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Antibacterial Activities of Three Species of Mangrove Leaves Extract Against *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT: The increasing incidence of infections and drug resistance has led scientists to seek approaches towards medicinal plants that are potentially effective against many microorganisms. Therefore, this study was planned to assess the antibacterial activity of 3 mangrove species (Scyphipora hydrophylaceae C.F.Gaertn., Lumnitzera littorea (Jack) Voigt and Avicennia alba Blume) against Staphylococcus aureus and Escherichia coli bacteria. This study used the disc diffusion method to measure the diameter of inhibition at several concentrations of mangrove leaf ethanol extracts, namely 15%, 10%, 5%, 2.5%, 1.25% and 0.625% b/v as well as positive control cefadroxil for bacteria and DMSO as negative control. The test results showed that ethanol extracts of mangosteen leave Scyphipora hydrophylaceae C.F.Gaertn and Lumnitzera littorea (Jack) Voigt had a solid response to Escherichia coli bacteria and moderate potential against S. Aureus bacteria—no antibacterial activity against both test bacteria for Avicennia alba mangrove. This study concludes that Scyphipora hydrophylaceae C.F.Gaertn and Lumnitzera littorea (Jack) Voigt have potent antibacterial activity against Escherichia coli, but Avicennia alba has no antibacterial activity.

Keywords: **mangrove; antibacterial activity; drug-resistant; natural product.**

Introduction

Enterococcus faecium, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* SPP and *Escherichia coli* are some of the pathogenic groups that cause many incidents of resistance to antibiotics. The group of bacteria is often abbreviated as ESKAPEEc [1,2]. The incidence of multidrug resistance (MDR) to gram-positive bacteria and gram-negative bacteria makes it difficult to cure patients, so it is necessary to control the incidence of resistance as the primary goal of treatment to prevent infection [3,4].

Resistance, environmental problems, carcinogens, side effects and high medical costs are caused by antibiotics, so it is necessary to consider the use of medicinal materials of natural origin to replace synthetic antibiotics [5]. Plant-based products are the leading group of natural ingredients that have been the primary alternative for treating resistant infection causes for a long time [6]. In this case, resistance-resistant antibiotics must be found as an anti-infective treatment [7].

Siak Regency is one of the districts in Riau province; almost all parts of Siak Regency are covered by forests, including mangrove forests [8]. The research results [9] show that most people in the Siak district area have not optimally utilized mangrove plants. The use of mangrove plants is solely from the tree trunk, so the number of mangrove forests in Siak district has decreased due to the conversion of mangrove land to residential, industrial and port development.

Antibacterial research on mangroves in Indonesia, especially in Siak Regency, is still limited, while mangrove plants are widespread in Indonesia [10]. The purpose of this study was to determine the antibacterial activity of ethanol leaf extracts of three mangrove species, namely Scyphipora hydrophylaceae C.F.Gaertn., Lumnitzera littorea (Jack) Voigt and Avicennia alba Blume taken in

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Sungai Apit District, Siak Regency, Riau Province against Staphylococcus aureus bacteria as Gram-positive bacteria and Escherichia coli as Gram-negative bacteria by disc diffusion method so that it can provide information in the pharmaceutical field and can be helpful in the development of pharmaceutical preparations.

Method

Tools and Materials

The tools used in this study are a rotary evaporator (Buchi, Switzerland), distillation apparatus (Buchi), glassware (Pyrex), aluminium foil, autoclave (Gea), dark bottle, Petri dish, hot plate, incubator (Memert, Germany), vernier calliper, blender, scissors, Ose needle, gauze, cotton, paper disc (Whatman No.42, Germany), parchment paper, filter paper, Laminar Air Flow (JSCB-900SL, Korea), oven (Memert), tweezers, micropipette (Nesco), drip plate, spatial, vortex (As One) and analytical balance (Shimadzu).

