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Antibacterial Activity *Rhizophora stylosa* and *Avicennia marina* of Mangrove Fruit Extraction on *Vibriosis* of Mangrove Crab Larvae (*Scylla Serrata* Forsskal)

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***Abstract*— *The stability of mangrove crabs (Scylla serrata Forsska^{0}l) production were still constrained, especially the high mortality in the larval stage caused by vibriosis infection in the form of vibrio bacterial attack, so natural extracts as antibacterial such as R.stylosa and A.marina were needed. The purpose of this study was to assess the antibacterial activity R.Stylosa and A.marina of mangrove fruit extracts through in-vitro as the cause vibriosis of mangrove crab larvae. (Scylla serrata F). On fruit sample and extraction of active ingredients R.Stylosa and A.marina fruit were the initial stages of the study, then continued with bacterial isolation, soaked calculation of% and antibacterial activity test for antibacterial on vibrio bacteria. The results of the soaked calculation of% R.stylosa at the concentration of 2 mg / disk / 50 µl, the highest obtained in metanol was 12.92% and A.marina was 8.61% in metanol; The results of antibacterial activity based on inhibition zone area, for the highest R.stylosa in solvent metanol (11.40 mm) , in V.harveyi bacteria, then followed by 7.18 mm in V. alginolyticus bacteria, then 7.03 mm in V.paraemolyticus bacteria, where as the results of antibacterial activity based on inhibition zone area ,for the highest A.marina in the Chloroform solvent (21.09mm), in V.harveyi bacteria, then followed 19.08mm in V. paraemolyticus bacteria, and 11.80mm in V.alginolyticus bacteria. Obstacle zones obtained categorized as moderate to very high (> 11- <15mm = moderate; height = 15-20 mm; and very high => 20mm) based on the description at Zainuddin (2006) so that they met the requirements to be applied through in-vivo.*

***Keywords*— *R.stylosa, A.marina, Antibacterial, Vibriosis, Scylla Serrata*.**

I. INTRODUCTION

One of the roles of mangroves is as an antibacterial active ingredient, because mangrove plants, such as *R.stylosa* and *A.marina* contain antibacterial compounds such as *alkaloids, flavonoids, phenols, terpenoids, steroids and saponins*. This class of compounds is the ingredient of modern medicines (Janah, 2017; Pratama, 2014; and Eryanti, 1999), so it is expected to be used for testing the production of antibiotics against *Vibrio* sp bacteria in tackling *vibriosis* in crab larvae that are of economic importance in aquaculture, this is interesting to study because ecologically there is a strong interaction between mangrove crabs as cultivants and mangroves as habitats, and educationally this research is expected to contribute in order to help increase productivity in the fishing industry sector, especially in crab cultivation.

The increasing demand for mangrove crabs stimulates farmers to cultivate mangrove crabs, both in hatcheries and enlargement in ponds associated with the mangrove environment. The limited population of mangrove crabs and the amount that is affected by the season and the high cost of feeding is one of the obstacles in the maintenance of crabs (Lateef *et.al*., 2008). The main obstacle in aquaculture activities is diseases in biota (Hatmanti, 2003). Lavilla and Pena (2004) state that bacterial infections attack all crab stages, both larvae and adult crabs. Jithendran *et.al*. (2010) stated that *Vibrio harveyi* 10^5 - 10^7 CFU /ml pathogenic in the mangrove crab zoea stage. Irianto (2007) explains that *Vibrio* sp. is a primary pathogen in marine and brackish culture.

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According to Ashofa and Prayitno (2014), *Vibrio* sp. also a secondary pathogen, meaning *Vibrio* sp.infect after the attack of other diseases such as protozoa or other diseases. Furthermore, it was added that the maintenance of mangrove crabs (*Scylla serrata Forsskal*) still often decreased production due to the presence of *vibriosis* in the form of an attack of vibrio bacterial infection. The mortality of mangrove crab larvae has been stated by many researchers, one of the causes is bacterial vibrio infection (Putri and Prayitno, 2015). Various types of Vibrio bacteria can cause 100% mortal in mangrove crabs, especially in the larval stage to adult size (Taplur *et al*. 2011).

