

In Vitro Antibacterial Activity of Sponge *Acanthella cavernosa* Against *Vibrio harveyi*

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ABSTRACT

V. harveyi is known as one of the causative agent in marine aquaculture. In this study, the extract from the sponge *Acanthella cavernosa* was tested for in vitro activity against *Vibrio harveyi*, marine bacteria. Disc diffusion method was used to carry out the antibacterial assay. Aqueous and methanol extracts of *A. cavernosa* at various concentrations (1, 3, 5, 7 and 9 mg mL⁻¹) exhibited anti-*V. harveyi* activity with inhibition zone diameters in the range of (8.07 ± 0.75 mm – 10.70 ± 0.10 mm) and (8.23 ± 0.38 mm – 16.77 ± 0.35 mm), respectively. Methanol extract showed more inhibitory effect against *V. harveyi* compared to the aqueous extract. The extract concentration of 9 mg mL⁻¹ exhibited maximum anti-*V. harveyi* activity in this study. It is the first report for the antibacterial activity of extract of *A. cavernosa* against *V. harveyi*. These results suggested that *A. cavernosa* is a potential source of alternative substance for controlling *V. harveyi* in shrimp farming.

Keywords: *Acanthella cavernosa*, Anti-*Vibrio harveyi* activity, disc diffusion method

INTRODUCTION

Black tiger shrimp (*Penaeus monodon*) has been known as a major important species in ASEAN and worldwide [1]. Shrimp farming have become a significant aquaculture activity in the tropics countries. In 1995, the ASEAN Member Countries produced about 558,000 metric tons of *P. monodon*, about 78% of the total world production of shrimp [2]. In 2008, Indonesia has produced 630,000 tons of shrimps and prawns, of which 410,000 tons through aquaculture. A number of 150,000 tons were exported with a value of 1.1 billion US\$, making shrimp one of the most valuable export products of Indonesia. However, the shrimp industry is susceptible. In 2009, the production dropped 10% [3]. Bacterial diseases have become major problem in the shrimp farming in Indonesia and other Asean countries [4]. Based on farm level surveys in 16 Asian countries in 1998, revealed that disease and environment-related problems have caused annual losses of more than USD 3,000 million to aquaculture production [2].

Vibrio harveyi is recognized as an important pathogen with causing mass mortalities in shrimp hatcheries [5]. In the intensive culture of *P. monodon*, virulent strains of *V. harveyi* cause devastating mortality in the hatchery stage. The disease phenomenon caused by these strains is commonly referred to luminous vibriosis [6]. In 1991, vibriosis spreading was reported in Eastern Java, Indonesia resulting in a decreased of 70% larval production and it is estimated that more than USD 85 million was lost [4].

Vibrios represent a large proportion of bacterial population of marine environment and act as pathogenic primary and secondary invaders of shrimps in the culture system [7]. Nowadays, using antibiotics on shrimp farmings not only for treatment but also used to prevent diseases. Even though use antibiotics leads to develop of drug resistant pathogenic bacterial strains [8], using chemical antibiotic in all shrimp farming is still popular in Indonesia.

The issue of chemical residues present in shrimp, particularly antibiotics, has been thrown into sharp relief by reports of residues of antibacterial being detected in shrimps imported into the EU from Vietnam, Thailand and Indonesia [9]. The resistance of *V. harveyi* to antibiotics might be caused by the use of various antibiotics in Indonesia aquaculture [10]. Based on these issues, addition of chemical antibiotics to control *V. harveyi* has become less effective [8] and affect product quality and consumer safety.

Due to increase of antibiotic resistant of marine bacteria *V. harveyi*, there has been the urgency to find new drug against these pathogenic bacteria [11]. Therefore, we conducted screening to explore the antibacterial activity from marine sponge to develop alternative treatment for bacterial diseases control in shrimp culture and found that *Acanthella cavernosa* has potential antibacterial activity. *A. cavernosa* have been reported as producers of several bioactive potential [12]. Chang et al. [13] reported that *Acanthella* sp. produce kalihinol-A for antibiotic source. However, the in vitro activity of *A. cavernosa* against bacterial disease in aquaculture is little known. Therefore, the aim of this study was to investigate the natural antibiotic from *A. cavernosa* against bacterium in marine aquaculture, *V. Harveyi*

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MATERIALS AND METHODS

Vibrio harveyi strain and medium

V. harveyi was obtained from Fish Quarantine Centre, Juanda, Surabaya, East Java, Indonesia. The bacterium was cultured in Nutrient Broth (NB) Medium, 13 g L⁻¹, Oxoid and Thiosulfate Citrate Bilesalt Sucrose Agar (TCBSA), 88 g L⁻¹, Oxoid. The bacterial stock cultures were maintained at 4 °C.

