Potency of mangrove *Rhizophora mucronata* as bactericide for vibrio causing tiger shrimp disease

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Abstract. Tiger shrimp diseases have been occurred in Indonesian brackishwater ponds for two decades and considered as one of the factors causing mass mortality of the cultured shrimp and making big loss for the farmers. *Vibrio harveyi* is considered as the main causative agent of this disease. The natural substance is proposed to kill or inhibit the growth of pathogenic vibrios. In order to know the potency of mangrove *Rhizophora mucronata* as a bactericide for *V. harveyi*, research had been conducted at the Research Institute for Coastal Aquaculture (BPPBAP) from February to May 2013. This research consists of several steps, i.e. 1) collection of plant; 2) drying of the plant; 3) making powder of plant; 4) extraction of plant/herb; 5) qualitative bioassay; and 6) quantitative bioassay. The results showed that qualitatively all part of the *R. mucronata* collected from Bone regency had activity against *V. harveyi*, but the leaf part of this mangrove collected from Maros regency did not have this activity, and neither the leaf or bark part of this mangrove collected from Pangkep regency. The values of Minimum Inhibition Concentration (MIC) on *V. harveyi* were ranged between 1-10.000 mg/L depending on which part of the plant and the origin of the mangrove. The root part of the plant had higher activity against *V. harveyi* than other parts of the plant. *Rhizophora mucronata* collected from Bone regency had the highest activity against *V. harveyi* than that collected from Maros and Pangkep regencies.

1. Introduction

Until now, tiger shrimp is still considered as the main product of Indonesian aquaculture. Besides that, white leg shrimp, milkfish, groupers, rabbitfishes, and seaweed are also important to fulfill the Marine and Fisheries Ministry target to become the biggest aquaculture producer in 2015. Through the Blue Revolution Policy, it was hope that the increase of aquaculture products should be 353% of that in 2010 [1]. However, several problems have been occurred, including shrimp diseases that happened in the shrimp hatchery and the culture ponds.

As we know that the shrimp diseases are the resultant of the weak shrimp stocked in the culture ponds, the virulence of pathogenic organisms including *Vibrio harveyi*, and the worse of the pond water quality [2]. The shrimp disease caused by this *V. harveyi* is then called as vibriosis. In general, vibrios in the shrimp is mainly caused by one or some strains of pathogenic vibrios like *Vibrio harveyi*, *V. alginolyticus*, *V. parahaemolyticus*, *V. damsela*, *V. vulnificus*, *V. penaeicida*, and *V. fischeri* [3–5]. Vibrios also cause diseases in marine fishes and bivalves [6], and even corals and sponges [7].
**V. harveyi** is considered as an opportunistic pathogen that attacks the shrimp when the culture water quality is getting worse. Larval stages of the shrimp-like zoea, mysis, and early postlarvae are usually more susceptible to *V. harveyi* than the older stage, so that mass mortality of the shrimp larvae oftenly occur in the shrimp hatchery.

To encounter vibriosis in the cultured tiger shrimp, some farmers use chemicals and antibiotics. Actually, this is the wrong solution because this antibiotic will make the resistant Vibrios species and accumulation of the antibiotic in the shrimp flesh [8]. Vibriosis should be prevented by environmental hygiene, vaccination, immunostimulant, and using antibacterial substances [9].

The use of the natural product of the mangrove plants for insecticide had been reported. Some mangrove plants have bioactive substances like saponins and tannins that are useful for bacteria eradication. Tannin can be used for protein denaturation and prevent the growth of bacteria. Saponins is a bioactive substance of the plant that can be used for bactericide [10].

In order to know the potency of mangrove *Rhizophora mucronata* as a bactericide for *V. harveyi* causing tiger shrimp disease, screening of the extract activity, column chromatography, and purification of the substances had been done. Antimicrobial activity of the plant extract w detected by 96-microwell plate [9].

### 2. Materials And Methods

#### 2.1. Collection of Mangrove plant, *Rhizophora mucronata*

Mangrove plants, *R. mucronata* were collected from the brackish water pond area in Bone, Maros, and Pangkep regencies of South Sulawesi province. Mangrove plant parts included leaf, flower, fruit, fruit cover, bark, and root was collected using plant scissors, knife, and ax (Figure 1). These collected plant samples were stored in a black plastic bag and brought to the Fish Health Management Laboratory of the Research Institute for Coastal Aquaculture (BPPBAP) for the bioactive isolation work.

