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EFFECTS OF GREY MANGROVE LEAF EXTRACT (*Avicennia marina*) ON THE GROWTH OF *Staphylococcus aureus*

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ABSTRACT

The emergence and spread of methicillin-resistant *Staphylococcus aureus* in recent decades complicates the antibiotic therapy. The grey mangrove (*Avicennia marina*) has flavonoid, terpenoid, saponin, phenolic, tannin, and alkaloid chemical compounds that have antimicrobial activity, thus potentially inhibiting *Staphylococcus aureus*. This study aims to examine the effects of the grey mangrove (*Avicennia marina*) leaf extracts on the growth of *Staphylococcus aureus*. This research uses laboratory experimental design with disc diffusion method to test the inhibition of *Staphylococcus aureus* bacteria growth in Mueller-Hinton medium. There were 6 treatment groups, namely negative control of 0.2% DMSO, positive control of amoxicillin 30 µg, *Avicennia marina* leaf extract at concentration of 25%, 50%, 75%, and 100%. The results of this research showed that *Avicennia marina* leaf extract produces inhibition zone of 7.06 mm, 8.51 mm, 10.07 mm, 13.29 mm at concentration of 25%, 50%, 75%, and 100%. Meanwhile, positive control produces inhibiton zone of 23.65 mm, and negative control has no inhibition zone. The statistical tests using one-way ANOVA resulted in a significance value of less than 0.05 ($p<\alpha$). The conclusion of this study showed that the grey mangrove (*Avicennia marina*) leaf extract at 100% concentration is the most effective treatment group in inhibiting the growth of *Staphylococcus aureus*, although the inhibition was not much better when compared to the inhibition of the positive control group.

Introduction

Staphylococcus aureus infection is the most common cause of localized purulent lesions in humans. *Staphylococcus aureus* infects about 30% of the total human population on earth. Some of the diseases it can cause are infectious skin diseases such as folliculitis, furuncle, impetigo, purulent abscess, staphylococcal scalded skin

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syndrome and also systemic diseases including pneumonia, empyema, osteomyelitis, acute endocarditis, and catheter-related bacteremia. In addition, this microorganism also forms an enterotoxin that has great potential to contaminate food and cause poisoning (Kumar, 2012; Tong et al., 2015).

After the utilization of penicillin antibiotics in the early 1940s, it was not long before strains of *Staphylococcus aureus* began to emerge the resistance capabilities to penicillin, thus impairing the antibacterial action of the drug (Foster, 2017). The emergence and spread of methicillin-resistant *Staphylococcus aureus* (MRSA) in recent decades has become a major problem in all parts of the world and has made antibiotic therapy for *Staphylococcus aureus* infections increasingly difficult. In a study conducted in four hospitals located in Sumatra, Java and Bali, the prevalence of MRSA was 6.6%, lower than in most other countries, but higher than northern European countries. The prevalence of Hospital-Acquired MRSA in China has reached 50.4%. In the United States, the mortality rate of MRSA infection has surpassed AIDS, Parkinson's disease, and homicide cases (Santosaningsih et al., 2014; 2016; 2018). Many treatments have failed due to the rise of multidrug-resistant bacteria. This has developed into a major health problem for all humans. Therefore, the discovery of new antibiotics is a significant goal for now. The pharmaceutical industry still requires natural materials as one of the main components in new drug compounds. Most of the antibacterial chemicals discovered to date are derived from plants and microorganisms (Balouiri et al., 2016).

The grey mangrove plant (*Avicennia marina*) has a wide potential benefit. Previous studies have shown that *Avicennia marina* contains chemical compounds namely saponins, flavonoids, terpenoids, phenolics, tannins, naphtoquinones, alkaloids which have anti-inflammatory, antioxidant, antimicrobial, antiviral, anthelmintic, antimalarial, antidiabetic, and anticancer activities. The bark, fruit, and leaves can be used to treat skin diseases such as fish stings, dermatophytosis, furuncles, skin ulcers, abscesses, scabies, burns, reduce rheumatic pain, and even have antimalarial effects (ElDohaji et al., 2020). Based on the great potential of *Avicennia marina* mangroves and the threat of bacterial resistance, the authors wants to know the effect of grey mangrove (*Avicennia marina*) leaf extract on the growth of *Staphylococcus aureus*.

