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Sensitivity Test of Pedada (*Sonneratia alba*) Leaf Extract Against the Growth of *Staphylococcus aureus, Escherichia coli,* and *Aeromonas hydrophila* Bacteria

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ABSTRACT

Sonneratia alba (Pedada) is one of the potential mangroves as a natural antibacterial because it contains secondary metabolites, such as flavonoids, saponins, steroids, tannins, and terpenoids. This study aimed to determine the inhibitory power of Pedada leaf extract in inhibiting the growth of *Staphylococcus aureus*, *Escherichia coli,* and *Aeromonas hydrophila*. The research method used is experimental, including sample preparation, extraction, and sensitivity tests using the Kirby-Bauer disc method. This study used 100%, 50%, 25%, and 12.5% extract doses, positive control (Oxytetracycline), and negative control (a quade). The results showed that Pedada leaf extract could inhibit the growth of pathogenic bacteria; 100% concentration was the concentration with the widest inhibition zone diameter of *S. aureus* 15.48 mm, *E. coli* 13.37 mm, and *A. hydrophila* 13.88 mm. Based on this study's results, the best extract concentration for inhibiting bacterial growth is 100% concentration.

Keywords: Pathogenic Bacteria, *Sonneratia alba*, Sensitivity Test

1. INTRODUCTION

Mangrove is one type of plant that is quite numerous in the territory of Indonesia, one of which is *Sonneratia alba* mangrove (Rina et al., 2024). This plant's leaves contain various phytochemical compounds, including flavonoids, alkaloids, saponins, and tannins, which have antimicrobial activity that can inhibit the growth of various pathogenic microorganisms (Sinubu et al., 2022). Phytochemical compounds are compounds that are naturally found in plants and have biological functions in the field of health and medicine (Kulkarni & Manohara, 2024)

Several bacterial diseases in fish caused by pathogenic bacteria often pose a significant threat to fisheries. Some examples of the types of diseases caused by bacteria include. MAS, *Calibacillosis*, and *Staphylococcus disease*. These bacterial infections can spread rapidly, cause high mortality, affect the quality of fish production, and significantly reduce yields (Aisyah & Mulyani, 2024). *Staphylococcus* is a disease that can cause inflammation of the skin and body tissues of fish and infection of internal organs. This infection usually occurs in stressed fish, especially in poorly managed aquaculture environments (Vaiyapuri et al., 2019).

Escherichia coli in fish can cause Colibacillosis, a severe systemic infection. The disease affects various organs of the fish, including the digestive tract, kidneys, and respiratory organs, causing symptoms such as swelling, internal bleeding, and loss of appetite. This infectious disease is often associated with poor aquaculture management practices, such as poor water quality (Liao et al., 2021). *Aeromonas hydrophila* is the leading cause of *Motile Aeromonas Septicemia* (MAS) in fish; this disease can cause symptoms such as skin ulcers, inflammation of internal organs, and sudden death. *A. hydrophila* can infect almost all fish species, especially under stress conditions and poor water quality. MAS is one of the fisheries' most dangerous bacterial diseases (Sinubu et al., 2022).

Various ways can be used to prevent disease attacks in fish farming, such as antibiotics. However, antibiotics can hurt other organisms and humans as consumers (Aisyah & Mulyani, 2024). Therefore, finding alternative treatments that are more environmentally friendly and safe for fish health is important. Extracts from various plants, including

mangroves such as Sonneratia alba, can act as antibacterials and overcome this problem without harmful side effects (Manuhuttu & Saimima, 2021). Based on this, researchers researched the use of S. alba leaf extract to prevent diseases caused by bacteria *S. aureus, E. coli*, and *A. hydrophila*. This study aimed to determine the inhibition of Pedada leaf extract on the growth of bacteria *S. aureus, E. coli*, and *A. hydrophila*.

2. RESEARCH METHOD

Time and Place

The research took place from October 2024 to January 2025. Sampling was carried out at Lhok Bubon Beach, Samatiga Subdistrict, West Aceh District, and analysis of inhibition zone observations was carried out at the Marine Microbiology Laboratory, Faculty of Fisheries and Marine, Universitas Riau.