The materials used in this study were Muhler Hinton Agar (MHA) media (Merck), Nutrient Broth (NB) media (Merck), Potato Dextrose Agar (PDA) media (Merck), Cefadroxil antibiotic disk (HJ), 70% alcohol, 96% ethanol, Dimethyl Sulfoxide (DMSO) (Merck), physiological NaCl solution, distilled water, 2N sulfuric acid, concentrated hydrochloric acid, 1% iron (III) chloride, chloroform, ammonia chloroform, magnesium metal, Lieberman-Bouchard reagent and Mayer reagent. The bacteria used in this study were Staphylococcus aureus (ATCC 12600) and Escherichia coli (ATCC 25922).

Plant Determination

Three leaf samples of mangrove plants were randomly sampled from Sungai Apit District, Siak Regency, Riau Province and then named mangrove samples A, B and C. Furthermore, the samples were identified at Andalas University Herbarium (ANDA) Padang City; each sample was identified as Scyphipora hydrophylaceae C.F.Gaertn, Lumnitzera littorea (Jack) Voigt and Avicennia alba Blume.

Sample Preparation

Samples of mangrove leaves, each as much as 508g Scyphipora hydrophylaceae C.F.Gaertn, 453g Lumnitzera littorea (Jack) Voigt, and 586g Avicennia alba Blume made into simplisia mangrove leaf powder. Furthermore, each sample was remacerated with 70% ethanol. With a solvent ratio of 1 4. The extract was carried out by immersing the model in a dark bottle containing ethanol solvent for three days while stirring occasionally and storing it in a place protected from light. Then filtering was done, and the pulp was macerated again for three days. Repetition in the same way is done three times so that the ethanol extract of mangrove leaves is obtained. The maceration results were collected and concentrated with a rotary evaporator until a thick extract was obtained.

Phytochemical Screening

Flavonoid

The extract weighed 0.5 g, and 5 mL of 95% ethanol was added. Then, take 2 mL, add 0.1 g of magnesium powder, and add ten drops of HCl P from the side of the tube. Shake gently; if a red or orange colour forms, it indicates the presence of flavonoids.

Saponin

The extract was weighed at 0.5 g and shaken with 10 mL of water (if necessary in a water bath). A positive reaction is indicated by stable foam that does not disappear when hydrochloric acid is added.

Table 1. Ha phytochemical testing results of ethanol extract of mangrove leaves

Secondary Metabolites	Mangrove Species		
	Scyphipora hydrophylaceae C.F.Gaertn.	*Lumnitzera littorea* (Jack) Voigt	*Avicennia alba Blume*
Alkaloid	+	+	+
Flavonoid	+	+	+
Fenolik	+	+	+
Saponin	+	+	+
Tannin	+	+	+
Terpenoid	+	+	+
Steroid	+	+	+

Table 2. Hasil pengujian antibakteri ekstrak etanol daun *Scyphipora hydrophylaceae* C.F.Gaertn

Bakteri Uji	Perlakuan	Zona Hambat (mm)			
		R1	R2	R3	*Rata-Rata ± Standar Deviasi*
Staphylococcus aureus	Kontrol Positif	24,9	25,9	26	25,6±0,61
	Kontrol Negatif	0	0	0	0
	15%	9,1	9,4	8,9	9,13±0,25
	10%	7,8	7,6	8	7,8±0,2
	5%	6,7	7,1	6,9	6,9±0,2
	2,5%	5,9	6,1	5,3	5,76±0,43
	1,25%	5,1	4,8	4,7	4,86±0,21
	0,625%	0	0	0	0
Escherichia coli	Kontrol Positif	23	28,9	29,3	27,06±3,5
	Kontrol Negatif	0	0	0	0
	15%	13,9	14,1	13,7	13,9±0,2
	10%	12,3	12,1	11,8	12,01±0,25
	5%	11,2	10,9	10,7	10,9±0,25
	2,5%	10	9,3	9,7	9,7±0,35
	1,25%	9,1	8,7	8,9	8,9±0,2
	0,625%	8,1	7,5	7,8	7,8±0,3

