Journal of Aquaculture and Fish Health Vol. 9(2) - June 2020
DOI : 10.20473/jafh.v9i2.16211

In Vivo Test of *Rhizophora mucronata* Mangrove Extract From Pangandaran Coast Towards Nile Tilapia *Oreochromis niloticus* infected by *Vibrio harveyi*

Yeni Mulyani[1], Kiki Haetami[2], Lesta Krismawati Baeha[3], Sulastri Arsad[4] and Fiddy Semba Prasetiya[1,5*]

[1]Marine Science Department, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran, Jl. Raya Bandung Sumedang, Jatinangor, Sumedang 45363, Indonesia
[2]Fisheries Department, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran, Jl. Raya Bandung Sumedang, Jatinangor, Sumedang 45363, Indonesia
[3]Marine Science Study Programme, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran, Jl. Raya Bandung Sumedang, Jatinangor, Sumedang 45363, Indonesia
[4]Study Programme of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, Jl. Veteran No. 16, Ketawanggede, Malang 65145, Indonesia
[5]Research Center of Biotechnology and Bioinformatics, Universitas Padjadjaran, Jl. Raya Bandung Sumedang, Jatinangor, Sumedang 45363, Indonesia

*Correspondence :
fiddy.semba.prasetiya@unpad.ac.id

Received : 2019-11-21
Accepted : 2020-01-27

Keywords :
Rhizophora mucronata, Mangrove extract, Tilapia fish, Vibrio harveyi, Antibacterial activities

Abstract

This study aims to determine the effectiveness of *Rhizophora mucronata*'s bark extract in curing *Vibrio harveyi* infection in Nile tilapia fish by challenge testing (*in vivo* test). It was conducted in October 2018-May 2019 at the Central Laboratory and Building-4 of Aquaculture and Hatchery Laboratory in the Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran. This research used microdilution method to determine the Minimum Inhibitory Concentration (MIC) value of *R.mucronata*'s bark extracts, and experimental laboratory method with a Completely Randomized Design (CRD) model for challenge testing (*in vivo*) which consisted of 5 treatments and 3 replicates where the given treatment varied in concentrations. These various concentrations started from A (0 ppm/control), B (16 ppm), C (32 ppm), D (48 ppm), and E (64 ppm). The observed parameters were the inhibitory antibacterial activity for MIC and survival rate, as well as clinical symptoms and water quality for the challenge test. Survival Rate data of Nile tilapia seedlings was analyzed by regression analysis. The observation results of MIC, MBC values, clinical symptoms, and water quality were analyzed descriptively. The results showed the Minimum Inhibitory Concentration value of *R. mucronata* bark extract at a concentration of 6,250 ppm while its Minimum Bacteriocidal Concentration value at 50,000 ppm. The fish tolerance test results towards the extract (LC50 test) are 64 ppm. Differences in treatment produced results that have significant effects on survival rate. The use of *R. mucronate* bark extract at a dose of 64 ppm resulted in the highest survival rate of Nile tilapia fish at 76.66%.

INTRODUCTION

Mangroves are known to be sources of secondary metabolites (Mulyani *et al*., 2013). Arumugam *et al*. (2014) stated that *Rhizophora mucronata* is a medicinal mangrove plant commonly known as red mangrove. This mangrove species has important active metabolite compounds such as alkaloids, terpenoids, steroids, tannins, quinones, saponins, flavonoids, glycosides, and phenols. This supports the fact that those mangroves have high potentials as plants that contain plenty of benefits. *R. mucronate* is also one of the mangroves that have the potential as a primary ingredient in making natural antibacterials and has also been widely used by coastal communities for herbal medicine. One of the uses of mangrove plants as a natural medication is to prevent diseases against bacterial attacks in aquaculture production, especially nile tilapia aquaculture.

