

## Characterization of *Avicennia marina* Leaves Fraction as Antibacterial of *Aeromonas salmonicida*

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**ABSTRACT:** *Avicennia marina* is one of a kind of mangrove species that could be used as a potential antibacterial. The purified fractions of *Avicennia marina* leaves extract has not been widely studied and it could be expected to be used as antibacterial against *Aeromonas salmonicida* causing furunculosis disease in freshwater fish farming. Separation of the extract fraction done by using column chromatography. It has been found that F4-b fraction showed the best antibacterial activity against *Aeromonas salmonicida* which obtained by the eluent at the ratio of ethyl acetate: ethanol was 70:30. The results of identification fraction F4-b with UV-Vis and FTIR suspected that the compound which acts as an antibacterial against *Aeromonas salmonicida* was terpenoid. Terpenoid can react with porin (trans membrane protein) on outer membrane of *Aeromonas salmonicida* and damage cell wall of bacteria.

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### INTRODUCTION

*Aeromonas salmonicida* is a gram negative bacteria that cause *furunculosis* disease in various species of freshwater and brackish fish. These bacteria often infect economically valuable fish such as salmon, carp, cod, carpandeels [1]. Visible symptoms of infection due to *Aeromonas salmonicida* are the furuncle, haemorrhage, and ulcer [2].

*Avicennia marina* contain terpenoids, steroids, flavonols, and the glucosides compounds [3]. *Avicennia marina* can also be used as larvicidal for *Aedes aegypti* [4] and have capability of inhibiting multi-drug resistant *Staphylococcus aureus* (MRSA) [5]. However, the using of fractions *A. marina* leaves extract has not been widely used as an alternative natural antibacterial to overcome furunculosis disease in freshwater aquaculture activities.

### MATERIAL AND METHODS

#### Extraction

*Avicennia marina* leaves were obtained from Rungkut Surabaya. The leaves cleaned under running water, then rinsed with distilled water. Afterwards, mangrove leaves crushed with a blender. Extraction done by using maceration method with a solvent-rise sequentially by polarity from n-hexane, ethyl acetate and ethanol [6] (1: 3 w/v) during 24 hours at room temperature (30°C). The filtrate then evaporated with a vacuum evaporator at 40°C.

#### Fraction Separation of *Avicennia marina* Leaves Extract

Fractionation of antibacterial compounds from *Avicennia marina* leaves extract performed by column chromatography using 70-230 mesh silica gel as a stationary phase while ethyl acetate and extracting solvent which has the most effective inhibitory power used as mobile phase with a ratio of 100: 0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0: 100 [7, 8].

#### Evaluation of *Avicennia marina* Leaves Fraction Inhibiting for *Aeromonas salmonicida*

Kirby-Bauer method was chosen to determine the antibacterial activity of *Avicennia marina* leaf fraction for *Aeromonas salmonicida*. Antibacterial sensitivity known by measuring the inhibition zone or a clear zone formed around the paper discs (diameter 6 mm) which already contain antimicrobial substances [9]. All fractions that obtained from chromatography column were tested for *Aeromonas salmonicida* inhibitory.

### Identification of *Avicennia marina* Leaf Extract Fraction

The fraction from column chromatography that showed the best inhibition activity for *Aeromonas salmonicida* was identified by UV-Vis and FTIR.

## RESULTS AND DISCUSSION

### Antibacterial Activity of *Avicennia marina* Leaves Fraction against *A. salmonicida*

Antibacterial activity of *Avicennia marina* leaves crude extract against *Aeromonas salmonicida* can be seen in Table 1.

*Avicennia marina* leaves crude extract showed that ethanol extract was the most effective extract for inhibiting *Aeromonas salmonicida* so it continued to be separated by column chromatography. There were 15 fractions of *Avicennia marina* leaves extracts from column chromatography but only 6 fractions that showed antibacterial activity. Antimicrobial activity from each fraction can be seen in Table 2.

