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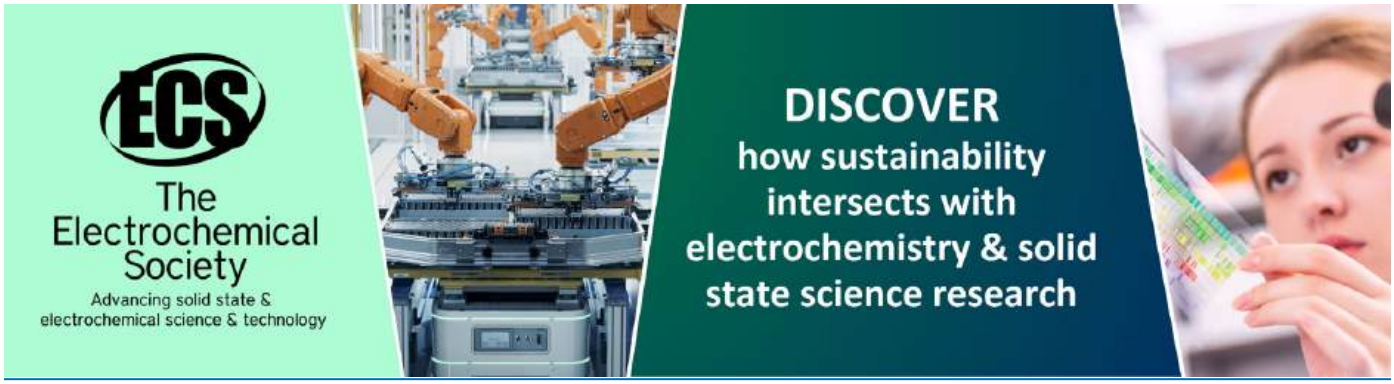

The Potency of Antibacterial Activity of *Avicennia officinalis* Leaf Extract Originating from Ambon Bay, Maluku, Indonesia

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Abstract. Mangroves are known as perennial plants that thrive and adapt in tropical coastal areas affected by tidal cycles. Mangroves serve ecological functions such as protecting the coast from wind, currents, and sea waves. Besides its ecological functions, mangrove leaf known well has an activity as antibacterial. The objective of this study was to determine the yield, phytochemical component contained and antibacterial strength of *Avicennia officinalis* leaf extract, originating from Ambon Bay against *Staphylococcus aureus* and *Escherichia coli*. The result showed that *Avicennia officinalis* leave extracts methanol extract generated an inhibition zone 14.97±0.41 mm against *Staphylococcus aureus* and 12.01±0.06 mm against *Escherichia coli*. Methanol solvent exhibits the highest antibacterial activity against *Staphylococcus aureus*, followed by ethyl acetate and n-hexane extracts. However, *Escherichia coli* showed more resistance to the *Avicennia officinalis* leaf extract.

Key words: Antibacterial activity, *Avicennia officinalis*, inhibition zone, Ambon Bay.

1. Introduction.

In Ambon Bay, mangrove forests constitute an essential ecosystem for supporting the development and protection of the Ambon City. Mangroves are perennial plants that have adapted to flourish in the wetlands of tropical and subtropical coastal regions impacted by tidal cycle[1]. Mangroves serve ecological functions such as protecting the coast from wind, currents, and sea waves. They also serve as habitats, feeding grounds, breeding and growth sites, as well as nesting places for aquatic organisms.

Mangrove fruit has many benefits that are directly related to human life on land, and can be developed as an alternative food source. Apart from being useful as a source of food and fulfilling other needs, mangroves also function as medicinal plants, making them valuable for traditional medicine. Most parts of the mangrove are useful as medicinal extracts, and many parts of the mangrove have been used by coastal communities for natural medicinal purposes[2].

Mangroves used as medical materials because they contain alkaloids, phenols, flavonoids, and saponins [3]. These substances are produced as secondary metabolites or originate from symbiotic microorganisms (endophytes). Mangroves used for medicinal purposes come from the fruit, leaves, bark, and roots [4].

