The Performance of Mangrove (Rhizophora apiculata) Leaf Extract to Treat Vibriosis

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RESEARCH ARTICLE

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The Performance of Mangrove (*Rhizophora apiculata*) Leaf Extract to Treat Vibriosis *(Vibrio harveyi)* in Mud Crab (*Scylla serrata*)

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Mud crabs (*Scylla* serrata) is one of the most economic crabs in Central Java. However, mortality was reached 80% during fattening due to bacterial infection. Meantime, mangrove leaf contain bacteriostatic and bactericidal such as saponin, flavonoid, tanin, alkaloid, steroid, terpenoid and phenol that might effective to control bacterial infection. This study was carried out to observe the performance of mangrove leaf extract to treat vibriosis in mud crabs at various concentration *in vitro* and *in vivo*. *In vitro* study demonstrated that mangrove leaf extract at concentration of 300 mg/l to 900 mg/l were able to prevent *Vibrio harveyi* growth and formed clear ring between 11.37–13.67 mm. Forty eight mud crabs at body weight of $40,11 \pm 2,53$ g were injected with a bacterial *V. harveyi* at density of 10^6 CFU/ml in between the leg join. Then immersed into mangrove leaf extract at concentration of 0 mg/l, 300 mg/l, 600 mg/l, 900 mg/l for 30 minutes. Post injection of bacterial Vibrio crabs showed clinical signs such as wide apart of swimming and walking legs, blackened carapace, white spots, and red spots. Immersion of mangrove leaf extract post injection could improved survival rate, healing and histopathological features of experimental crabs. Therefore, it can be concluded that the use of mangrove leaf extract at 900 mg/l demonstrated best survival rate, histopathological feature and recovery after 14 days post infection.

Keywords: *Scylla serrata*, Mangrove Leaf, *Vibrio harveyi*, Survival Rate.

1. INTRODUCTION

Central Java is one producer of soft shell Mud crabs (*Scylla serrata*), producing more than 800 tonnes in 2010 and significantly declined to 351 tonnes in 2011.[13] This significant decreased of the mud crabs production partly due to over exploitation and disease. Vibrios were commonly found as a normal microflora of mud crabs.[18] Its also demonstrated that *V. Harveyi* infected mud crabs could be further infected by white spot syndrome virus.[21] Further research conducted by Chen et al., and Des Rosa and Johny[2,4] stated that vibriosis become a concern in mud crabs culture. Furthermore Lavila-Pitogo and De la Pena[10] discovered that the cause of vibriosis in Iloilo, The Philippines was *Vibrio vulnificus, V. parahemolyticus, V. splendidus, and V. Orientalis*. Similar study conducted Sarjito[24] demonstrated that *V. harveyi, V. fischeri and V. Ordalii* were involved in the vibriosis outbreaks of mud crabs in Semarang Bay, Central Java. According Jithendran et al.,[7] the clinical signs of crabs infected by bacteria *Vibrio* spp. showed black spots or brown spots on the carapace as well as erosion and melanization (dark brown to black colour) in the infected area. A similar symptomes reported by Sarjito et al.[24] such as gills opening and dark carapace, injuries on the claw, ventralabdomen, and carapace. Slow movement and low feeding response, frequently rise to the surface, and produce bubbles. The above bacteria could cause 100% mortality on mud crab, especially on the larval stage.[27]

Various chemicals and antibiotics have been applied to prevent and cure the disease. However, the results were unsuccesfull. Moreover, the use of antibiotics could lead pathogens resistance, polluted the environment, and endanger consumer health.[6,30] Therefore, the use of natural ingredient become an alternative way to handle that problem. One of the natural ingredient was mangrove leaves extract (*Rhizophoraapiculata*). Several mangrove species content bioactive compound that potentially being used for natural remedies.[21] Mangrove leaves (*R. apiculata*) extract was reported inhibited the growth of bacteria because of its bioactive compounds such as alkaloids, tannins, steroids, saponins, phenols, glycosides, flavonoids and terpenoids which proved enable to reduce the growth of bacteria. Moreover, it also functionated as an antiseptic, anti-virus, anti-inflammatory, and anti-bacterial remedy.[19,22,24] Flavanoids was the bio compound that able to prevent inflamation.[11] The aims of this study was to find an effective dose of mangrove leaf extract on the

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Table I. **Antibacterial activity of methanol *R. apiculata* leaf extract against bacterial *Vibrio harveyi*.**

		Diameter of inhibition (mm)			
Pathogen	Replication	Control (PBS)	300 mg/l	600 mg/l	900 mg/l
Vibrio harveyi	1	0	11.1	13.4	13.8
	2	0	11.6	13.0	13.4
	3	0	11.4	13.1	13.8
Average			11.37	13.17	13.67

Table II. **Phytochemical screening of *R. apiculata* leaf extract.**

No.	Component	Colour indication	Result
1.	Saponin	Brown and foams	Positive (+)
2.	Flavonoid	Red on surface	Positive (+)
3.	Kuinon	Black	Negative (−)
4.	Tanin	Black	Positive (+)
5.	Alkaloid	Bright-red	Positive (+)
6.	Steroid	Brown	Negative (−)
7.	Terpenoid	Red-brown	Positive (+)
8.	Phenol	Red-black	Positive (+)

survival rate, hepatopancreatic histopathological feature of mud crab infected by *V. harveyi*.