Materials and Methods

1. Preparation of mangrove leaf extract

Avicennia marina leaves were obtained from the mangrove cultivation and ecotourism area in Wonorejo, Surabaya. Mangrove leaves that have been collected must be washed with running water until clean and then drained until there is no water, then dried in an oven at a temperature of ± 40°C until the leaves become brittle. Mangrove leaves that have been dried are cut into small sizes, then mashed using a blender and sieving. Extraction of mangrove leaves using maceration method by soaking them in 96% ethanol solvent for about three days at room temperature while stirring occasionally. The extract was filtered and then extracted again with a new 96% ethanol solvent. Perform the filtration process again after 24 hours. The extract obtained will be concentrated by evaporating the solvent using a vacuum rotary evaporator at low temperature. This extract is then concentrated by evaporating the solvent using thin film technique (Egra et al., 2019; Azwanida Nn, 2015; Sharief Md & Maheswara Rao, 2014).

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Table 1. The results of inhibition zone diameter measurement

Treatments	Diameter of inhibition zone (mm)					Strength of inhibition
	I	II	III	IV	Average diameter	
C– (0.2% DMSO)[a]	0	0	0	0	0	Weak
25%	6.53	7.17	8.30	6.23	7.06	Moderate
50%	8.79	8.55	9.93	6.76	8.51	Moderate
75%	10.65	9.56	11.20	8.87	10.07	Strong
100%	12.99	12.87	15.40	11.90	13.29	Strong
C+ (amoxicillin 30 µg)[b]	21.04	23.06	23.44	27.04	23.69	Very strong

[a] negative control
[b] positive control

2. Preparation of the extract concentrate series

The extraction of *Avicennia marina* leaf produces the extract at a concentration of 100%. The extract will then be diluted with 0.2% DMSO to obtain concentrations of 25%, 50%, and 75% using the simple dilution formula : $M_1 \times V_1 = M_2 \times V_2$.

3. Antibacterial activity test of mangrove leaf extract

Staphylococcus aureus bacteria were obtained at the Microbiology Laboratory of the Faculty of Medicine, Hang Tuah University, Surabaya. The Kirby-Bauer diffusion method used to test the inhibition zone of bacteria growth. It requires 4 culture samples in each treatment groups and applies the random sampling method. *Staphylococcus aureus* was planted on Mueller-Hinton agar medium using sterile cotton buds, then paper disc contained mangrove leaf extract at concentration of 25%, 50%, 75%, and 100% were placed on the surface of the media. Paper disc contained 0.2% DMSO (negative control) and amoxicillin 30 µg (positive control) were also placed on. The plates then incubated at 37°C for 24 hours and measured the inhibition zone formed by bacteria after 24 hours of incubation.

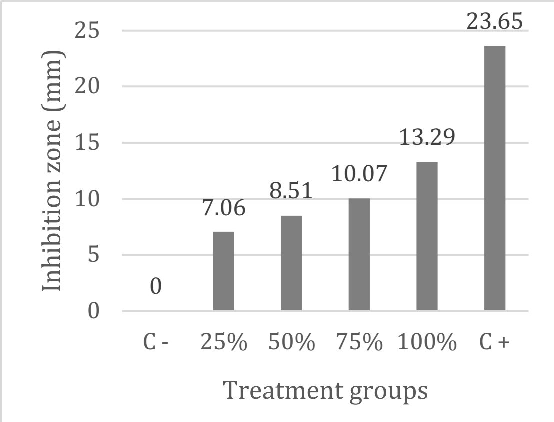

Figure 1. The comparison of the inhibition zone

Results and Discussion

The result of this study showed that mangrove extract at concentration of 25%, 50%, 75%, and 100% had an average inhibition zone diameter of 7.06 mm, 8.51 mm, 10.07 mm, and 13.29 mm. Meanwhile positive control (amoxicillin) had an average inhibition zone diameter of 23.65 mm and negative control (0.2% DMSO) has no inhibition zone. Based on Figure 1, there is a comparison of the inhibition zone between concentration groups and it can be concluded that the greater the concentration value of the extract, the greater the diameter of the inhibition zone formed, vice versa. The greater the concentration of extracts in the media, the more extract content is dissolved and diffused into bacterial cells, disrupting colony formation or even killing the bacteria. It can be concluded that

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extracts with large concentrations have better and more effective antibacterial activity performance (Andries et al., 2014).