![Figure 1](image1.jpg)

**Figure 1.** *Rhizophora mucronata* (A), Cutting mangrove root (B)

#### 2.2. Preparation of Mangrove Plant Herbs

Collected plants were cleaned and separated by the plant parts, i.e., leaf, flower, fruit, fruit cover, bark, and root. All of these plant parts were cutted in small size before dried (Figure 2). Drying of the plant parts was done by putting on the plastic tray separately, numbered sequentially, and air-dried for about two weeks or until fully dried. The dried plant herbs were ground into powder form, then stored in clipped plastic, labeled, and numbered sequentially.

![Figure 2](image2.jpg)

**Figure 2.** Cutting mangrove plant root, *R. mucronata*
2.3. Extraction of the plant herbs
Each plant powder was weighted for 5 g and extracted using the maceration method in a glass Erlenmeyer. The plant powder was added with 80% methanol until it was submerged, then stirred thoroughly. The glass Erlenmeyer was then covered with aluminum foil and stored for 24-h at room temperature. After 24-h, the methanol extract was filtered through Whatman No. 1 filter paper fitted in a Buchner funnel using suction and residue was submerged again with 80% methanol until three days or colorless and filtered every 24-h again. The filtrates obtained were combined and collected for concentration under reduced pressure by a rotary evaporator (Buchi type). If some methanol is still there (not too dry), the methanol evaporation should be done manually using tissue paper and small air hole.

2.4. Qualitative bioassay of the plant herbs for bactericide of V. harveyi
Antibacterial activity of the R. mucronata methanol extract was carried out using “High Throughput Screening. Each methanol extract obtained was weighted for 10 mg separately, then dissolved into 1 mL 10% DMSO. Antibacterial activity of the methanol extract was tested in 96-microwell plate [9].

2.5. Determination of Minimum Inhibition Concentration (MIC)
The determination of MIC was done using a 96-microwell plate [9] for the plant parts which had an antibacterial activity for V. harveyi. Each methanol extract was diluted to get the final concentrations of 0, 1, 10, 100 and 1000 mg/L.

3. Results And Discussions

3.1. Qualitative Bioassay of the Plant Herbs For Bactericide of Vibrio harveyi
Table 1 shows that each plant part (leaf, flower, fruit, fruit cover, bark, and root) and each origin (Bone, Maros, and Pangkep regencies) had different antibacterial activity against V. harveyi.

| No. | Mangrove plant part | Origin of mangrove |
|-----|---------------------|--------------------|
|     |                     | Bone   | Maros  | Pangkep |
| 1   | Leaf                | Positive | Negative | Negative |
| 2   | Flower              | Positive | (-)    | (-)     |
| 3   | Fruit               | Positive | Positive | Positive |
| 4   | Fruit cover         | Positive | Positive | Positive |
| 5   | Bark                | Positive | Positive | Negative |
| 6   | Root                | Positive | Positive | Positive |

(-) no samples

This qualitative bioassay showed that 13 of 16 samples of mangrove R. mucronata had antibacterial activity against V. harveyi. Antibacterial activity of the plant part was indicated by no color change of the bacterial culture media after incubation for 24-h in 96-microwell plate at room temperature using cell growth indicator (MTT). This result showed that basically, R. mucronata contains antibacterial activity but different activities for each part of the plant and the origin of the plant. This might be related to the environment of the origin of R. mucronata. This mangrove could grow in a wide range of water salinity. So that water salinity of the mangrove environment might cause the secondary metabolic of R. mucronata.

Feliatra (2000) reported that some species of mangrove plants have antimicrobial activity against Vibrio spp. The sensitivity of the Vibrio spp was shown by clear zone area surrounding paper disk containing mangrove extract on the bacteria culture agar media.
Secondary metabolic is defined as substances produced by microorganisms like bacteria, plants, and insects that are not used for their primary needs (growth and reproduction) but used for their existences in the environment. Some secondary metabolic produced by plants include alkaloid, terpenoid, and flavonoid. Secondary metabolic substances are directly affected by the worse environment condition and the available associated organisms like predators, competitors, and pathogenic microorganisms who compete for the living space and food.

