

# Potential of methanol extract from the stem bark of mangrove *Rhizophora mucronata* against bacteria *Escherichia coli* and *Aeromonas hydrophilla*

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**Abstract.** *Escherichia coli* and *Aeromonas hydrophilla* are well known pathogenic bacteria causing diseases in both human and animals. Since the popular antibacterial drugs in the market lead to resistance, other alternatives antibacterial need to be searched from the active ingredient found in marine and terrestrial vegetation. The potential use of secondary metabolites from marine vegetation is currently being developed. One of marine vegetation that has been expected to have antibacterial activity is mangrove. This study aims to provide information related to the phytochemical and antibacterial activity of methanol extract of the bark of mangrove *Rhizophora mucronata*. The phytochemical screening and disc testing methods were used in this study. The results showed that the methanol extract of *R. mucronata* contained phenolics, alkaloids, and terpenoids. Furthermore, the positive outcome of active antibacterial potential of the methanol extract against *E. coli* and *A. hydrophilla* has been indicated by inhibition of discs in a clear zone at 7,56 and 7,00 mm.

## 1. Introduction

Mangrove is an estuary plant living in a high salinity habitat, influenced by temperature, wind, wave, and ocean current. This extreme environmental condition leads the plant to produce various secondary metabolite compounds that function as a protective effect to surrounding environment. The secondary metabolite compounds contained in these mangroves can potentially be developed as antibacterial, antiviral, and antioxidant agents. [1] explains that mangrove plants in Indonesia are the largest in the world, both in terms of territory ( $\pm 42,550 \text{ km}^2$ ) and number of species ( $\pm 45$  species). On the basis of this mangrove, Indonesia certainly has a high species diversity and possibly potential as a medicinal material.

*Escherichia coli* and *Aeromonas hydrophilla* are pathogenic bacteria causing various diseases in human, fish and other animals. The use of antibiotic drugs such as chloramphenicol has been proven to cause resistance to these bacteria. Therefore, it is necessary to develop alternative antibiotics as antibacterial agents derived from terrestrial and aquatic plants.

One of the alternative aquatic plants that have antibacterial potentials is mangrove plant. Research on antibacterial activity of mangrove plants has been shown on the leaves extract of *Avicennia marina* and *R. stylosa* living in Sudanese waters. These plants demonstrated the potential reactions to inhibit the activity of *E. coli* and *Candida albicans*. The antibacterial activity of the plants was suspected



because of the secondary metabolite compounds; tannins, alkaloids, flavonoids, steroids, and glycosides contained in extract of *A. marina* and *R. stylosa* [2].

One of the mangroves living in East-coast Shore of Surabaya (Pamurbaya), East Java, Indonesia is *R. mucronata*. This mangrove species is a true mangrove commonly found and ethno-botanically utilized some parts of the plant as a pain reliever and natural wood dye by the surrounding community [3]. This study focused on finding alternative utilization of methanol extract of plant stem bark of *R. mucronata* in inhibiting the activity of *E. coli* and *A. hydrophilla* bacteria.



**Figure 1.** *Rhizophora mucronata*

## 2. Method

### 2.1. Materials Research

Dry samples of mangrove rod bark of *R. mucronata* were collected from Pamurbaya area, Surabaya, East Java. The taxonomic test of *R. mucronata* sampling was conducted at Purwodadi Botanical Garden. The chemicals used in the extraction process include pro-analyzed (p.a) and technical organic solvents which has been distilled such as methanol, ethyl acetate, ethanol and n-hexane. The materials for antimicrobial assays include agar media, *E. coli* and *A. hydrophilla* bacteria.

### 2.2. Extraction

Methanol solvent was used in extraction of *R. mucronata*'s stem bark. This solvent was chosen because it is a universal solvent which can dissolve all the active ingredients of secondary metabolite compounds.

### 2.3. Antibacterial Bioactivity Test

The method used in the antimicrobial test is the disc diffusion method (agar diffusion method) with pour order.

