MIC (Minimum Inhibition Concentration) test of metanol extract on rhizophora stylosa and chloroform Avicennia marina against vibriosis in mangrove crab larvae (Scylla serrata forsskal)

Burhanuddin1, A Saru2, A Rantetondok3 and E N Zainuddin3

1Graduate School, Hasanuddin University, Makassar, Indonesia.
2Faculty of Marine and Fishery Science Study Program of Marine Science, Hasanuddin University, Makassar, Indonesia.
3Faculty of Marine and Fishery Science Study Program of Aquaculture, Hasanuddin University, Makassar, Indonesia.

Email: h.burhanuddin@unismuh.ac.id

Abstract. At the level of severe attack, the type of vibrio bacteria cause 100% death in mangrove crab larvae, it can be overcome by using mangrove fruit extracts. Research information on the antibacterial activity of R.Stylosa and A.marina mangrove fruit extracts that cause vibriosis of mangrove crab larvae (Scylla serrata F), including further research on the minimum concentration of mangrove fruit extracts that still able to inhibit the growth of disease-causing bacteria in mangrove crab larvae, considering the use of medication / extracts at high doses is not safe for the early stages of mangrove crab larvae. The purpose of this study was to determine the minimum concentration of mangrove extracts (metanol R.stylosa and Chloroform A.marina) that can inhibit vibriosis in mangrove crab larvae (S. serrata). This study systematically began with fruit sample collection, extraction of R.Stylosa and A.marina active ingredients, bacterial isolation, % of extract calculation and antibacterial activity test for vibrio bacteria, then the minimum mangrove extract concentration or MIC (Minimum inhibition Concentration) test. The MIC test results of metanol extract of R.stylosa fruit were found at concentrations of 1000 ug / mL, where the minimum categorized concentration with an area of diameter inhibition zone, and the MIC test results of A.marina chloroform extract were found at concentrations of 250 ug/mL, which the minimum categorized concentration with an area of diameters of inhibition of 8.26 ug / mL. The MIC test value is still categorized as safe to be applied to mangrove crab larvae (S. serrata).

1. Introduction
The hatchery has succeeded in seeding mangrove crabs but has not been able to meet the need for crab seeds because it still faces various problems. One of the problems faced is an effort to increase the survival rate of the larval stage, especially in the zoea and megalopa stages. Various studies have been carried out, but the survival rate obtained is remain less. Jantrarotai et.al., (2002) reported the survival rates of zoea stadia to megalopa ranging from 22.91% [1], Karim (2013) 15-21%, 49%; 15.67-31.50%, 29.42-48.42% and 21% [2-4], It is suspected that the decline in mangrove crab production was mainly due to the spread of disease in crab culture. The disease caused by Vibrio bacterial infection or called vibriosis is one of the diseases that often occurs in the cultivation of mangrove crabs [5].Types of bacteria in mangrove crabs have been reported by Lavilla and De la Pena (2004), namely the discovery of V.vulnificus, V. parahemolyticus, V.splendidus, and V.orientalis in Iloilo, Philippines. Sarjito et.al., (2014) also reported that [6] V.harveyi, V.fischeri and V.ordalii were found in mangrove crabs in Semarang, Central Java, while V.alginolyticus and V.cholera were
found by Taplur et al., (2011) in crab movement (blue swimming crab) from Terengganu waters, Malaysia. Kareho et al., (2019) reported that before being given A. marina, there were identified V. alginolyticus bacteria in the digestive tract of crab Uca spp [7]. Furthermore [8], it was explained that at severe attack rate, this type of bacteria can cause 100% death in mangrove crabs, especially in zoea stadia larvae to megalopa, related to this case, then it is a reason for conducting research on the use of natural materials such as mangroves (Rhizophora spp) and fires (Avicennia spp) in order to overcome the attack of Vibrio sp bacteria on mangrove crabs [9], especially in larval stages. Burhanuddin et al. (2019) has conducted a preliminary study of the in vitro test on the antibacterial activity of the R. Stylosa and A. marina mangrove fruit extract in vitro causing vibriosis of mangrove crab larvae (Scylla serrata F) [10]. Antibacterial properties of A. marina and R. stylosa extracts because they contain secondary metabolite compounds such as alkaloids, steroids, tannins and glycosides [11]. Further research on the minimum concentration of mangrove extract which is still able to inhibit the growth of vibrio-causing bacteria in mangrove crab larvae is very much needed, considering the use of medication at high doses was less safe for the early stages of mangrove crab larvae.

2. Material and Methods

2.1. Materials

The tools used in the study are incubators, micropipets, autoclaves, fume hoods, digital scales, Vortex, refrigerators, jars, needle ose, petri dishes, test tubes, bunsen, basins, trays, aeration hoses, blowers, aersators, 1000 mL measuring cups, funnel, 100 mL measuring cup, 10 mL pipette, 1000 mL and 500 mL erlenmeyer flask, measuring flask, tube rack, mask and gloves.

The materials used in this study were mangrove fruit extract, Solvent chlorofm and methanol, Sea water Complete (SWC) media, Tryptic Soy Agar (TSA), Tryptic soy Broth (TSB), Bacteria V.harveyi of BPPBAP Takalar,, 70% alcohol, distilled water sterile, paper disks, cotton, thiosulfate, chlorine, matches, sterile sea water, label paper, tissues, aluminum foil, Artemia salina and washing soap.

2.2. Preliminary Test

Preliminary Test of this study as stated by Burhanuddin et al. (2019) include; fruit sample collection, extraction, determination of extract percentage, and antibacterial activity test [10].