The recent investigations on the *Avicennia marina* crude leaf extract accumulated from the Red Sea, Egypt, showed the numerous bioactive substances and favourable antimicrobial activity against various

fish and human pathogens namely; *Pseudomonas fluorescens, Staphylococcus epidermidis, Bacillus subtilis, Escherichia coli, Klebsiela pneumoniae,* and *Bordetella pertussis* [5]. Therefore, this extract can be used in aquaculture, environmental, and pharmaceutical applications.

It was also reported that crude extract derived from various mangrove species including *Avicennia marina, Rhizophora macromata, Rhizophora mucronatz Sonneratia caseolaris* and *Brwigieera cylindrical* leaves, exhibit significant antioxidant and antimicrobial properties [6] [7]. Additionally, bioactive components such as terpenoids, alkaloids, tannins, steroids, saponins and steroids has also been found in crude extracts of *Suaeda maritima* leave which function as antimicrobial and scavenging activity [8].

Based on the information above, many mangrove leaf extracts have been studied, however, there hasn't been any investigation on the bioactive potential of *Avicennia officinalis* leaves in Ambon Bay. Therefore, this study was carried out to determine the antibacterial potency of *Avicennia officinalis*leaf extract originating from Ambon Bay against pathogenic bacteria.

2. Materials and methods.

2.1. Sampling collection.

Avicennia officinalis leaves were collected from Waiheru Village water, Ambon Bay. Mature and healthy leaves were collected, cleaned using running water to remove dirt especially remain sand sticking to the leaves. The leaves then dried at 40 ºC using oven for 36 hours, dry leaves sliced into small pieces, 1 cm or even smaller, then blend into powder.

2.2. Materials and equipments.

Materials consisted of: Mangrove leaves of *Avicennia officinalis*, nutrient agar (Merck), distilled water, n-hexane (Merck), ethyl acetate (Merck), methanol (Merck), DMSO, ampicillin (Generic), bacterial isolates of *Escherichia coli* and *Staphylococcus aureus*. Equipment consisted of: Laminar air flow, autoclave, analytical balance, petri dishes, test tubes, Erlenmeyer flasks, incubator, oven, needle holder, bunsen burner, micropipette, rotary vacuum evaporator (Buchi).

2.3. Preparation of Mangrove Leafe extract.

The extraction of mangrove leaves was performed using a modified version of the procedure described in reference [9] . The powdered mangrove leaf was extracted using three solvents: methanol, ethyl acetate, and n-hexane, by using the following procedure:

Each of the three 1 L Erlenmeyer flasks is filled with 50 g of mangrove leaf powder. Afterwards, 500 mL of solvents, specifically methanol, ethyl acetate, and n-hexane, were poured in to each of the three flasks, respectively. The extraction process was conducted for a duration of three days utilizing a shaker incubator at ambient temperature. The crude extract was produced by evaporating the solvent filtrate using a rotary vacuum evaporator.

2.4. Phytochemical analysis[10].

Phytochemical analysis was conducted qualitatively in order to know the bioactive components present in a substance. Phytochemical analysis includes the determination of compounds consisted of: flavonoids, saponins, tannins, polyphenols, triterpenoids, and steroids of the crude extract of mangrove leaves.

2.4.1. Flavonoid test. 1 mL crude extract was filled in the test tube, followed by the addition of small amount of magnesium powder and a few drops of concentrated HCl. The presence of flavonoid was indicated by the appearance of orange, pink, or red-colored solution.

2.4.2. Saponin test. The sample solution about 2 mL was placed in a test tube, then shaken for a few minutes. The presence of saponin indicated by the formation of stable foam lasting for 15 minutes or more.

2.4.3. Polyphenol Test. The sample solution 1 mL was placed in the test tube, then a few drops of 5% ferric chloride solution ($FeCl_3$) are added. Polyphenol indicated by the occurrences of a brown precipitate.