This research aimed to create a natural antibacterial formula in the form of *R.Stylosa* and *A.marina* mangrove fruit extracts for disease prevention in mangrove crab larvae which is expected to contribute to increased production, environmentally friendly and sustainable. While the specific purpose of this study is to examine the antibacterial activity of *R.stylosa* and *A.marina* mangrove fruit extracts in vitro against vibrio bacteria that cause vibriosis in mangrove crab larvae (*S.serrata F*). The specific target to be achieved in this research is the determination of % soaked and antibacterial activity in In-*vitro* *R.stylosa* and *A.marina* mangrove fruit extracts by maceration or immersion in multilevel solvents based on their level of polarity (n-hexane, chloroform, metanol, and water) to overcome the problem of *vibriosis* in the cultivation of mangrove crabs, especially in the larval stage.

II. METHODS AND MATERIAL

Sample Preparation

R.stylosa and *A.marina* mangroves were collected from the area around the coast of Kuri Caddi, Maros, South Sulawesi. About 8 kg of *R.stylosa* and *A.marina* mangroves then in scaled wet weight, collected in a fresh state then dried at a temperature of less than 40°C by using sunlight. After drying the mangroves were scaled again and obtained a dry weight of 4 kg each *R.stylosa* and *A.marina* blended until smooth, to get the weight of powder each 300g *R.stylosa* and *A.marina*

Mangrove Fruit Extraction

Extraction was carried out using multilevel maceration method on two samples of simplicia powder (derived from mangrove fruit derivatives of *R.stylosa* and *A. marina* species). The extraction process was carried out by kinetic maceration method (assisted with stirrer) for 24 hours at room temperature for each solvent. The solvents used were successively starting from non-polar (n-hexane), semi-polar (chloroform), and polar (methanol and water) solvents. A total of 300g of simplicia was divided 50g each into 6 erlenmeyer flasks, then each of the erlenmeyers contained with simplicia was soaked with 300ml of solvent (1: 6) and extracted on a magnetic stirrer. This extraction was carried out for 24 hours and repeated for 3 times. After being extracted with n-hexane solvent, the simplicia pulp was dried before being macerated with chloroform solvent, until the water solvent.After the extraction process was done , the organic solvent was evaporated by vacuum using Rotavor. For water solvents, a freeze dryer was carried out until the extract was obtained. The results of the thick extract were weighed and stored at cold temperatures until they would be used for testing and to calculate the extract a soaked stuff. This extraction method refered to the extraction method Zainuddin (2006).

To calculate the extract yield used the formula:

$$\% \text{ Yield} = \frac{\text{Extract weight}}{\text{Weight of powder biomass}} \times 100\%$$

The extraction procedure of *R.stylosa* and *A.marina* mangroves by using multilevel solvents (n-hexane, chloroform, metnaol, and water) based on polarity can be seen in the following figure:

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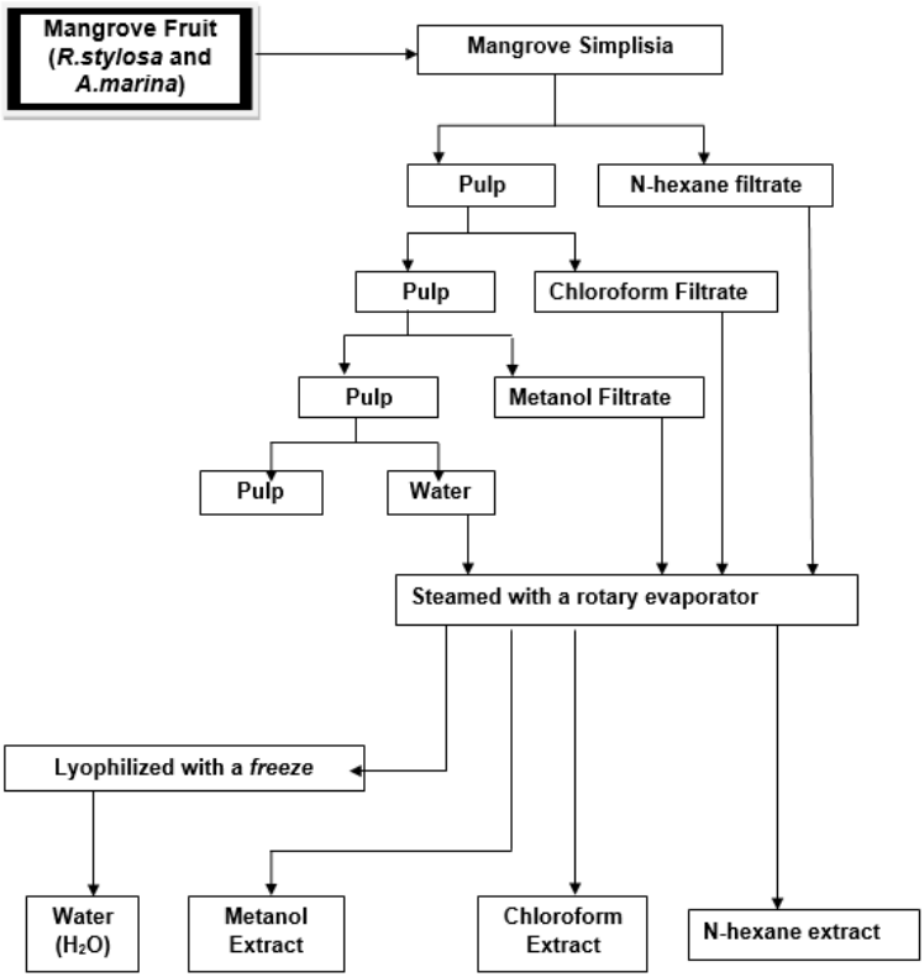