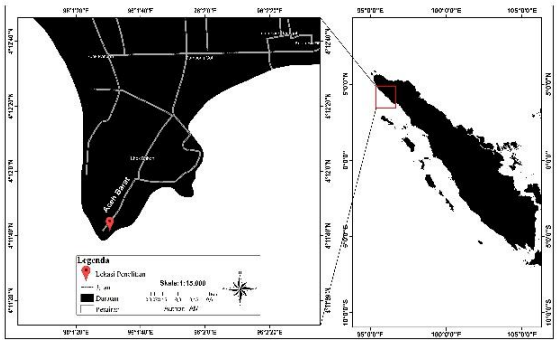


Figure 1. Research location

Method

The method used was experimental, and the doses used in this study were 100%, 50%, 25%, 12.5%, control + (Oxytetracycline), and control (distilled water). Steps in the sensitivity test include sample preparation, extraction (maceration), and antibacterial sensitivity testing using the Kirby-Bauer disc method. To reduce the level of error, three repetitions are carried out (Ramadhani et al., 2022).

Procedures

Preparation of Pedada Leaf Extract (*Sonneratia alba*)

This process can be obtained through the following steps: First, the third and fourth leaves of S. alba are taken from the shoots and leaflets. Then, the leaves are washed and dried indoors for 7-10 days. The dried leaves are pureed using a blender. The grinding results are then soaked (maceration) using 96% ethanol solvent for 24 hours (Nusaibah et al., 2022). The filtrate from soaking was filtered using filter paper, then the filtrate was evaporated using a Rotary Evaporator at 80ºC to separate the solvent and extract.

Rejuvenation of Test Bacterial Isolates

Staphylococcus aureus, Escherichia coli, and *A. hydrophila* bacteria used in this study came from the Marine Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau. Isolates were then cultured on NA (Natrium Agar) media and incubated in an incubator for 18-24 hours at 37ºC. Preparation of bacterial suspensions by inoculating bacteria using an ose on NB (Natrium Broth) media and incubating in an incubator for 18 hours at 37ºC (Kurniawan et al., 2022).

Zone of Inhibition Observation

The zone of inhibition of *S. alba* leaf extract against *S. aureus, E. coli,* and A. hydrophila bacteria was observed based on the Kirby-Bauer disc method. This test measures the inhibition zone around the disc placed on agar media. The stage in the sensitivity test is that the disc paper is given a concentration solution of *S. alba* leaf extract (100%, 50%, 25%, 12.5%), Oxytetracycline as a positive control and distilled water as a negative control (Ramadhani et al., 2022). The concentration of the extract was dripped using a 100µl micropipette. Furthermore, the bacterial suspension was spread on NA media, and blank discs were placed on as many as six pieces in 1 Petri dish. Then, the media was incubated at 37ºC for 24 hours. The test results were determined by measuring the diameter of the clear zone around the disc paper using a calliper.

3. RESULT AND DISCUSSION

Inhibition of *S. alba* Leaf Extract

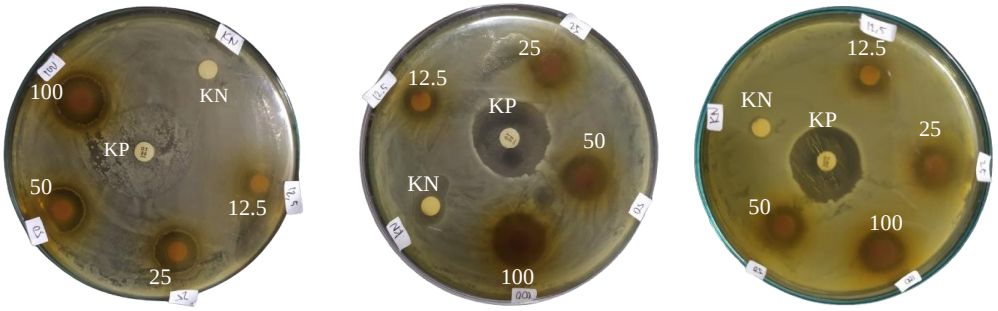
The results of measuring the diameter of the inhibition zone of *S. alba* leaf extract against bacteria *S. aureus, E. coli*, and *A. hydrophila* at concentrations of 100%, 50%, 25%, 12.5%, Oxytetracycline, and distilled water as a control. The sensitivity test results showed that *S. alba* leaf extract has antibacterial activity against *S. aureus, E. coli*, and *A. hydrophila* (Table 1).