2.4.4. Alkaloid Test. The sample solution 1 mL was placed into a test tube, then 2-3 drops of Dragendorff's reagent are added. The presence of alkaloid indicated by the occurrence of the orange precipitate.

2.4.5. Steroid and Triterpenoid Test. The sample solution 1 mL was added with 3.5 drops of chloroform, followed by 3-5 drops of acetic acid anhydride and 10 drops of concentrated sulfuric acid. Positive steroid test is indicated by the color change of the solution to blue or green. While, the presence of triterpenoid presence marked by the color change of the solution to brown or reddish-brown.

2.5. Antibacteri assay.

The test to determine the antibacterial activity of *Avicennia officinalis* leaf extract was carried out according to [11]

2.5.1. Preparation of Positive Control Dilution.. Ampicillin was used as the positive control. Positif control was prepared in a dilution by concentration 100 mg/mL. Ampicillin belongs to a broad-spectrum group that can inhibit both gram-positive and gram-negative bacteria.

2.5.2. Preparation of Test Bacteria. The test bacteria used are *Escherichia coli* and *Staphylococcus aureus*. The test bacteria were cultured on Nutrient Agar and incubated at a temperature of 37ºC for 24 hours. Subsequently, the bacteria were collected and introduced into a physiological saline solution containing 0.9% NaCl. The density was adjusted to 1×10^9 (adjusted to the Hi-Media McFarland No.4 standard solution). Subsequently, a dilution series was prepared up to 1×10^7.

2.5.3. Antibacterial test. Up to 20 mL of Nutrient Agar were poured into a petri dish and allowed to solidify, ready for use in testing against pathogenic bacteria. A bacterial inoculum suspension of 0.1 mL was poured and evenly spread. Discs containing the extract were positioned on the surface of the Nutrient Agar. Ampicillin (100 mg/ml), and 5% DMSO was used as a positive and negative control, respectively. The petri dishes were placed in an incubator at a temperature of 37°C for 24 hours. Afterwards, inhibition zones were then observed and measured in millimeters. Each test in this experiment was repeated three times in order to ensure consistency.

3. Results and Discussion.

The yield of *Avicennia officinalis* leaf extract increases in proportion to the polarity of the solvent used. The polarity increases in the order of n-hexane, ethyl acetate, and methanol (Table 1). This indicates that polar and semi-polar compounds are the most abundant in the leaf extract of *Avicennia officinalis*. Polar solvents effectively can extract polar secondary metabolites such as quaternary alkaloids, phenolic components, carotenoids, tannins, sugars, amino acids, and glycosides [10]. This was also reported an increase in the yield of *Avicennia sp* leaf extract with the increasing polarity of the solvent [12].

Table 1. Yield of *Avicennia officinalis* mangrove leaf extract.

Solvent	**Yield %**
Methanol	28.40
Ethyl Acetate	10.21
n-hexan	9.65

The qualitative test results in Table 2 indicated the presence of steroid, triterpenoid, flavonoid, alkaloid, polyphenol, and saponin compounds in the extract of *Avicennia officinalis* leaves. This aligns with the findings of [13], which revealed the presence of phytochemical constituents such as alkaloids, tannins, flavonoids, and sugars in mangrove leaf extracts, commonly obtained in plant extracts. Similarly, [14] reported that flavonoids and alkaloids are common groups of compounds found in plant leaf extracts. [15]reported that the hydroalcoholic extract of *Avicennia marina* leaves contains phytosterols, flavonoids, tannins, and phenols. The methanol fraction of *Avicennia officinalis* leaves is also reported to contain active components such as steroids, tannins, and flavonoids [16]. Flavonoids are phenolic structures containing a complex carbonyl group with soluble proteins [17]. Meanwhile, [18] discovered the presence of pentanoic acid, decanoic acid, diethylhydroxylamine, pyrrolidine, octadecyl isocyanate, thiazolidinone, and flavonoids within the methanol extract of *A. marina*. [19] also demonstrated the presence of terpenoids, phenols, steroids, tannins, glycosides, and saponins in the ethyl acetate extract of *A. marina* and *Rhizophora mucronata* leaves. Recently, [20] confirmed the presence of fatty acids and their esters, as well as terpenoids and carotenoids as major constituents in *A. marina* extract.

