Effect of Root Extract of Avicennia Alba and Rhizophora Apiculata and Their Minimum Inhibitory Concentration on Vibrio Sp (MC2P5) Cause Shrimp Vibriosis Disease

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Effect of Root Extract of *Avicennia Alba* and *Rhizophora Apiculata* and Their Minimum Inhibitory Concentration on *Vibrio* Sp (MC_2P_5) Cause Shrimp Vibriosis Disease

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Abstract – Effect of root extract of *Avicennia alba* and *Rhizophora apiculata* on *Vibrio* sp (MC_2P_5) has made an effort to control vibriosis disease in black tiger shrimp (*Penaeus monodon* Fabr.). The roots of *Avicennia alba* and *Rhizophora apiculata* from Sungsang mangrove areas, Banyuasin South Sumatera. This study aims to gain root extract *Avicennia alba* and *Rhizophora apiculata* that can be used to control *Vibrio* sp (MC_2P_5) that cause disease in black tiger shrimp, and determine the minimum inhibitory concentration (MIC). The design used was a randomized block design (RBD) factorial with two factors, namely the type of extract (*Avicennia alba* and *Rhizophora apiculata*) and extract concentrations (0, 60, 120, 180, and 240 ppm). The results showed that the extracts of the most widely obtained from the methanol extract of the roots of *Avicennia alba* was 1.77 grams and methanol extract of *Rhizophora apiculata* was 4.85 grams. The average diameter of the largest inhibition zones obtained from treatment of the methanol extract of the roots of *Avicennia alba* of 13.60 mm. MIC of methanol extract of the roots of *Avicennia alba* against *Vibrio* sp (MC_2P_5) was 16 ppm with an average diameter of clear zone was 8.76 ± 0.68 mm.

1. INTRODUCTION

Shrimp crop failures that occur in shrimp ponds in Indonesia became a phenomenon which is very detrimental to fish farmers. Crop failure is usually caused by the bacterium *Vibrio* attack which resulted in the death of shrimp in a short time and in large numbers. The shrimp were infected with *Vibrio* generally characterized by clinical symptoms, where the shrimp look weak, dark red or pale, red antennae and swimming legs. *Vibrio* are pathogens that infect and cause disease in the current weak shrimp conditions and extreme environmental factors [1].

Vibrio harveyi continues to cause chronic mortality by 30% in Australia including *Penaeus monodon* larvae and post larvae under conditions of stress. The problem is caused by secondary vibriosis are common. *Vibrio harveyi* appears to release exotoxins and can lead to death of 80-100% in Australia at the hatchery *P. monodon* [2]. Prevention of the spread of bacterial diseases needs to be done early, for example by using antibiotics [1]., but the use of antibiotics continuously and improper dosage will result in bacteria becoming resistant [3]. The use of natural anti-bacterial is still limited because of the information regarding the type, effectiveness and how utility yet. Among the natural ingredients that are known as antibacterial is a mangrove. Mangroves in addition can improve the fertility waters also produce active compounds such as saponins, flavonoids and alcohol octacyl active as antimicrobial compounds [4]. Excessive use of antibiotics cause certain bacteria resistant. Species of microorganisms have high levels of vulnerability to substance different antibiotics and the vulnerability may change during the treatment period. Therefore it is necessary to test susceptibility of microorganisms to antibiotics. The susceptibility of microorganisms to antibiotics can be determined by the tube dilution technique and technique cup paper plate. The method is to assign the smallest number of antibiotic required to inhibit the growth of organisms in vitro, the number is also called the MIC (minimum inhibitory concentration).

So far only known as a support mangrove fisheries activities that provide habitat and as a supplier of nutrients, but from research known some mangrove plants produce secondary metabolites that are antibacterial. *Avicennia* and *Rhizophora* sp sp is a mangrove plant that can be found around the pond. According [5] extracts of some plants such as *Rhizophora stylosa* mangrove, *Soneratia griffithii, Kandelia candel, Aegiceras floridum* and *Excoecaria agallocha*, has been shown to inhibit the growth of bacteria *Staphyloccoccus aureus* because it produces secondary metabolites that can inhibit the growth of microbes can even kill microorganisms. This compound can control the populations of pathogenic microorganisms in the waters around the mangrove forests. Therefore it is necessary to do research on anti-vibrio isolation of secondary metabolites of mangrove *A. alba* and *R. apiculata*. Mangrove-containing compounds such as

alkaloids, flavonoids, phenols, terpenoids, steroids and saponins. This class of compounds is an ingredient of modern medicine[6].

This research aimed to obtain a secondary metabolite extract of the roots of *A. alba* and *R. apiculata* anti- *Vibrio* sp cause disease in black tiger shrimp vibriosis and to determine the value of the MIC of the extract obtained.

2. METHODS

2.1.Sampling

Root samples that will be derived from plant extracts mangrove *A. alba* and *R. apiculata* in Breech Estuary mangrove forests, Banyuasin South Sumatra Province. The roots of *A. alba* and R. *apiculata* taken part submerged approximately 10 cm and 3-4 cm in diameter.