Sample of *Acanthella cavernosa*

The fresh sample of *Acanthella cavernosa* (Figure 1) was collected at Ketapang Coast, Banyuwangi, and East Java, Indonesia in February-March 2008.

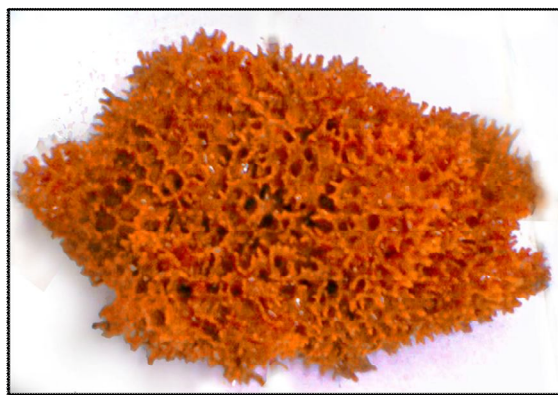


Figure 1 Morphology of *A. Cavernosa*

Preparation of Sponge *A. cavernosa* Extract

The sponge sample *A. cavernosa* were thoroughly washed with distilled water. Then fresh sample of sponge (100 g) was allowed to thaw, cut into small pieces, and was macerated with 80% methanol or water (100 ml) for three days and then filtered. The filtered was evaporated to a thick residue at 40° C and stored at 4 °C in a refrigerator for further use as crude aqueous and methanol extracts.

Antibacterial Sensitivity Assay

The disc diffusion method was used to carry out the antibacterial sensitivity assay. Assay discs were treated at the following concentrations (1 mg mL⁻¹, 3 mg mL⁻¹, 5 mg mL⁻¹, 7 mg mL⁻¹ and 9 mg mL⁻¹ for both aqueous and methanol extracts). The extracts with different concentration were impregnated on to sterile filter paper disc with the diameter 6 mm (Whatman No. 1) and placed on to the TCBSA medium which were previously swabbed with *V. harveyi* with bacterial density of 10⁶ cells mL⁻¹. All the plates were incubated for 24 h at 37 °C. Control disc was maintained, without the extracts. All concentration was performed by triplicate. The antibacterial sensitivity was interpreted from the size of the diameter of zone inhibition measured to the nearest millimeter [mm] as observed from the clear zones surrounding the discs [11].

RESULTS AND DISCUSSION

In vitro antibacterial sensitivities of the aqueous and methanol crude extracts of marine sponge *A. cavernosa* at different concentrations (1 mg mL⁻¹, 3 mg mL⁻¹, 5 mg mL⁻¹, 7 mg mL⁻¹ and 9 mg mL⁻¹) were tested against marine bacteria *V. harveyi* and its potency was measured as the diameters of the zone of inhibition (Table 1 and Table 2). The results showed that the crude aqueous and methanol extracts inhibit the growth of *V. harveyi*. All concentrations of aqueous and methanol extracts showed inhibitory effects in the range of (8.07 ± 0.75 mm – 10.70 ± 0.10 mm) and (8.23 ± 0.38 mm – 16.77 ± 0.35 mm), respectively. The crude extracts of *A. cavernosa* at different concentrations exhibited activity against *V. harveyi* indicated by clear inhibition zone surrounding the discs. *A. cavernosa* have been reported as the source of several bioactive substances including kalihinol A for antimalarial and antibacterial activity [12] [13]. Bugni *et al.* [14], reported that eight kalihinol type diterpenes were isolated from two Philippine *A. cavernosa* as potential bacterial folate biosynthesis inhibitors. Bergquist and Bedford [15] proposed that antibacterial activity to gram-negative bacteria suggests that active constituents in sponges act to increase the efficiency of capture and digestion of particulate materials by causing a clumping of susceptible bacteria.

