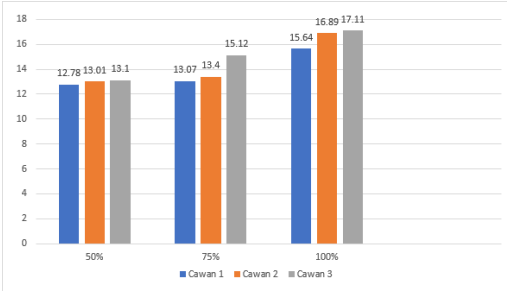

Figure 1. Comparison of the Three Concentrations

Figure 1 shows a significant upward trend, indicating that the higher the concentration of the extract, the larger the inhibition zone produced.

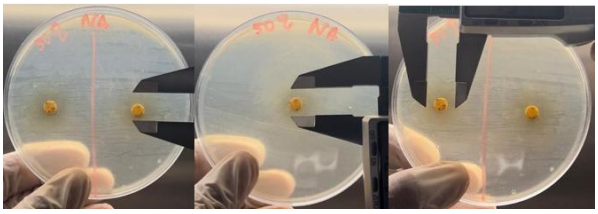

Figure 2. Sensitivity Test Results of Bilimbi Leaf *(Averrhoa bilimbi L.)* Extract at 50% Concentration

Figure 2 shows varying inhibition zone diameters, but all three samples share the same interpretation, categorized as intermediate.

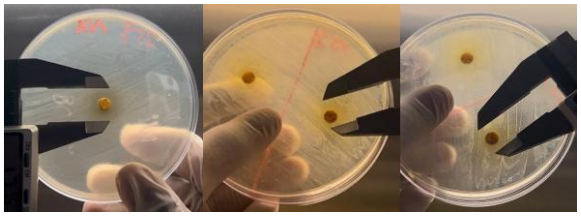

Figure 3. Sensitivity Test Results of Bilimbi Leaf (*Averrhoa bilimbi L.*) Extract at 75% Concentration

Figure 3 shows varying inhibition zone diameters with two interpretations: intermediate and sensitive.

Figure 4. Sensitivity Test Results of Bilimbi Leaf *(Averrhoa bilimbi L.)* Extract at 100% Concentration

Figure 4 shows varying inhibition zone diameters, but all three samples share the same interpretation, classified as sensitive.

3.2.Discussion

Based on the results of this study, it was found that the higher the concentration of bilimbi leaf extract (*Averrhoa bilimbi L.),* the larger the inhibition zone produced. The formation of the clear inhibition zone caused by the bilimbi leaf extract is attributed to the presence of antibacterial compounds such as flavonoids, triterpenoids, alkaloids, and tannins contained in the extract. These antibacterial compounds have different mechanisms of action. Flavonoids, which contain phenolic compounds, effectively inhibit the growth of viruses, bacteria, and fungi by forming complexes and denaturing soluble proteins. This process disrupts the permeability of the bacterial cell wall, leading to the leakage of intracellular bacterial components. Triterpenoids form strong polymer bonds that damage porins, which are channels for the entry and exit of substances in the bacterial cell. The damage to porins causes nutrient deprivation, thereby inhibiting bacterial growth. Alkaloids, then, interfere with the synthesis of peptidoglycan, resulting in the improper formation of the bacterial cell wall. Since the cell wall regulates the bacterial reproductive system, disruption in its formation leads to bacterial death. Lastly, tannins have the ability to prevent plasma coagulation, which interferes with the formation of bacterial body structures, ultimately causing bacterial lysis (Agastia et al., 2021; Habibi, 2017; Hana Putri Gerung & Antasionasti, 2021; Indratama & Yenita, 2019; Rahman et al., 2017; Simanullang et al., 2021).