Alkaloid

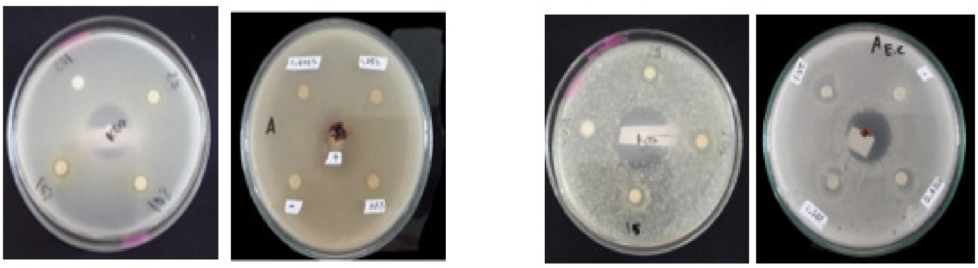
The extract was dissolved in several drops of 2 N sulfuric acid, stirred, and tested with alkaloid reagents: Mayer's reagent, Dragendorff's reagent, and Bouchardat's reagent. Positive results were shown in Mayer's reagent; a white precipitate was formed; in Dragendroff's reagent, a red to orange residue was formed; and in Bouchardat's reagent, a yellowish brown precipitate was formed.

Tanin

The extract is extracted with ethanol, filtered, and the filtrate is taken. To a test solution, 10% gelatin is added; if it contains tannin, a white precipitate solution is formed. To the following sample, NaCl-gelatin is added to the test solution (1% gelatin solution in 10% NaCl solution); if it contains tannin, a white precipitate solution is formed. A few drops of 3% $FeCl_3$ were added to the sample. If the solution includes a blue-black colour, it contains hydrolyzed tannins; if a blue-green colour forms, it contains condensed tannins.

Triterpenoids dan Steroids

0.1 g of extract was added with three drops of acetic anhydrous solution and one drop of concentrated H2SO4. Positive results are shown in red (triterpenoids) and green (steroids).



Staphylococcus aureus *Escherichia coli*

Figure 1. Uji aktivitas antibakteri ekstrak etanol daun mangrove *Scyphipora hydrophylaceae* C.F.Gaertn

Table 3. Hasil pengujian antibakteri ekstrak etanol daun *Lumnitzera littorea* (Jack) Voigt

Bakteri Uji	Perlakuan	Zona Hambat (mm)			
		R1	R2	R3	*Rata-Rata ± Standar Deviasi*
Staphylococcus aureus	Kontrol Positif	24,9	25,1	24,7	24,9±0,2
	Kontrol Negatif	0	0	0	0
	15%	10,2	9,8	10,1	10,03±0,21
	10%	9,1	8,6	8,9	8,87±0,25
	5%	7,3	7,1	7,1	7,16±0,12
	2,5%	5,9	5,3	6,1	5,76±0,42
	1,25%	0	0	0	0
	0,625%	0	0	0	0
Escherichia coli	Kontrol Positif	25,6	24,9	25,1	25,2±0,36
	Kontrol Negatif	0	0	0	0
	15%	14,2	13,9	12,8	13,63±0,73
	10%	11,5	12,3	11,2	11,67±0,56
	5%	10,3	9,9	9,7	9,97±0,31
	2,5%	9,3	9,1	8,8	9,07±0,25
	1,25%	8,1	8,1	7,8	8±0,17
	0,625%	0	0	0	0

Antibacterial Activity Testing

Bacteria were rejuvenated first; then, a microbial suspension was made. Ethanol extracts of mangrove leaves were made into solutions with concentrations of 15%, 10%, 5%, 2.5%, 1.25% and 0.625% b/v using DMSO solvent. The test bacterial suspension, as much as 0.3 mL, was put into a Petri dish, and then 15 mL of MHA media was homogenized and allowed to solidify. The test solution with each concentration was taken as much as 10 μL, dripped on the disc paper, and placed on the inoculum media. They were incubated for 24 hours at 37˚C. Microbial growth was observed, and the clear zone formed around the disk was measured using a calliper. For comparison, blank discs were dripped with 10 μL DMSO for negative control and cefadroxil 0.01% for positive control.