3.2. Minimum Inhibition Concentration (MIC) of the mangrove plant on Vibrio harveyi

Table 2 shows that different plant parts and the mangrove origins had different Minimum Inhibition Concentration (MIC) of R. mucronata methanol extract on V. harveyi.

Table 2. Minimum Inhibition Concentration (MIC) of mangrove Rhizophora mucronata collected from different regencies on bactericide against Vibrio harveyi (mg/L)

| No. | Mangrove plant part | Bone (mg/L) | Maros (mg/L) | Pangkep (mg/L) |
|-----|---------------------|-------------|--------------|----------------|
| 1   | Leaf                | 1,000       | Negative     | Negative       |
| 2   | Flower              | 100         | (-)          | (-)            |
| 3   | Fruit               | 100         | 10,000       | 10,000         |
| 4   | Fruit cover         | 1           | 10,000       | 10,000         |
| 5   | Bark                | 100         | 100          | Negative       |
| 6   | Root                | 1           | 10,000       | 10,000         |

(-) no samples

Using 96-microwell plate method, it was shown in Table 2 that R. mucronata methanol extract from Bone regency had stronger antibacterial activity than Maros and Pangkep regencies. Based on the plant parts, the root part and the fruit cover part of the mangrove plant collected from Bone had 1 mg/L in MIC test on V. harveyi. This means that those parts of the Bone origin had stronger antibacterial activity than the other plant parts of the Bone origin and the other samples, including from the same plant parts but from Maros and Pangkep regencies. Plant leaf methanol extract of Maros and Pangkep origin and plant bark of Pangkep origin did not have any antibacterial activity against V. harveyi (Table 2). Plant root methanol extract of R. mucronata collected from Bone had a strong antibacterial activity against V. harveyi indicated by its MIC of 1 mg/L shown by no color change in the bacterial culture media after added with bacterial growth indicator. While mangrove plant leaf methanol extract of Maros and Pangkep did not have any antibacterial activity shown by the color change to blue or purple [9]. The lower value of MIC shown by mangrove methanol extract means a strong antibacterial activity against V. harveyi.

To inhibit bacterial growth, certain concentration of an antimicrobial substance is needed for bacterial cell wall destruction. Antimicrobial substances could be a bacteriostatic or a bactericide if their role as bacteriostatic, then below the MIC of this substance will cause the normal growth of the pathogenic bacteria. Mechanism of antibacterial activity usually inhibits the cell wall permeability, genetic system, enzyme activity, and increasing the essential nutrient Jawetz et al. (2001) [11] reported that the inhibition of the bacterial growth and the killing bacteria is caused by inhibition of synthesizing protein due to the bioactive substances. Mechanism of antibacterial inhibition on the pathogenic bacteria could be through bacterial cell wall destruction that causes lysis or inhibition of synthesizing bacterial cell wall, changing cytoplasmic membrane permeability that causes nutrient losses from the cell wall, denaturation of cell protein, and destruction of metabolic system in the cell through inhibition of intracellular enzyme activity [12].

Water environment conditions and biotic organisms have a big role in antibacterial activity substances produced by secondary metabolic of the mangrove plants. The triterpenoid substance is produced by the mangrove plant in order to protect mangrove from the high salinity. Low water
salinity close to freshwater, or very high salinity will enhance some secondary metabolic substances like triterpenoids for salinity stress and phenolic for oxidative stress.

Yasmon (2000) [13] mentioned that mangrove extract had antibacterial activity against Vibrio parahamolyticus in muddy and seawater media. It was also reported that mangrove leaf was more effective than mangrove fruit and bark. Mangrove Sonneratia ova and had more antibacterial activity against Vibrio parahaemolyticus in the plant leaf than mangrove fruit and bark. However, until now, it is uncertain bioactive substances in that mangrove that inhibit V. parahaemolyticus.

4. Conclusion
Based on the above experiment, it is concluded that mangrove Rizophera mucronata has anti Vibrio harveyi activity. Among 16 samples of the plant parts, the plant root and the plant fruit cover methanol extract of R. mucronata collected from Bone regency have the highest antibacterial activity against Vibrios causing tiger shrimp disease indicated by the MIC value of 1 mg/L. But the plant leaf methanol extract from the mangrove collected from Maros and Pangkep regencies did not have any antibacterial activity against Vibrio harveyi. The MIC values of R. mucronata methanol extract were 1-10,000 mg/L.

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