The bilimbi leaf extract *(Averrhoa bilimbi L.)* at 50% concentration produced an inhibition zone categorized as intermediate, while at 75% concentration, two interpretations were obtained: intermediate and sensitive. At 100% concentration, the inhibition zone was classified as sensitive. Factors influencing the inhibition zone include suboptimal antibiotic absorption, bacterial population size, antibacterial concentration, culture media composition, incubation time, and temperature. The researcher encountered difficulties in evenly spreading the bacteria, which is an indicator of the bacterial population density, on Nutrient Agar medium. The inoculation process requires dropping the bacterial suspension and evenly spreading it using a Drigalsky spreader before incubation. This procedure affects the results and antibacterial activity of the bilimbi leaf extract *(Averrhoa bilimbi L.)* (Martsiningsih et al., 2023). It is important to note that one critical factor enabling Staphylococcus epidermidis, a coagulase-negative species, to survive in harsh environments is biofilm production. Biofilm formation begins with initial adhesion to foreign surfaces or endothelium, leading to accumulation into a multicellular structure. Once formed, biofilms protect bacteria against host defenses, causing clinical manifestations and antibiotic resistance (Agung Dhimasena Widyananda et al., 2021).

Therefore, bilimbi leaf (*Averrhoa bilimbi L.)* can be considered a potential herbal option to inhibit bacterial activity. This is supported by research conducted by Zarwinda. I, et al., (2021) who tested the inhibitory effect of ethanol extract of bilimbi leaves on the growth of *Staphylococcus epidermidis* using the disc diffusion method. Their study showed that the inhibition zones increased with rising extract

concentrations, with measured zones of 15 mm at 100%, 12 mm at 75%, 11 mm at 50%, and 10 mm at 25% concentrations. These results demonstrate that ethanol extract of bilimbi leaves is effective in inhibiting *Staphylococcus epidermidis*, with the largest inhibition zone observed at 100% concentration (15 mm), which falls under the sensitive categor (Zarwinda et al., 2021)

4. Conclusion

Based on the sensitivity test of bilimbi leaf extract *(Averrhoa bilimbi L.)* against the growth of *Staphylococcus epidermidis*, it can be concluded that the inhibition zone on the growth of *Staphylococcus epidermidis* with 50% concentration of bilimbi leaf extract is classified as intermediate. At 75% concentration, the inhibition zone showed both intermediate and sensitive categories, while at 100% concentration, the inhibition zone was classified as sensitive. Comparison of the inhibition zones among the three concentrations indicated that the 100% concentration produced a larger inhibition zone than the other two concentrations. Further studies are recommended to test the effectiveness of bilimbi leaf extract against bacteria with the same gram classification as *Staphylococcus epidermidis*, to perform bacterial growth tests using an anaerobic jar for better bacterial cultivation, and to conduct phytochemical analysis of bilimbi leaves to identify the compounds present in the extract. The implications of this study suggest that bilimbi leaf extract has potential as a natural antibacterial agent against *Staphylococcus epidermidis*, especially at higher concentrations which show significant inhibitory effects. This contributes importantly to the search for alternative antibacterial agents derived from natural sources, particularly amid rising antibiotic resistance cases. The use of traditional medicinal plants like bilimbi offers a sustainable and affordable solution, supporting the development of herbal medicines based on local wisdom. This research also opens opportunities for further development in phytotherapy and pharmaceutical biotechnology.

The opportunities for further research include testing the extract against other Gram-positive bacteria to determine the antibacterial spectrum coverage. Additionally, it is recommended to use an anaerobic jar during *Staphylococcus epidermidis* growth tests to optimize incubation conditions. Phytochemical analysis is also necessary to identify the active compounds responsible for the antibacterial activity, which will help elucidate the extract's mechanism of action. Further studies may also involve toxicity tests and in vivo experiments to assess the safety and efficacy of the extract within living organisms. Moreover, developing herbal product formulations based on bilimbi leaf extract, such as ointments or antiseptic solutions, represents a promising research direction. Comparative studies with synthetic antibiotics are also important to evaluate the potential of this extract as an alternative or complementary therapy in bacterial infection treatment.

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