### 2.4. GC-MS Analysis of Methanol Extract *R. mucronata*

Methanol extract of *R. mucronata*'s stem bark was analyzed using GC-MS HP 6890. 1  $\mu$ L sample was injected into GC-MS operated using capillary model number agilent column 19091S-433 HP-5MS 5% Phenyl Methyl Siloxane, 30 m long, diameter 250  $\mu$ m, and 0.25  $\mu$ m thickness. The oven temperature was programmed between 100-220  $^{\circ}$  C with a temperature rise rate of 15  $^{\circ}$  C / min, a 10.5 psi pressure helium carrying gas, a total rate of 140 mL / min and a split ratio of 1:50.

## 3. Results

*R. mucronata* is one of the many mangroves living in the East-coast of Surabaya. The utilization of mangrove stem part of *R. mucronata* in this research was based on chemotaxonomic study of secondary metabolite compounds from the mangrove. The extraction process of secondary metabolite

compound from *R. mucronata*'s bark was performed by maceration method using methanol solvent at room temperature.

A total of 1.005 kg *Rhizophoramucronata*'s bark powder was proceeded in maceration method by immersing the powder with methanol solvent for 3 (three) days. The extraction obtained as much as 235.0543 g brown paste-shaped. The antibacterial activity of methanol extract of *R. mucronata* in this research was conducted to measure the ability of the extract in controlling microorganism / bacterium of *A. hydrophilla* and *E. coli*. Chloramphenicol 1% was used as comparator. The 1% dosage of chloramphenicol is the usual dosage in antibacterial (antibiotic) drugs production.

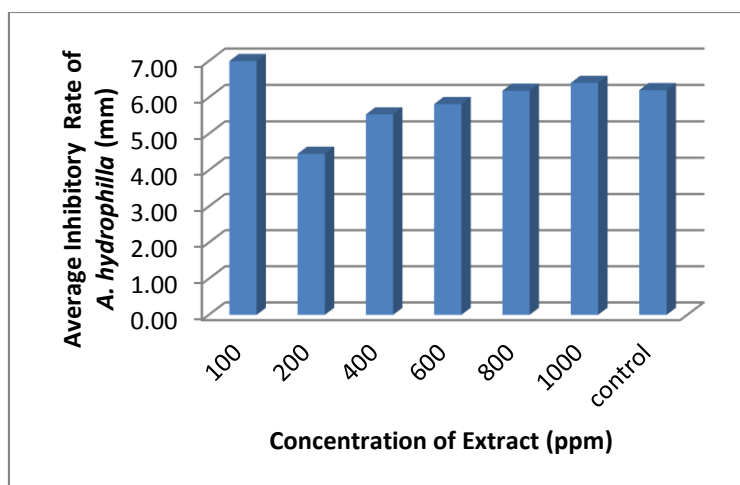
### 3.1. Antibacterial activity of extract on *A. hydrophilla*

*A. hydrophilla* is one of the bacteria causing disease in fish. The existence of these bacteria cause the fish quickly suffered to the protein damage so that it quickly experience decay. The magnitude of the methanol extract inhibition zone against *A. hydrophilla* test bacteria was presented in Table 1.

**Table 1.** Inhibition zone diameter of methanol extract of *R. mucronata* against *A. hydrophilla*

No	Extract content (ppm)	Inhibitory rate (mm)			Average (mm)
		1	2	3	
1	100	6.65	6.55	7.8	7.00
2	200	4.45	4.15	4.75	4.45
3	400	7.65	5.2	3.75	5.53
4	600	4.05	6.8	6.6	5.81
5	800	5.95	6.85	5.75	6.18
6	1000	7.2	5.95	6.05	6.4
7	Chloramphenicol	6.25	5.05	7.3	6.28

Based on Table 1, each sample test clearly has an inhibitory activity against *A. hydrophilla*. The largest inhibitory rate was at 100 ppm of methanol extract with average inhibitory of 7.00 mm. Compared with chloramphenicol, 100 ppm of methanol extract has greater inhibitory rate. The comparison of inhibitory rate of methanol extract at various concentrations was presented in Fig. 2.