Results and Discussion

This study was conducted to see the antibacterial activity of ethanol extracts of leaves of several mangrove species, namely *Scyphipora hydrophylaceae* C.F.Gaertn (sample A), *Lumnitzera littorea* (Jack) Voigt (sample B) and *Avicennia alba* Blume (Sample C) against *Staphylococcus aureus* bacteria as Gram-positive bacteria and *Escherichia coli* as Gram bacteria. The solvent used for maceration is 70% ethanol. The extract yields were 24.2% from 123g of Scyphipora hydrophylaceae C.F.Gaertn extract, 43% from 196.8g of Lumnitzera littorea (Jack) Voigt extract and 15.15% from

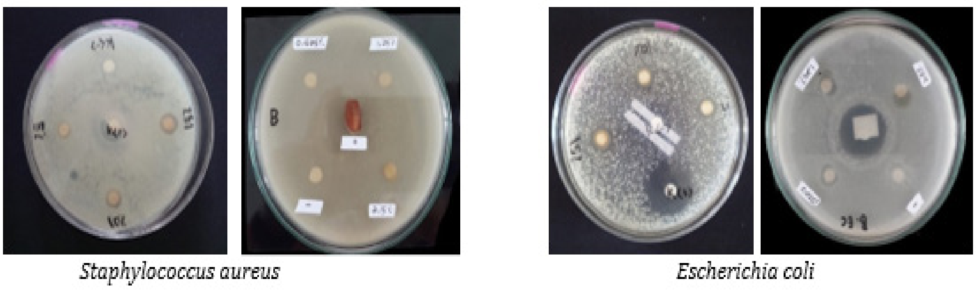


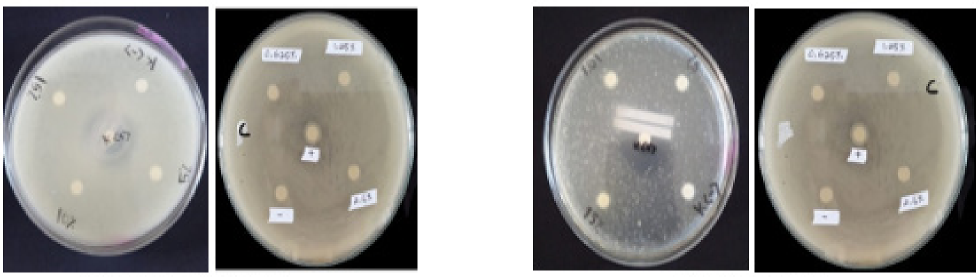
Figure 2. Uji aktivitas antibakteri ekstrak etanol daun mangrove *Lumnitzera littorea* (Jack) Voigt

Table 4. Hasil pengujian antibakteri ekstrak etanol Daun *Avicennia alba* Blume

Bakteri Uji	Perlakuan	Zona Hambat (mm)			
		R1	R2	R3	*Rata-Rata ± Standar Deviasi*
Staphylococcus aureus	Kontrol Positif	24,7	25,2	24,9	24,93±0,25
	Kontrol Negatif	0	0	0	0
	15%	0	0	0	0
	10%	0	0	0	0
	5%	0	0	0	0
	2,5%	0	0	0	0
	1,25%	0	0	0	0
	0,625%	0	0	0	0
Escherichia coli	Kontrol Positif	29,1	28,9	29,3	29,1±0,2
	Kontrol Negatif	0	0	0	0
	15%	0	0	0	0
	10%	0	0	0	0
	5%	0	0	0	0
	2,5%	0	0	0	0
	1,25%	0	0	0	0
	0,625%	0	0	0	0

88.8g of Avicennia alba Blume extract. The results of phytochemical tests on ethanol extracts of the leaves of each mangrove sample can be seen in Table 1. The results show that all mangrove samples contain all secondary metabolites, namely alkaloids, phenolics, flavonoids, saponins, terpenoids and steroids; this indicates that mangrove plants can be used as a source of medicinal materials from nature.