2. MATERIALS AND METHOD

This research was design experimentaly using Completely Randomized Design with 4 treatments namely mangrove leaf extract 0 mg/l, 300 mg/l, 600 mg/l and 900 mg/l. Data of survival rate of experimental crabs was analysed by analysis of variance whilst data of clinical signs and histopatology were analysed descriptively. The experiment was conducted in the fish health laboratory, faculty of Fisheries and Marine Science, Diponegoro University.

The mangrove leafs (*Rhizophora apiculata*) around 1 kg were collected from Kendal District. They firstly cleaned, washed and dried at room temperature. Then soaked in methyl alcohol (methanol), macerated, and filtered to obtain extract solution. The solution was mixed and concentrated by Rotary Vacuum Evaporator at a temperature of 50 °C to obtain the concentrated extract.[1]

The performance of mangrove leaf extract against bacterial pathogen *Vibrio harveyi in vitro*[23] was conducted using agar disk diffusion method. Antibacterial assay plates were prepared by pouring 20 ml of Zobell marine agar and allowed to solidify. The 0,2 ml culture of *Vibrio harveyi* in the logarithmic phase at concentration 10^8 cells/ml was spread on to agar medium. Blank paper disk (6 mm; Advantec, Toyo Roshi, Ltd., Japan) were impregnated with 15 μl of mangrove extracts at concentration of 300 mg/l, 600 mg/l, 900 mg/l and in PBS as negative control respectively. The plates were then incubated at 34 °C for 48 hours. Each sample was tested in triplicate and the diameter (mm) of the clear zone was measured.[25] Phytochemical test was carried out to identified bio compound of mangrove leaf extracts using standard procedures for qualitative determination of phytochemical constituents.[17]

Fourty eight mud crabs (*S. Serrata*) at average of 40.11 ± 2.53 g were used in this experiment. The treatments consisted of 4 concentration of mangrove leaf extracts and replicated 3 times. Each crabs were kept individually in the wooden shelter in Aquarium. The crabs were injected intramuscularly with bacterial

Fig. 1. Clinical signs of mud crab post infection (a, b) and post bathing (c, d), where (a) red on swimming leg, (b) blackened carapace (c) recovery of red legs, (d) normal carapace (greenish).

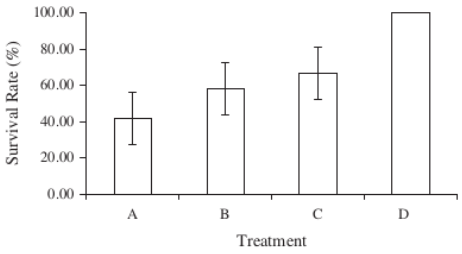

Fig. 2. Diagram of survivalrate of infected mud crabs (*S. serrata*) post-immersion (A) 0 mg/l; (B) 300 mg/l; (C) 600 mg/l; (D) 900 mg/l of magrove leaf extract.

pathogen *Vibrio harveyi* at concentration of 10^5 cfu/ml. After the appearance of moribund signs, crabs were then long-bathed at various concentration of mangrove leaf extract (*Rhizophora apiculata*) namely 0 mg/l, 300 mg/l, 600 mg/l, 900 mg/l fro 30 minutes. Clinical signs, survival rate, and histopathological feature of hepatopancreas were observed. Water quality was maintained at pH 8.3, salinity 25 ppt, dissolved oxygen 5–5.9 mg/l and water temperature 27–28 °C.

3. RESULTS AND DISCUSSION

The *in vitro* study of mangrove leaf extract (*Rhizophora apiculata*) indicated that the mangrove leaf extract possessed intermediate antibacterial activities against *Vibrio harveyi*, producing clear zone at diameter between 11.37–13.67 mm at all concentrations (300 mg/l, 600 mg/l and 900 mg/l) as indicated in Table I.