**Figure 2.** Comparison of concentration test against *A. hydrophilla* inhibition zone

According to [4], the formation of inhibition zone is a measurement of the strength of an antimicrobial agent against the examined bacteria. The resistance around the disc depends on the absorption capacity of the used active ingredient. If the antibacterial agent positively inhibit, the bacterial growth will stop. This condition was indicated by the clear circle around the disc that is not overgrown with bacteria after incubated for 18-24 hours.

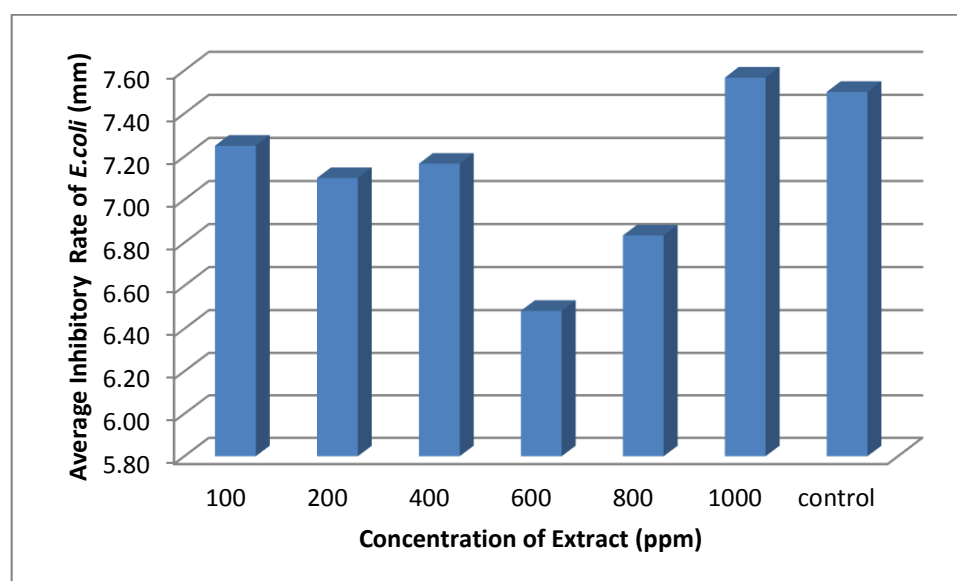
### 3.2. Antibacterial activity of extract on *E. coli*

*E. coli* is one of the many types of bacteria found in fresh water. Consuming the bacterial-contaminated food or beverages can cause gastrointestinal diseases. The magnitude of methanol extract inhibition zone against *E. coli* was shown in Table 2.

**Table 2.** Inhibition zone diameter of extract methanol of *R. mucronata* bark against *E. coli*

No	Concentration	Inhibitory rate (mm)			Average (mm)
		1	2	3	
1	100	8.65	6.15	6.95	7,23
2	200	5.05	7.8	8.45	7,1
3	400	8.15	6.85	6.5	7.16
4	600	6.3	7.7	5.45	6.48
5	800	8.25	6.5	5.75	6.8
6	1000	8.65	6.85	7.2	7.56
7	Chloramphenicol	8.15	7.95	6.4	7.56

Based on table 2, it was recorded that the extract has an inhibition zone diameter of  $> 6$  mm which means that the test material show positive activity in inhibiting the growth of *E. coli*. The highest concentration was seen at 1000 ppm with 7.56 ppm inhibition as well as chloramphenicol positive control was. Inhibitory rate comparison of methanol extract at various concentrations is shown in Fig. 3.