The strength of the inhibition can be classified into 4 categories, namely weak (diameter<5 mm), moderate (5 mm<diameter<10 mm), strong (10 mm<diameter<20 mm), very strong (diameter>20 mm) (Kasenda, 2016). Based on those classification, it is known that the extract concentration of 75% and 100% is classified as strong category. Extract concentration of 25% and 50% classified as moderate, and positive control considered has very strong inhibiton. The group that had the largest inhibition zone diameter was the group with a concentration of 100% with an average inhibition zone diameter of 13.29 mm, although the inhibition was not much better when compared to the inhibition of the positive control group.

The statistical tests using one-way ANOVA on SPSS resulted in a significance value of less than 0.05 ($p<\alpha$). It can be concluded that there is an effect of mangrove leaf extract on the growth of *Staphylococcus aureus*. The inhibitory zone of the mangrove leaf extract at 100% concentration was significantly different from other treatment groups, according to statistical analysis using spss.

Avicennia marina leaf extract contains phytochemicals that play a role in antibacterial activity to inhibit the growth of *Staphylococcus aureus*. These substances include triterpenoids, alkaloids, phenols, flavonoids, saponins, and tannins (Sumartini, 2021). There are several mechanisms of a substance being able to inhibit the growth of a bacteria, ranging from changing the permeability of the cell membrane, destroying the cell membrane by inhibiting or modifying its formation, causing the release of nutrients from the cell, changing the shape of the structure of proteins and nucleic acids, inhibiting enzyme activity, and inhibiting the synthesis of nucleic acids and proteins (Cholifah et al., 2020).

Triterpenoid compounds could kill bacteria by forming strong polymer bonds with the outside of transmembrane proteins called porins so that porins become damaged and cause reduced cell membrane permeability, bacteria are lacking in nutrients and could kill bacteria (Herliany et al., 2018). Alkaloids have antibacterial activity because they can inhibit the formation of proteins and nucleic acids in bacteria, damaging membranes through the mechanism of inhibiting the formation of bacterial membranes and modifying membrane permeability that triggers the lysis and death of bacteria. Furthermore, secondary metabolites can go deeper and damage the bacterial membrane (Nimah et al., 2012; Yan et al., 2021). Through the formation of hydrogen bonds, phenol compounds and their derivatives can easily form protein complexes. The H^+ ions produced by these complexes can cause damage to the phosphate groups contained in the bacterial membrane. This can cause damage to phospholipid molecules which makes the bacterial membrane unable to maintain its shape (Santosaningsih et al., 2014). Flavonoids could kill bacteria by making complex compounds with extracellular proteins and soluble proteins to inhibit motility and destroy bacterial walls by breaking peptidoglycan bonds, resulting in the release of intracellular chemicals (Egra et al., 2019; Ibrahim et al., 2022). Saponin compounds cause surface tension to become weaker, resulting in greater permeability or cell leakage and release of intracellular chemicals. If the situation continues, the bacterial cells may lyse and die (Herliany et al., 2018). Tannins have a role in inhibiting the enzymes reverse transcriptase and DNA

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topoisomerase, thus preventing the formation of bacterial cells. Meanwhile, protein transport and enzyme performance are disabled due to the antimicrobial action of tannins (Egra et al., 2019; Herliany et al., 2018; Ngajow et al., 2013). With these various antimicrobial mechanisms, it is possible to inhibit the growth of *S. aureus*.

Conclusions

The grey mangrove (*Avicennia marina*) leaf extract has a significant effect on inhibiting the growth of Staphylococcus aureus. *Avicennia marina* leaf extract at 100% concentration is the most effective treatment group in inhibiting the growth of *Staphylococcus aureus*, although the inhibition was not much better when compared to the inhibition of the positive control group.

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