Fig.1: Mangrove Fruit Extraction Procedure

Isolation of *vibrio* spp

The results of vibriosis bacterial isolates were obtained from the results of the bacterial culture of Takalar Brackish Aquaculture Research Center, then pure culture and isolated in the laboratory of Parasites and Fish Diseases Hasanuddin University (Isolation of bacteria from the media of crab larvae maintenance was carried out by taking 1 mL of media water then diluted with 9 mL of solution physiological saline (0.85% NaCl) and then 0.1 mL are inoculated on TCBSA (Thiosul phate Cetoate Bile Sucrose Agar) plates, incubated for 48 hours.The bacteria that grow on petri dishes are isolated and purified based on the shape and color of the colonies on TSA media (Tryoptic Soy Agar) slant, incubated for 4 hours, propagated to NB (Nutrient Broth) and used for inhibition zone test).

Antibacterial Activity Testing

Antibacterial activity testing was carried out at the Fish Parasite and Disease Laboratory of the Faculty of Marine and Fisheries, Hasanuddin University, using disk diffusion method (Zainuddin, 2006). The four extract solvents (n-hexane, chloroform, methanol, and water) were weighed at a concentration of 2 mg / disk / 50 µl, then put into an ependorf tube and dissolved with each solvent. Then homogeneous using vortex and ready to be tested. Vibriosis pathogenic bacterial isolates were cultured again in an oblique TSA medium, then incubated for 24 hours. As a positive control ciprofloxacin was used. The negative control used is the solvent used for extraction (n-hexane, chloroform, methanol and water).

The making of a microbial suspension test was carried out by taking 1 ose needle of pure culture bacteria,

then put it in a test tube containing 2 ml of 0.9% physiological NaCL solution, then divortexed and put as much as 200 μl into six bottles containing 20 mL of warm TSA media and flattened with a circular motion so that the bacteria evenly distributed. After that, the agar medium in a still liquid bottle is poured into a Petri dish and allowed to condense.

Bacterial inhibitory activity is indicated by the presence of inhibition zones (clear zones / halo zones) around the paper disc. The diameter of the bacterial growth inhibition zone is measured in mm and made a quantitative measure for the size of the inhibitory zone (Figure 2) and (Table 1).