Based on the concentration of the extract, the highest zone was at a concentration of 100% and the lowest at a concentration of 12.5%

(Purba et al., 2022). The decrease in extract concentration is directly proportional to the decline in inhibition zone (Sugiaman et al., 2023). Ramadhani et al. (2022) stated that substances with more potent antibacterial activity can produce a larger inhibition zone. In other words, the higher the extract concentration used as an antibacterial, the more effective it is in inhibiting bacterial growth. Antibacterial compounds in *S. alba* leaf extract work by damaging the bacterial cell wall and inhibiting metabolic processes, which ultimately cause cell death (Yudha et al. 2013).

Table 1. Measurement of the diameter of the zone of inhibition of *S. alba* leaf extract against the growth of bacteria *S. aureus*, E. coli and *A. hydrophila*

Extract Concentration	Mean Inhibition Zone Diameter (mm) and St.Dev		
S. alba (%)	*S. aureus*	*E. coli*	*A. hydrophila*
100	15.48±0.702	14.37±1.275	13.88±0.604
50	13.45±0.936	10.48±1.732	12.97±0.510
25	11.33±0.104	10.77±0.236	11.97±0.548
12.5	10.52±0.583	9.42±0.927	11.37±0.748
K+	29.00	29.00	28.00
K-	0	0	0



Staphylococcus aureus *Escherichia coli* *Aeromonas hydrophila*

Figure 2. Clear zone diameter on bacterial growth media

Sonneratia alba has the potential to be an antibacterial and has been tested to inhibit pathogenic bacteria. S. alba extract contains various phytochemical compounds, including flavonoids, tannins, saponins, and triterpenoids (Ramadhani et al., 2022). This mangrove plant has antibacterial properties caused by the content of bioactive compounds. Langi et al. (2022) revealed that *S. alba* extract contains phytochemicals such as tannins, flavonoids, saponins, triterpenoids, and alkaloids. Extracts from *S. alba* have bacteriostatic properties and can prevent or slow the growth of bacteria (Sinurat et al., 2019).

The difference in activity of antibacterial compounds against these three bacteria is due to differences in cell walls. *S. aureus* is a Gram-positive bacterium with a single, simple cell wall structure, while *E. coli* and *A. hydrophila* are Gram-negative bacteria with a more complex cell wall structure (Manuhuttu & Saimima, 2021), so the difference in the inhibition zone area of *S. aureus* is greater than that of *E. coli* and *A. hydrophila*. Fitri & Rahayu (2020) stated that the constituent of the *S. aureus* bacterial cell wall is a simple layer, allowing substances that have the potential to damage bacterial cells to enter easily. The cell wall of *E. coli* bacteria consists of three layers, namely the outer membrane, which functions as a molecular filter; this membrane is asymmetrical and is composed of a phospholipid, lipo-polysaccharide, lipoprotein, and protein layers, making it difficult for molecules from a wide area to penetrate it (Ulfah, 2020).

Several studies have been conducted on *S. alba* leaf extracts and various *S. alba* leaf extraction methods. Dotulong et al. (2018) explained that young leaves of *S. alba* mangrove extracted with methanol and ethanol by Soxhlet and maceration methods contain bioactive components such as phenols, flavonoids, tannins, and alkaloids, which have naturally been shown to have antioxidant properties. Linggama et al. (2019) mentioned that young leaves of *S. alba* extracted with hot water have

excellent antibacterial properties in inhibiting the growth of *S. aureus* and *E. coli.* Ibrahim et al. (2019) stated that the extraction of young S. alba leaves using the maceration method effectively inhibited the growth of S. *aureus, E. coli*, and *A. hydrophila* bacteria.

4. CONCLUSION

The *S. alba* leaf extract effectively inhibits the growth of *S. aureus*, *E. coli*, and *A. hydrophila* bacteria, with inhibition zones classified as strong. A concentration of 100% is the concentration with the highest inhibition zone of 15.48 mm *S. aureus*, 14.37mm E. coli, and 13.88 mm *A. hydrophila*. In comparison, 12.5% concentration is the lowest inhibition zone area with a diameter of 10.59 mm for *S. aureus*, 9.42 mm for *E. coli*, and 11.37 mm for *A. hydrophila*.

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