This study revealed that the mangrove leaf extract (*R. Apiculata*) was intermediately effective as an antibacterial agent against*V. Harveyi*, because according to *Clinical and Laboratory Standards Institute*[3] effectiveness of antibacterial according to clear zone formation devided into three namely clear zone ≤12 mm (*Resistance*), 13–16 mm (*Intermediate*), and ≥17 mm (*Sensitive*). It was reported that secondary metabolites in the mangrove seed were involvrd in the antibacterial activities of *Vibrio harveyi*.[12,19] The highest antibacterial activities were believed to be the presence of high content of secondary metabolites, such as saponin, flavanoid, tanin, alkaloids, terpenoids, and phenol (Table II). Moreover, plant-derived compounds that potentially exhibited antibacterial activity include phenolics, phenolic acids, quinones, saponins, flavonoids, tannins, terpenoids, and alkaloids.[9]

Infected mud crabs showed abnormal behaviour and morphological changes whitin 30–60 minutes post injection of *V. harveyi*, such as passive movement, wide apart of swimming and walking legs, blackened carapace, and appearance of red spots on swimming legs (Figs. 1(a, b)). It was reported that the presence of white spots on the carapace, emit bubbles, slowing and passive movement, and opening of crab gills were some clinical signs of mud crabs infected by bacterial *V. harveyi*.[5,15] Post long-bath, it was clear that redish colour at swimming legs gradually disappeared, carapace colour became greenish and mud crabs were started to response to feed (Figs. 1(c, d)).

After 14 days post injection no mortality was found in the crabs immersed in mangrove leaf extract at concentration

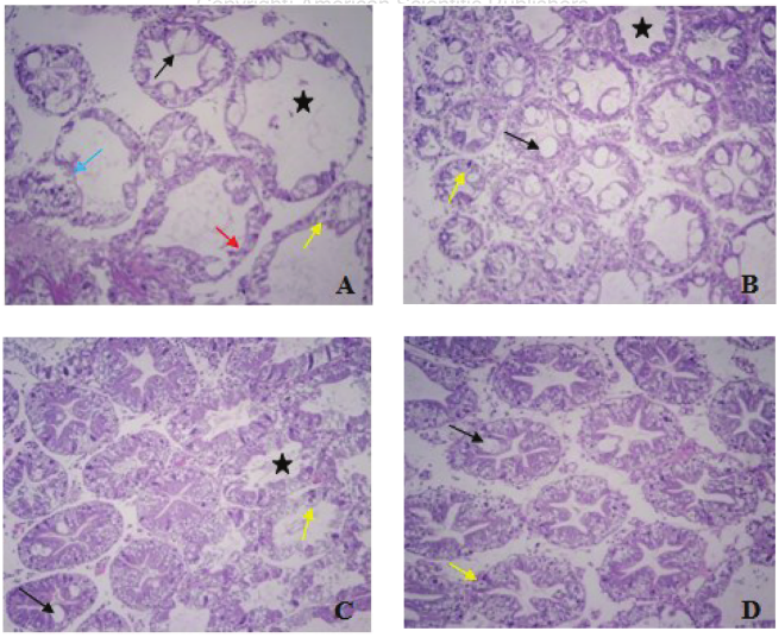

Fig. 3. Cross section of Mud Crabs Hepatopancreas (*Scylla*serrata) Post Immersion in Mangrove leaf extract. HE stain, 100 magnification, and (A) 0 ppm; (B) 300 ppm; (C) 600 ppm; dan (D) 900 ppm ↙: Bolitas ↙: Lesion ↙: Vakuolosis ↙: Nekrosis ⋆: Atrofi.

900 mg/L. Whilst infected mud crabs that immersed in 0 mg/L, 300 mg/l and 600 mg/l mangrove leaf extract only survived 41.6%, 58.33%, 66.67% and 100% respectively. This was indicated that the use of 900 mg/l mangrove leaf extract cured bacterial infection and prevent further mortality (Fig. 2). Similar research also indicated that leaf mangrove extract (*Aegiceras comiculatum*) was able to inhibit *V. Harveyi* and *V. Parahaemolyticus*.[28] Other researcher stated that extract mangrove *Avicennia* sp and *Sonneratia* sp were also able to prevent WSSV infection.[29]

Histopathological study demonstrated in general, hepatopancreas of experimental mud crabs were normal (Fig. 3). However, mild necrosis and vacuolalization were found in this study and it might not solely due to *V. Harveyi* artifical infection. Necrosis of the cells could be caused by biological agents such as viruses, bacteria, fungi, and parasites or chemical agents as well as the disruption of the blood supply to the tissues of the body, resulting in shrinkage of nucleus as a whole.[26] Previous research reported that there were necrosis in hepatopancreas of *Penaeussemisulcatus*,[14] and juvenile vannamei shrimp (*Litopenaeusvannamei*).[16] Vacuolisation was an empty holes due to the damage of hepatocytes and nucleus and cytoplasm were disappeared. Vakuolisis were empty holes cells that occured due to accumulation of fat in the hepatopancreas tubules, nor buildup of toxic materials or lack of oxygen.[8] Those research confirmed that vacuolisis in this research was due to non bacterial infection.

4. CONCLUSION

Based on the research results above, it can be concluded that mangrove leaf extract (*R. apiculata*) contained bioactive compounds that strongly inhibited bacterial pathogen. At concentration of 900 mg/l demonstrated the best performance to combat bacterial infection of *V. Harveyi* provided 100% survival rate of mud crabs (*S. serrata*) up to 14 days of recovery period. There was no spesific histopathlogical feature related to bacterial infection.

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