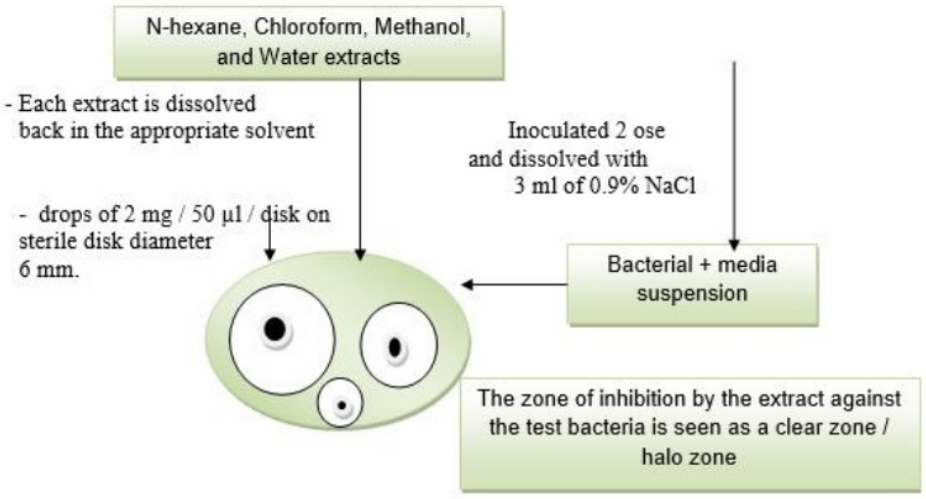

Fig.2: Inhibition Zone Test Procedure

Table 1. Inhibition power levels based on measurements of inhibition zone diameters

No	Inhibitory Zone Diameter	Information
1	>20 mm	Very High
2	15 – 20 mm	High
3	>11 - <15 mm	Medium
4	≤10 mm	Low

III. RESULTS AND DISCUSSION

Percentage of Yield

The yield is the ratio of the amount of extract obtained from an ingredient to the initial weight of the ingredients, the results of the calculation of % extract of *R.stylosa* and *A.marina* are presented in Table 2.

Tabel 2.The percentage yield of R.stylosa and A.marinaextracts with multilevel solvents.

No	Sample	Powder weight (bs) (g)	Ekstract	Extract weight (be) (g)	%yield
01.	*Avicennia marina*	300	n-hexane Chloroform Methanol Water	1,916 3,125 25,824 1,545	0,64 1,04 8,61 0,52
02.	*Rhizophora stylosa*	300	n-heksan Clorofrom Methanol Water	4,200 1,912 38,774 2,124	1,40 0,64 12,92 0,71

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The calculation result of R.stylosa% at a concentration of 2 mg / disk / 50 µl, the highest was obtained in methanol is 12.92% and A.marina as much is 8.61% in methanol as solvent. The high percentage of rendeman obtained in polar methanol solvents is because methanol as a polar solvent has an alcohol functional group in the form of a hydroxyl group that has a high boiling point and low molecular weight which has high solubility in water, thus causing the solubility of the extract to be high. The higher yield yield shows that more bioactive compounds are contained in an ingredient (Rohmansyah 2011).

Anti-bacterial Activity of *R.stylosa* Extract

The antibacterial activity of R.stylosa fruit extracts based on the average-diameter inhibition zone of various *R.stylosa* extract solvents against the bacteria *V. alginolyticus*, *V.harveyii* and *V. parahaemolyticus* are presented in Table 3

Table 3. Average diameter of inhibition zones of various solvents of R.stylosa extract against bacteria V.alginolyticus, V.harveyii and V.parahaemolyticus

Solvent Extract	Inhibit zone against - (mm)		
	V. alginolyticus	*V. harveyii*	*V. parahaemolyticus*
n-heksana	6.00± 0.20[a]	6.00±0.10[a]	6.00±0.05[a]
Cloroform	6.00± 0.10[a]	7.06±0.28[a]	6.93±0.39[a]
Metanol	7.18± 0.35[a]	11.40±6.71[b]	7.03±0.57[a]
Water	6.00± 0.25[a]	6.00± 0.10[a]	6.00±0.15[a]

Note: The average value followed by different letters means that it is significantly different in the Tukey test of 0.01 level (P <0.05).

Tukey test results (0.01) in Table 3 show that the methanol extract solvent showed the highest inhibitory zone diameter of 11.40 mm, but it was not significantly different from other solvents. The results of the average inhibition zone diameter (Table 4) of the 4 extracts tested showed that the highest inhibition zone diameter was obtained in methanol extract (11.40mm) against V.harveyi bacteria which were categorized as moderate inhibition levels (> 11- < 15mm), followed by methanol extract (7.18mm) against V. alginolyticus bacteria, and methanol extract (7.03mm) against V.pharaemolyticus bacteria which are categorized in the level of weak inhibition (≤10 mm) according to what was stated Zainuddin, (2006). This can be explained that the polar methanol solvent has the highest inhibitory zone capability, because it has an alcohol functional group in the form of a hydroxyl group that has a high boiling point and high water solubility (due to the hydrogen bonding between alcohol and water) so it has the ability explore the active ingredient extract is greater than other solvents.