2.3. Methods

Determination of the minimum inhibitory concentration was carried out at the Fish Parasite and Disease Laboratory, Department of Fisheries, Faculty of Marine and Fisheries, Hasanuddin University. This stage was carried out after getting the results of antibacterial activity tests (based on the method of Schegel and Schmidt, 1994) [12]. Active extracts of mangroves that inhibit bacteria with the largest inhibitory diameter that were followed by a minimum inhibitory test (MIC). The KHM test in this study was carried out on Vibrio harveyi bacteria by using a simplified extract that had been obtained from the results of previous tests.

Schegel and Schmidt (1994) stated that there were two methods used in testing MIC (Minimum Inhibitory Concentration) [12] or KHM, namely the dilution tube technique (tube dilution technique) and the agar diffusion method (agar diffusion method). In the dilution tube technique, several series of tubes prepared containing a culture medium have been inoculated with microorganisms to be tested and given antimicrobial substances with varying concentrations. Antimicrobial activity is determined by the turbidity seen in the tube. Furthermore [13], stated that in this method used an erlenmeyer filled with liquid media and a certain number of bacteria tested, then each erlenmeyer was filled with compounds tested and incubated at 37 ° C for 24 hours. The lowest concentration of compounds that give clearer yields to cultures is the minimum inhibitory content of these compounds.

The method used was the agar diffusion method (agar diffusion method) or liquid dilution using a 96 well microtiter container with the broth dilution method [14]. Bacterial suspension was prepared by dissolving one bacterial loop in 2 mL of 0.9% NaCl solution. Then 50 μL of this solution was inoculated in 50 mL TSB and incubated overnight in an incubator shaker. The extract concentration used begun from 2 mg/mL and diluted with a multiple of 0.5 times. The concentration of antibacterial mangrove fruit extract in the MIC/MIC test was 2000 μg /ml, 1000 μg /ml, 500 μg /ml, 250 μg /ml, 125 μg/ml, 62.5 μg /ml, 31.25 μg/ml, 15,625 μg/ml, and 7,8125 μg/ml [14].
The minimum inhibitory concentration test (MIC test) referred to the bacterial inhibitory activity test indicated by the presence of an inhibitory zone (clear zone/halo zone) around the paper disc. The diameter of the bacterial growth inhibition zone was measured in mm and made a quantitative measure for the size of the inhibition zone.

Each Extract is dissolved in the appropriate solvent

Put about 2 mg/50 µl/disk on sterile disks of diameter 6 mm

The disk containing the extract is placed on surface

Inoculated 2 ose and dissolved with 3 ml NaCl 0,9%

Suspension of V. harveyi + media

Inhibition Zone by the extraction with clear zona

Figure 1. Minimum Inhibition Zone Test Procedure (MIC)

2.4. Data Analysis
Research data were tested descriptively.

3. Results and discussion

3.1. MIC Test (Minimum Inhibition Concentration)
Before doing the in-vivo test, the MIC test was first performed with the aim of knowing the minimum dose of A.marina chloroform extract and R.stylosa methanol extract which can inhibit the growth of V.harveyi bacteria.

MIC test was measured based on the area of the zone of inhibition formed around the disk / disc containing the extract. MIC test results of A.marina chloroform extract were presented in Figure 2.

Figure 2. Test chart of MIC on Bacteria V. harveyi Extract of Chloroform A. marina
In Figures 2 and 3, it can be seen that the MIC test results of Chloroform A.marina extract against Vibrio harveyii bacteria that were tested at extract concentrations of 2000–7.8 µg / mL had the ability to inhibit the growth of Vibrio harveyi bacteria at the concentrations of 250 µg / mL, MIC values found at a concentration of 250 µg / mL (average inhibitory zone = 8.26 µg / mL), MIC test results obtained in this study were at a concentration of 250 µg / mL, where the minimum categorized concentration with an area of diamate inhibition zone 8, 26 µg / mL. Concentrations below 250 µg / mL which is 125 µg / mL extract have no potential to inhibit the growth of Vibrio harveyi bacteria. This based on the statement [15] Antimicrobial or antibacterial properties of a compound of high activity against pathogenic bacteria then value of the lowest bacterial inhibition concentration (MIC), otherwise the inhibitory diameter is large.

For the minimum inhibition concentration test or MIC of R.stylosa methanol extract can be seen in Figure 4.

Figure 3. MIC test results of MIC test on Bacteria V.harveyi Extract of Chlorofom A.marina.

![MIC test results on Bacteria V.harveyi Extract of Chlorofom A.marina.](image)

Figure 4. MIC test results on V.harveyi bacteria Methanol Extract R.stylosa
In Figures 4 and 5, it appeared that the MIC test results of R.stylosa methanol extract on Vibrio harveyi bacteria were tested at extract concentrations of 2000 - 7.8 µg / mL, MIC test results obtained in this study were found at concentrations of 1000 ug / mL, which is a minimum categorized concentration with an area of 6.67ug / mL diameter inhibitory zone, because the concentrations below 1000 ug / mL that is 500 ug / mL the extract has no potential to inhibit the growth of Vibrio harveyi bacteria, this based on statement [15]. Antimicrobial properties or antibacterial is said to have a high activity against pathogenic bacteria if the value of the lowest bacterial inhibition concentration (MIC), but the inhibitory diameter is large.

4. Conclusion
The lowest concentration of mangrove extract (MIC) or MIC (Minimum Inhibition Concentration) which can inhibit vibriosis in A.marina chlorofin extract is 250ug / mL and in R.stylosa methanol extract is 1000ug / mL.

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