The results of testing ethanol extracts of mangrove leaves *Scyphipora hydrophylaceae* C.F.Gaertn; *Lumnitzera littorea* (Jack) Voigt and *Avicennia alba* Blume against bacteria *S. aureus* and *E. coli*, respectively, can be seen in tables 2, 3 and 4 through measurement of diameter of inhibition (DDH). According to [12], the effectiveness of an antibacterial can be seen from the inhibition zone formed and is divided into four groups based on the precise area began, namely weak response (diameter ≤5 mm), moderate (diameter 5-10 mm), substantial (diameter >10-20 mm), and solid (diameter ≥20 mm). It can be seen from the table that ethanol extracts of mangrove leaves *Scyphipora hydrophylaceae* C.F.Gaertn and Lumnitzera littorea (Jack) Voigt have a solid response to E. coli bacteria and moderate potential against S. aureus bacteria at a concentration of 15%. These results show that both mangrove species have antibacterial potential because they have a solid prospect to inhibit bacterial growth at a relatively small concentration of 15%. The ethanol extract sample of *Avicennia alba* Blume mangrove leaves did not



Staphylococcus aureus *Escherichia coli*

Figure 3. Uji aktivitas antibakteri ekstrak etanol daun mangrove *Avicennia alba* Blume

show antibacterial activity on both test bacteria.

It can also be seen from the measurement of the inhibition zone carried out on the ethanol extract samples of Scyphipora hydrophylaceae C.F.Gaertn and Lumnitzera littorea (Jack) Voigt leaves that the inhibition zone of Gram-negative bacteria is more significant when compared to Gram-positive bacteria. So, it can be concluded that the ethanol extracts of both mangrove leaf samples are more sensitive to Gram-negative bacteria.

This is to the theory that gram-positive bacteria are more resistant because they have a cell wall composed of a network with many pores and a thick peptidoglycan layer and are surrounded by a layer of ketoic acid. In contrast, gram-negative bacteria have a peptidoglycan layer on a thin cell wall and are surrounded by lipoproteins, lipopolysaccharides, phospholipids and some proteins. [11].

The theory says that the antibacterial effectiveness of mangrove leaves is due to the content of secondary metabolites of alkaloids, tannins, and flavonoids, which can be seen from preliminary phytochemical tests showing mangrove extracts contain all the secondary metabolites tested. [12]. Alkaloids are shown to have broad-spectrum antibacterial activity with sound antibacterial effects [13,14]. The antibacterial activity of alkaloids affects the bacterial cell membrane by affecting DNA function and inhibiting bacterial protein synthesis, including methicillin-resistant Staphylococcus aureus (MRSA)[15].

Several studies have shown that tannins have antimicrobial activity against Gram-positive and Negative bacteria. [16,17]. Tannins will pass through the bacterial cell wall and disrupt cell metabolism, resulting in bacterial death [18]. Other studies say that the antibacterial activity of tannins is assumed to be a consequence of their strong iron-binding ability to form complexes with iron from the bacterial growth medium since aerobic microorganisms require iron for many functions (e.g., reduction of DNA precursor ribonucleotides, formation of heme, iron, etc.) [19].

The antibacterial activity of flavonoids has also been proven by several studies [20-22]. Flavonoids were shown to affect three significant groups of proteins and enzymes that play essential roles in bacterial growth and metabolism: enzymes involved in DNA and protein metabolism (i.e., bacterial topoisomerases, helicases, DNA gyrases, and ribosomes); membrane proteins and enzymes involved to varying degrees in cellular transport, bioenergetics, maintenance homeostasis, and cell wall and lipid metabolism (i.e., efflux pumps and transporters, ATP synthase, cytochrome c, acyl carrier protein synthase); other targets involve beneficial effects against microbial pathogenesis, such as their actions against toxin and biofilm production [23].

Conclusion

Ethanol extracts of mangrove leaves of *Scyphipora hydrophylaceae* C.F.Gaertn and *Lumnitzera littorea* (Jack) Voigt had antibacterial solid activity against *Escherichia coli*, but *Avicennia* alba had no antibacterial activity.

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