2.2.Extraction of Secondary Metabolites of Mangrove Roots

Samples of the roots of mangrove plants taken approximately 1 kg then washed with distilled water. Then the samples were dried in an oven at a temperature of 70^{o}C to a constant weight. Subsequently the samples were destroyed by using the blender into a powder. Extraction of the sample preparation of mangrove roots, the root powder ratio: n-hexane is 1: 2. Powder was added a solution of n-hexane are macerated for 24 hours[7].

2.3.Antibacterial Activity Test

a. Determining antibacterial activity extract *R. apiculata* and *A. alba* against *Vibrio* sp

Determination of antibacterial activity of mangrove root extract conducted using a randomized block design (RBD) with factorial pattern. Factor 1: Type extract (E) : e1: n-hexane extract of *R. apiculata*, e2: ethyl acetate extract of *R. apiculata* , e3: methanol extract of *R. apiculata* , e4: n-hexane extract *A. alba* e5: ethyl acetate extract of *A. alba*, e6: methanol extract *A. alba*. Factor 2: Concentration of extract : control ;60; 120; 180; and 240 ppm with two replicatons. Antibacterial test performed by the Kirby-Bauer method or disc diffusion methods [8].

b. Determining the Minimum Inhibitory Concentration (MIC) Extract Against *Vibrio* sp(MC_2P_5)

MIC determination made by the disc diffusion methods [8] starts from a high to low concentration obtained from the results of the selection of antibacterial activity. This concentration is determined based on the results of further testing that the smallest concentration which has an average diameter of the largest inhibition zone. Lowest concentration obtained, made various concentration to 0, 10, 20, 30, 40, 50 and 60 ppm for methanol extract of *A. alba* on *Vibrio* sp (MC_2P_5). From concentration of 20 ppm were obtained to 0, 4, 8, 12, 16 and 20 ppm. Then determined the MIC values of the methanol extract of *A. alba* [modification 9].

3. RESULTS AND DISCUSSION

The weight of the final N-hexane, ethyl acetate, and methanol extract of roots *A. alba* root from 1 kg sample were 0.04, 0.39, and 1.77 grams, and extract of roots *A. alba* root were 0.32, 0.71, and 14.85 gram respectively.

Polarity properties of a material should be the same as the polarity of the solvent that can dissolve materials. As [10] to consider in the selection of the polarity of the solvent is the nature of the material. N-hexane non-polar have limitations in isolating polar compounds, as well as ethyl acetate which is semi-polar. In contrast to the methanol that are able to dissolve a wide range of polar and non-polar compounds. The use of solvent n-hexane and ethyl acetate is expected to attract secondary metabolites that have not attracted by methanol. Based on the solvent used, methanol produces extract heavier than the solvent n-hexane and ethyl acetate. This is presumably because the compounds contained in mangrove plants tend to be polar, so the polar methanol has no limitations in the isolated compounds on mangrove plants. Solvents are used depending on the nature of the component to be dissolved. In general methanol is the most widely used solvent in the process of isolation of organic compounds of natural materials, because almost can dissolve the entire class of secondary metabolites.

Antibacterial activity of extracts of roots of *R. apiculata* and *A. alba* against *Vibrio* sp (MC_2P_5)

Based on testing the antibacterial activity of Avicennia alba root extract made against Vibrio sp (MC_2P_5) results showed that the inhibition zone formed around the paper disc. According to [11] the diameter of inhibition zone is what determines the potential of an antibacterial compound. The broader the clear zone formed showed higher antibacterial activity produced by the extract.

Treatment of the methanol extract of *Avicennia alba* concentration of 60 ppm against *Vibrio* sp (MC2P5) showed inhibition zone diameter is 13.60 mm greatest but not significant with ethyl acetate extract treatment *R. apiculata* and *A. alba* concentration of 180 ppm (Table 1).

Table 1 Diamater inhibition zone (mm) of *R. apiculata* and *A. alba* root extract on *Vibrio* sp (MC_2P_5)

Extract types	Concentration (ppm)				
	0	60	120	180	240
N-hexane *R. apiculata*	0[a]	4,20 [bc]	9,48 [fgh]	5,90 [bcdef]	8,48 [defg]
Etyl acetate *R. apiculata*	0[a]	4,69 [bcd]	7,85 [cdefg]	10,24 [ghi]	7,61 [cdefg]
Methanol *R. apiculata*	0[a]	9,35 [efgh]	9,11 [efgh]	4,10 [bc]	12,59 [hi]
N-hexane *A. alba*	0[a]	6,15 [bcdefg]	9,60 [fgh]	5,37 [bcde]	9,15 [efgh]
Etyl acetate *A. alba*	0[a]	7,98 [cdefg]	0[a]	10,15 [ghi]	9,75 [fgh]
Methanol *A. alba*	0[a]	13,60 [i]	3,70 [b]	8,65 [efgh]	8,65 [efgh]