**Table 1.** Antimicrobial Activity of Various Extract of *Avicennia marina* Leaves for *Aeromonas salmonicida*

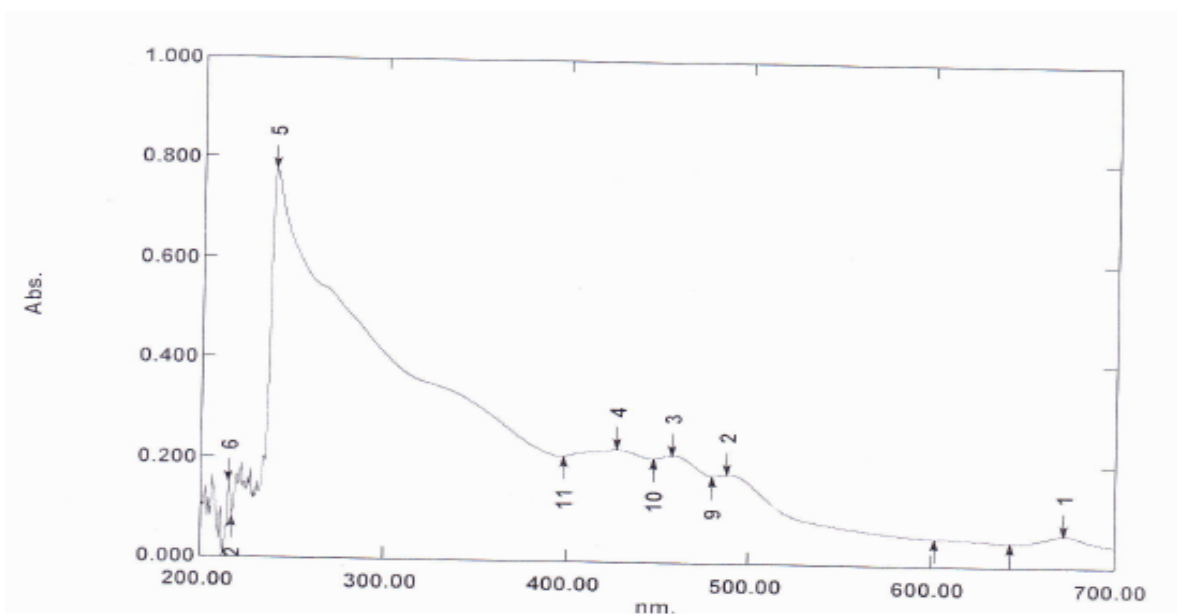
<i>Avicennia marina</i> Leaves Extract	Inhibition zone (mm)
n-hexane	9.1 ± 0.08
Ethyl acetat	8.6 ± 0.26
Ethanol	9.7 ± 0.33

**Table 2.** Antimicrobial Activity of *Avicennia marina* Leaves Fractions for *Aeromonas salmonicida*

Fraction Code	Eluent (ethyl asetat: ethanol)	Inhibition zone (mm)
F3-b	80:20	8 ± 0.2
F3-c	80:20	8.5 ± 0.1
F4-a	70:30	7.5 ± 0.26
F4-b	70:30	9 ± 0.2
F5-a	60:40	7.5 ± 0.26
F5-b	60:40	6.5 ± 0

### UV-Vis Spectrophotometer Identification

Identification of F4-B fraction from *Avicennia marina* leaves extract by UV-Vis spectrophotometer can be seen in Figure 1 and Table 3.



**Figure 1.** Spectra UV-Vis of Fraction F4-b from *Avicennia marina*

**Table 3.** Identification Spectra UV-Vis of Fraction F4-b from *Avicennia marina*

Wave Length Maximum of Fraction F4-b ( $\alpha$ max)	Wave Length Maximum from References	Suspected Compound
673	673 [10]	Chlorophyll a
488	481 [11]	$\beta$ -carotene
458	458 [12]	Diatoksantin Lutein Lycopene Crisantemaksantin
427	428 [12]	Mutatoxantin
239.5	239 [13]	$\beta$ -Sitosterol
215.50	215.5 [14]	Coumarin

### Infrared Spectrophotometer Identification

FTIR spectrophotometer showed that fraction of F4-B has strain C-H, -OH, C=O, and C-O. Fraction F4-B is believed to be terpenoid. Terpenoid has strain O-H, aliphatic C-H, C=O and C-O [15].

### DISCUSSION

Ethanol extract of *Avicennia marina* leaves showed maximum antibacterial activity against *Aeromonas salmonicida*, followed by n-hexane extract and ethyl acetat extract. This suggested that the bioactive compounds of *Avicennia marina* leaves contain more polar compound because the active component which act as antibacterial were more soluble in ethanol (polar solvent).

The results of identification fraction F4-b with UV-Vis and FTIR suspected that the compound which acts as an antibacterial against *Aeromonas salmonicida* was terpenoid Terpenoid is one of secondary metabolites that found in fraction F4-b from *Avicennia marina* leaves extract. It has a role in inhibition of *Aeromonas salmonicida*. The mechanism of terpenoid against *Aeromonas salmonicida* by reacting with porin (trans membrane protein) on outer membrane of *Aeromonas salmonicida* and form a strong bond polymers that cause damage porin. The damage of porin will reduce the permeability of cell wall of bacteria so that the bacteria growth is inhibited [16]. This study indicated that the use of the fraction from *Avicennia marina* leaves extract can be applied to treat diseases of farmed fish so that the use of chemical antibiotics that often applied to treat diseases of farmed fish can be avoided and the quality of the water environment can be maintained.

### CONCLUSION

Based on characterization of fraction F4-b compound from *Avicennia marina* leaves using UV-Vis and FTIR showed that terpenoid has a role in inhibition of *Aeromonas salmonicida*.

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