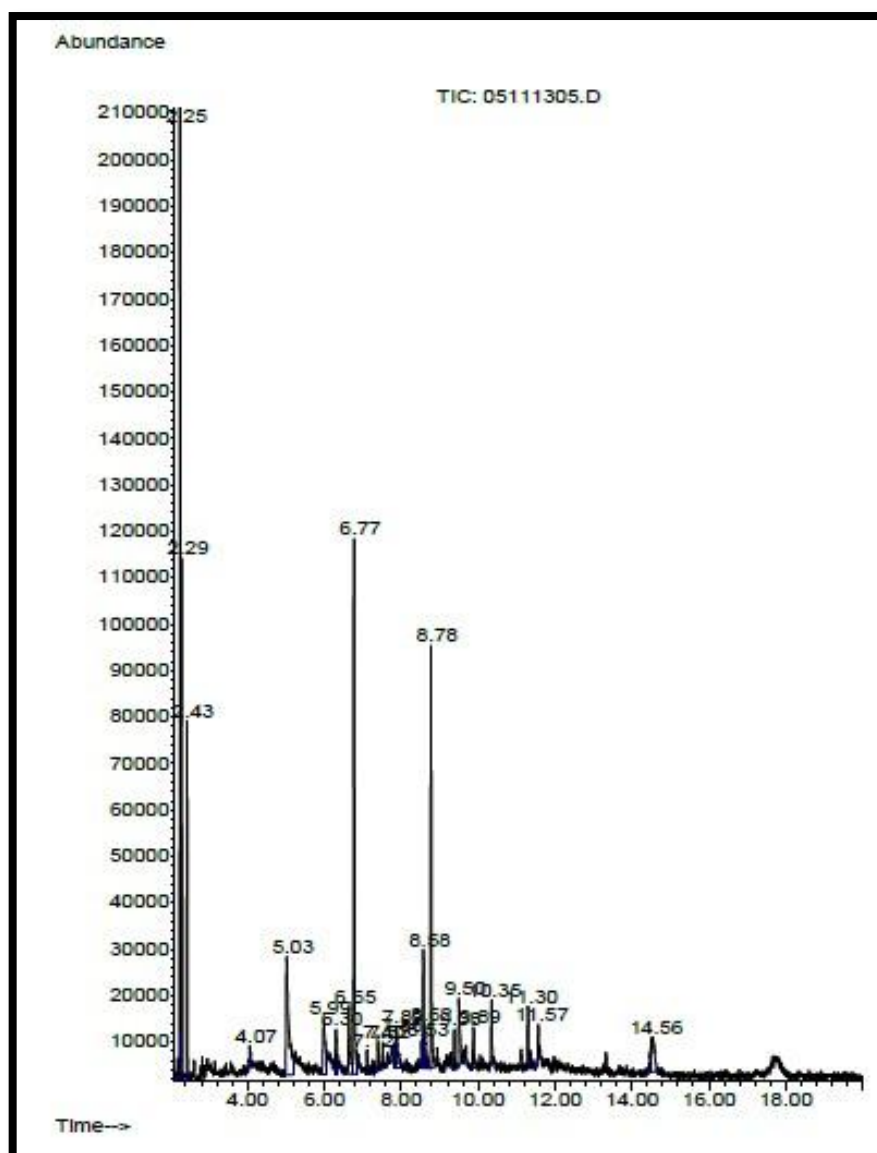
**Figure 3.** Comparison of concentration test against *E. coli* inhibition zone

The size of the inhibition areas produced by *R. mucronata* extract might be affected by the sensitivity of the organism, culture medium, incubation conditions and agar diffusion rate. This was in accordance with a statement that suggests factors affecting the speed of diffusion, they are; the concentration of microorganisms, the composition of the media, the incubation temperature and the incubation time [5]

The inhibitory zone differentiations produced by each extract can be affected by differences of the active ingredients content in each extract. The active compound will diffuse into the agar and will result in a concentration gradient. The highest concentration occurs in the area near the paper disc and decreases with increasing distance from the paper disc [5].

### 3.3. The content of secondary metabolite compounds of methanol extract of *R. mucronata*

Based on GC-MS chromatogram data (Fig. 4) it is known that there are 25 secondary metabolite compounds contained in methanol extract of *R. mucronata* stem bark.