Antibacterial Activity of *A.marina* Extract

The antibacterial activity of A. marina fruit extracts based on the average-diameter inhibition zones of various A. marina extract solvents against the bacteria *V. alginolyticus*, *V.harveyii* and *V. parahaemolyticus* is presented in Table 4.

Table 4. Average diameter of inhibition zones of various solvents of A.marina extract against bacteria V.alginolyticus, V.harveyii and V.parahaemolyticus.

Solvent Extract	Inhibit zone against- (mm)		
	V. alginolyticus	*V. harveyii*	*V.parahaemolyticus*
n-heksana	6.00±0.20[b]	6.00±0.15[b]	6.88±0.5[b]
Cloroform	11.8±1.87[a]	21.09±3.40[a]	19.08±1.24[a]
Methanol	6.00±0.20[b]	6.81± 0.26[b]	6.00± 0.36[b]
Water	6.00±0.10[b]	6.00 + 0.10[b]	6.00± 0.15[b]

Note: The average value followed by different letters means that it is significantly different in the Tukey test of 0.01 level (P <0.05)

The Tukey test results in Table 4 show that the chloroform extract solvent showed the best diameter of the inhibitory zone, significantly different from the other solvents. The results of the average inhibition zone diameter (Table 4) of the 4 extracts tested showed that the highest inhibition zone diameter was obtained in chloroform extract (21.09 mm) against *V.harveyi* bacteria, which were categorized in very high inhibitory levels (>

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20 mm), then followed by chlorofom extract (19.08 mm) against V. *pharaemolyticus* bacteria, which are categorized in high levels of inhibition (15-20 mm) and chlorofom extract (11.8 mm) against *V. alginolyticus* bacteria categorized in the level of moderate inhibition (> 11- <15mm) as stated by Zainuddin, (2006). Although the average inhibition zone of the above solvent is lower than the positive control solvent (Ciprofloxacin) with a inhibitory zone value of 16.39 mm, because Ciprofloxacin is a pure compound while the extract is still an impure compound because there are many other compounds contained therein, but its use is not recommended because it can make bacteria become resistant (invulnerable) as stated by Roza and Johnny, 1999).

A marina extract is one of the antibiotics that can be used in overcoming vibriosis in fish, shrimp, and crabs that have economic value in aquaculture efforts. This can be seen in Table 4, where the inhibition zone diameter in *A. marina* is extracted with chloroform showing a wider inhibition zone diameter than extraction with n-hexane, methanol and water solvents. Alam (2000) states that mangrove extract can suppress the growth rate of Vibrio harveyi.

Furthermore, the antibacterial activity of various extracts of A. marina and R.stylosa against V. algynoliticus (A), V.harveyii (B) and V. badaemolyticus (C) bacteria can be seen in Figure 3.

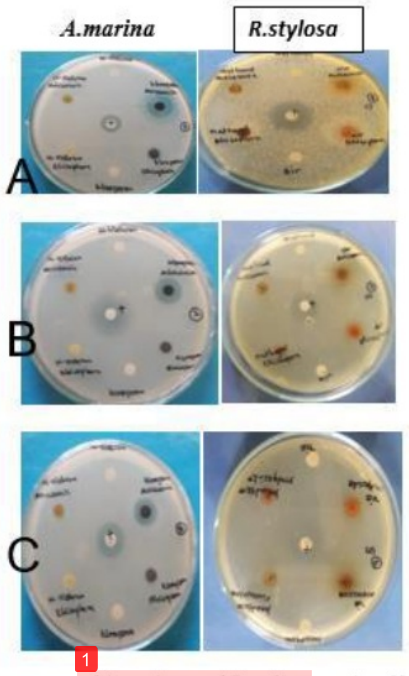

Fig.3. Antibacterial activity of various extracts of A.marina and R.stylosa against V. algynoliticus (A), V.harveyii (B) and V. parahaemolyticus (C) bacteria

IV. CONCLUSION

Polar methanol and chloroform extracts on *Rhizopora stylosa* and *Avicennia marina*are the finest solvent category in inhibition zone tests compared to other treatments (Indicators of high inhibition zone diameter and high% of extraction) so that it can be recommended for *In-vivo* application testing.

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