Table 1 Antibacterial activities of aqueous crude extract of *A. cavernosa*

Extract concentrations (mg mL ⁻¹)	Diameter of inhibition zone (mm)	Standar deviation (± mm)
1	8.07	0.75
3	8.87	0.21
5	9.23	0.51
7	9.77	0.35
9	10.70	0.10

Table 2 Antibacterial activities of methanol crude extract of *A. cavernosa*

Extract concentrations (mg mL ⁻¹)	Diameter of inhibition zone (mm)	Standar deviation (± mm)
1	8.23	0.38
3	10.37	0.59
5	12.20	0.40
7	14.23	0.55
9	16.77	0.35

Increasing extract concentration of *A. cavernosa* from 1 mg mL⁻¹ to 9 mg mL⁻¹ led to an increase of diameter of inhibition zone with the maximum inhibition zone of 10.70 ± 0.10 mm and 16.77 ± 0.35 mm were achieved from the highest concentration at 9 mg mL⁻¹ in both aqueous and methanol extracts. The result indicated that 9 mg mL⁻¹ was the best concentration in this experiment. This phenomenon agreed with Isnansetyo et al. [16], who reported that increasing *Geodia* sp. extract concentration from 1 to 4x minimum inhibitory concentrations (MICs) reduced the number of *V. harveyi* density from 2.39 x 10¹⁰ cells mL⁻¹ to 9.2 x 10³ cells mL⁻¹.

In all concentrations, methanol extract showed more effect against *V. harveyi* compared to the aqueous extract as shown in Figure 2. In accordance with the study of Karaman et al. [17], who showed that methanol extract of *Juniperus oxycedrus* inhibited the growth of 24 out of 56 bacteria species, however, *J. oxycedrus* aqueous extract showed no effect to 56 species of bacteria. Abu-Shanab et al. [18] also showed that methanol extract of the dried ripe berries of *Rhus coriaria* species inhibited all tested bacteria, however, its aqueous extract showed effect on 2 out of 5 tested bacteria. On the other hand, Nair et al. [19] reported that *Hibiscus rosasinensis* aqueous extract performed more effectively than methanol extract against tested bacteria.

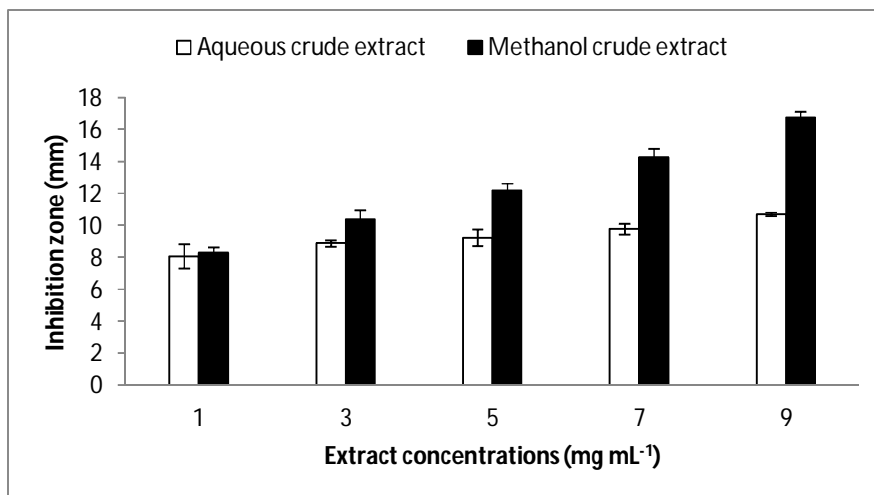


Figure 2 Inhibition zone diameters of *A. cavernosa* methanol and aqueous crude extracts

Bergquist and Bedford [15] have suggested that the antibacterial agents produced by sponges may have a role in enhancing the efficiency with which sponge retain bacterial food. It has been known that sponges produce secondary metabolites to repel and deter predators [20] and protection against some of these biotic

challenges [21]. Sponges produce a wide array of secondary metabolites ranging from derivatives of amino acids and nucleosides to macrolides, porphyrins, terpenoids, aliphatic cyclic peroxides and sterols [22].

CONCLUSION

The aqueous and methanol extracts of *A. cavernosa* showed a potential antibacterial activity against *V. harveyi*, a pathogenic bacterium in marine aquaculture. The maximum anti-*V. harveyi* activity was observed in methanol extract (16.77 ± 0.35 mm) compared to aqueous extract (10.70 ± 0.10 mm). We proposed to carry out the toxicity test of the extract before applied into shrimp culture.

ACKNOWLEDGEMENT

This study was financially supported in part by Faculty of Fisheries and Marine Science, University of Brawijaya, Indonesia. We sincerely grateful to Dr. Aida Sartimbul for her excellent support.

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