**Figure 4.** GC chromatogram of *R. mucronata* stem bark extract

The 25 secondary metabolite compounds contained in *R. mucronata* stem bark extract in accordance with the GC-MS tool literature are presented in Table 3.

**Table 3.** GC-MS literature of secondary metabolite compounds contained in *R. mucronata* bark extract

<i>No</i>	<i>Compound name</i>	<i>RT</i>	<i>% Area</i>
1	Benzene, ethyl- (CAS) \$EB \$...	2,25	16,00
2	Benzene, 1,3-dimethyl- (CAS) \$...	2,29	8,63
3	XYLENE	2,43	6,86
4	3-Cyclohexene-1-carboxaldehyde,...	4,06	0,78
5	Phenol, 2-ethoxy- (CAS) \$Guet...	5,03	9,05
6	1,2-Benzenediol, 4-methyl-	5,99	3,53
7	Phenol, 3-methyl-5-(1-methyleth...	6,30	2,01
8	Benzaldehyde, 4-hydroxy-	6,65	2,96
9	TRANS-2-(1-PENTENYL)FURAN	6,78	14,29
10	Vanilin	7,11	0,65
11	1,4-Benzenediamine, N,N-dimethyl-	7,40	0,87
12	Benzoic acid, 3-hydroxy-, methy...	7,53	0,82
13	Benzenamine, 3-azido-N,N-dimeth...	7,86	0,99
14	Benzoic acid, 3-hydroxy-	7,89	1,70
15	4-Methyl-2,5-dimethoxybenzaldehyde	8,53	0,73
16	Phloroglucinol \$1,3,5-Benzene..	8,58	4,06
17	Phenol, 4-(3-hydroxy-1-propenyl...	8,62	1,34
18	2-propyl-5-vinylthiophene	8,78	11,03
19	Phenol, 2-methoxy-4-(methoxymet...	9,38	1,06
20	2-Trimethylsilyl-1,3-dithiane	9,50	3,23
21	Phenol, 4-(3-hydroxy-1-propenyl...	9,89	1,45
22	2-Hydroxy-5-methylbenzaldehyde	10,35	1,87
23	Hexadecanoic acid, methyl ester...	11,30	1,75
24	2-Methoxy-5-amino-4,6-diphenylp...	11,57	0,63
25	Di-n-octyl phthalate	14,56	3,70

The above GC-MS analysis results provide information that in the stem bark of *R. mucronata* mangrove plant obtained from the East Coast coastline Surabaya is rich of secondary metabolite compounds of phenolic, alkaloid, and terpenoid groups. These compounds are a widely used chemical in the health field as antioxidant, antibacterial, and antibiotic agents.

The decrease of bacterial population used in the testing process at all concentrations given by the test compounds showed that *R. mucronata* extract was able to suppress the growth of *A. hydrophila* and *E. coli* indicated by the increasing of inhibition zone diameter. The methanol extract inhibition

zone against *A. hydrophila* and *E. coli* was higher than that of chloramphenicol. This means that the extract is active to kill bacteria and can be used as an alternative to natural chloramphenicol.

Internal factors decreasing the density or population of bacteria is caused by *R. mucronata* extract that produce secondary metabolite compounds contained alkaloid group, or its derivatives that become inhibitors for the growth of *A. hydrophila* and *E. coli*. [6] explained that the steroid group has antibacterial activity. Alkaloids and their derivatives are also widely used as antibiotics that are able to inhibit bacterial growth [7].

#### 4. Conclusions

The results of the research on the extract of methanol bark of *R. mucronata* obtained from the East Coast of Surabaya showed that the methanol extract was active as an antibacterial indicated by the inhibitory rate against *A. hydrophila* and *E. coli* of 7.00 and 7.56 mm. The activity of antibacterial extract is higher than that of the positive control of chloramphenicol, therefore it can be used as an alternative natural antibacterial agent. The 25 secondary metabolite compounds of phenolic, alkaloid, and terpenoid groups contained in *R. mucronata* stem bark extract. The active compounds that act as antibacterial agents are thought to belong to the alkaloids.

#### Acknowledgement

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