Notes : The numbers are followed by different letters indicate significant differences according to DNMRT 5%

A. alba extract can inhibit the growth of bacteria, is apparently due to the plant contains active compounds that act as antibacterial, such as alkaloids, saponins, glycosides, tannins, flavonoids. Research Floreal (2009) showed that the leaves of *Avicennia* sp plant contains alkaloids, saponins, glycosides, tannins, flavonoids in the leaves and sap are in smaller amounts. Triterpenoids found in all parts, especially the leaves and roots. Steroids are not found in all parts of the plant [12]. Extracts of some types of mangrove plants such as *Rhizophora stylosa*, *Soneratia griffithii*, *Kandelia candel*, *Aegiceras floridum* and *Excoecaria agallocha*, has been shown to inhibit the growth of bacteria *Staphyloccoccus aureus*. From the results of phytochemical studies note some *Kendelia candel* mangrove, *Aegiceras floridum*, *Rhizophora stylosa* and *Excoecaria agallocha* contain secondary metabolites found in various plant organs, especially the leaves, roots and seeds. For example, the root contains compounds oktakosil, stigmasterol, benzoksazolin-2-one, stigmasteril, glukopranosid, saponins and flavonoids [13].

Flavonoids as secondary metabolites produced by mangrove plants can inhibit the growth of bacteria. According [14] flavonoids as antibacterial compounds inhibit the growth and metabolism of bacteria by disrupting the cytoplasmic membrane of cells and denature proteins. Flavonoids can damage the cytoplasmic membrane which can cause leakage of important metabolites and enzyme systems inactivate bacteria. This damage allows the nucleotide and amino acid seeping out and prevent the entry of active ingredients into the cell, which can cause bacterial death [15].Tannins are one of the active ingredient that serves as an antimicrobial material contained in the mangroves. According to [16] activity of tannins associated with its ability to bind to the protein precipitate and encourage dehydration mucosal tissue. Allow the formation of a stronger protective layer, the cells move up (constrict). According to [17] *Vibrio* are gram-negative bacteria have a peptidoglycan content that may determine the shape of the cell and to provide the necessary rigidity to protect bacteria from osmotic preassure. Tannins have the ability to inhibit the synthesis of peptidoglycan so bacteria can not replicate.

Minimum Inhibitory Concentration (MIC) methanol extract of *A. alba* concentration of 60 ppm against *Vibrio* sp (MC_2

Further test results of Duncan's New Multiple Range Test (DNMRT) 5% methanol extract of *A. alba* known that concentration of 60 ppm is the smallest concentration that showed the ability to inhibit the growth of bacteria *Vibrio* sp (MC_2P_5). Based on the results Anova, the average diameter of inhibition zone methanol extract of *A.alba* of the bacterium *Vibrio* sp (MC_2P_5) showed that the concentration of the extract had a significant effect ($p < 0.05$) the results of further tests using test DNMRT 5% for the effectiveness of the extract concentration can be seen in Table 2.

Table 2 Test of the methanol extract of *Avicennia alba* against *Vibrio* sp (MC_2P_5)

The concentration of the methanol extract of *Avicennia alba* (ppm)	Average diameter of inhibition zone on *Vibrio* sp (MC_2P_5) (mm)
60	7,68[c]
50	7,40[c]
40	7,93[c]
30	6,58[b]
20	6,50[b]
10	0[a]
0	0[a]

Note: The numbers are followed by different letters indicate significant differences according to DNMRT 5%

Table 3 MIC methanol extract of *Avicennia alba* root on *Vibrio* sp (MC_2P_5)

Methanol extract *Avicennia alba* concentration (ppm)	Averageof inhibition zone on diameter *Vibrio* sp (MC_2P_5) (mm)
20	8,755±0,681
16	8,755±0,028
12	0
8	0
6	0
0	0

The concentration of 20 ppm is the smallest concentration that showed the ability to inhibit the growth of bacteria *Vibrio* sp (MC_2P_5) (Table 2). 12 the opinion of [11] concentration is an important factor in susceptibility test antibacterial material, because the higher the concentration, the greater the inhibition zone formed. Extract at a concentration of 16 ppm still can inhibit the activity of *Vibrio* sp (MC_2P_5), whereas the smaller concentration is 12 ppm, 8 ppm and 6 ppm of methanol extract of *A. alba* has been unable to inhibit the bacteria *Vibrio* sp (MC_2P_5). It can be determined that the value of the Minimum Inhibitory Concentration (MIC) of the methanol extract of *A. alba* on *Vibrio* sp (MC_2P_5) is the concentration of 16 ppm with an inhibition zone diameter average 8.75 ± 0.03 mm.

3. CONCLUSIONS

Methanol extract of *A. alba* and *R. apiculata* root obtained 1, 77 grams and 14.8 grams extract respectively. Methanol root extract of *Avicennia alba* was anti *Vibrio* sp (MC_2P_5) with MIC 16 ppm.

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