Table 2. Phytochemical compounds of *Avicennia officinalis* Leave extract

Phytochemical compounds	***Avicennia officinalis* Leave extract**		
	Methanol	**Ethyl Acetate**	**n-hexane**
Flavonoids	+	+	-
Alkaloids	+	+	+
Triterpenoids	-	+	-
Steroids	+	-	+
Polyphenols	+	+	+
Saponin	+	-	-

Antibacterial activity assay (Table 3) of *Avicennia officinalis* mangrove leaf extract against *Escherichia coli* and *Staphylococcus aureus* bacteria showed the inhibition zones ranging from 7.13±0.43 mm to 14.97±0.41 mm. Methanol extract exhibited the highest antibacterial activity against *Staphylococcus aureus* with a inhibition zone of 14.97±0.41 mm, followed by ethyl acetate and n-hexane extracts with inhibition zones of 13.23±0.22 mm and 11.43±0.22 mm, respectively. Meanwhile, *Escherichia coli* showed more resistance to *Avicennia officinalis* leaf extract, with a clear zone against n-hexane extract by 7.13±0.43 mm, followed by methanol and ethyl acetate extracts with clear zones of 12.01±0.06 mm and 13.20±0.05 mm, respectively.

Tabel 3. The Antibacterial Activities of *Avicennia officinalis* Leaf Extracts

***The extract of Avicennia officinalis* Leaves**	**The Inhibition Zone (mm) of Bacteria**	
	Escherichia coli	***Staphylococcus aureus***
Methanol	12.01±0.06	14.97±0.41
Ethyl Acetate	13.20±0.05	13.23±0.22
n-Hexsan	7.13±0.43	11.43±0.22
Control + (Ampicillin)	12.57±0.58	12.57±0.44
Control – (DMSO)	0	0

S. aureus is more susceptible to methanol extract, while *E. coli is* more susceptible to ethyl acetate extract. Overall, based on the inhibition zones of this research, the mangrove (*Avicennia officinalis*) leaf extract categorized as strong antibacterial potential, As it mention by [21], where the criteria for antibacterial efectivness were categorized as follows: an inhibition zone diameter of 5 mm or less is considered as weak, 5-10 mm is moderate, 10-20 mm is strong, and 20 mm or more is very strong.

It was stated that the ethyl acetate extract is the extract with the highest antibacterial activity because it exhibits the highest inhibition zone against various test bacteria [5]. [12] mentioned that the ethyl acetate extract has the highest antibacterial activity against *S. aureus* (16±1.15 mm) and *E. coli* (14±0.46 mm). Based on these results, there is a difference in the strength of antibacterial activity, possibly influenced by the origin of the mangrove, which could affect the variation in antibacterial activity.

4. Conclusions

The leaf extract of *Avicennia officinalis* demonstrates promising potential as an antibacterial agent, falling within the moderate to strong range. The antibacterial activity against *Staphylococcus aureus* is highest in the methanol solvent, followed by the ethyl acetate and n-hexane extracts. Nevertheless, *Escherichia coli* exhibits greater resistance to the leaf extract of *Avicennia officinalis*. The crude extract of *Avicennia officinalis* mangrove leaves can be categorized based on yield as follows: methanol extract has the highest yield, followed by ethyl acetate extract, and finally n-hexane extract with the lowest yield.

The *Avicennia officinalis* leaf extract is characterized by the presence of flavonoids, alkaloids, steroids, triterpenoids, polyphenols, and saponins. It is advisable to conduct additional tests to determine the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of the antibacterial properties of the crude mangrove leaf extract.

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