Nile tilapia (*Oreochromis niloticus*) is one of Indonesia's leading commodities that has vast potential to be developed in supporting national food security and economic security as well as improving people's welfare. Nile tilapia fish is a type of fish with high economic value where the need for seeds and fish consumption from year to year tends to increase continuously along with aquaculture expansion (Darwisito *et al*., 2008).

The presence of pathogenic bacteria in fish culture processes can cause enormous losses. The main pathogenic bacteria that often attack shrimps and fish, especially Nile tilapia, is *Vibrio* sp. (Rinawati, 2011). So far, countermeasures to bacterial attacks are generally done by administering antibiotics and chemicals.

Continuous distribution of antibiotics can cause pathogenic organisms to become resistant and more dangerous. Also, residues from antibiotics can pollute the aquatic environment, which causes water quality to decrease (Rinawati, 2011). One alternative that can be used is the bioactive compounds from mangroves. Feliatra (2000) demonstrated that several species of mangroves have antibacterial activities against *Vibrio* sp.

In vivo test was carried out to determine the extracts' abilities to subdue bacterial diseases on the tested animal directly. The extract's concentrations in *in vivo* test generally varied in a smaller range compared to the ones in *in vitro* test (Agung and Pringgenies, 2007). In the *in vivo* test, the animal was infected with pathogenic bacteria through various means such as injection and submersion. Antibiotics are usually given through food, immersion, or injection so that antibiotic residues can accumulate inside the fish (Cabello, 2006). According to Andrews (2001) in a study of antibacterial tests i.e., *Vibrio* sp. towards fish, he revealed that concentrations of 108 cells/ml within 24 hours could infect fish fry with a 100% mortality rate.

In preliminary research conducted by Pertiwi (2018), the largest clear zone diameter was obtained from an *R. mucronata* bark extract of a concentration of 50,000 ppm (4.36 mm). In the *in vivo* test, the LC_{50} test with treatments of 10 ppm, 25 ppm, 50 ppm, 75 ppm, and 100 ppm, each aquarium was filled with 10 tilapia seeds. In a concentration of 50-100 ppm, there was a 50-95% mortality of tilapia seeds. The analysis results, using the EPA Probit 1.5 program, produced a value of 64 ppm. This shows that the extract's safe limit concentration is 64 ppm and does not cause any toxicity in tilapia seeds, hence it is safe to be used as a concentration for treatments in the primary research. The present study aims to determine the effectiveness of *R. mucronata* bark extract in inhibiting *Vibrio harveyi* infection in Nile tilapia by *in vivo* test.

METHODOLOGY

Place and Time

This research was conducted in October 2018 - May 2019. Samples of *R. mucronata* bark were taken from Pangandaran Coast, Bojong Selaweh

District. The filtration evaporation was conducted at the Central Laboratory of Universitas Padjadjaran. The survival test was carried out in the Aquaculture and Hatchery Laboratory Building-4 Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran.

Research Material

Nile tilapia seeds were obtained from the Cibiru Fish Seed Center (*Balai Benih Ikan/*BBI) with lengths of 3-5 cm stocked in an aquarium measuring 60 x 40 x 40 cm. The aquarium was filled with water with a volume of 60 liters and was obtained from stocking up 10 tilapia fish seeds / 10 liters. The fish were adapted for 5 days then fasted for 24 hours. After starving, a lethal concentration test was carried out following the treatment of Nile tilapia seeds. In the water, during the tilapia seeds cultivation, they were given tuba root extracts under the treatment dose.

Observations of tested fish were carried out for 96 hours to see their response as they were exposed to tubal root extracts. The observed parameters in this study were the fish's survival rate and their blood.

Research Design

This research was conducted using experimental laboratory methods with a Completely Randomized Design (CRD) model for *in vivo* testing. The number of treatments in this study was (5) five, and each was repeated three times with the following conditions:

A = No submersion in mangrove extract (control); B = submerged with 25% mangrove extract from LC_{50} value; C = submerged with 50% mangrove extract from LC_{50} value; D = submerged with 75% mangrove extract from LC_{50} value; and E = immersed with 100% mangrove extract from LC_{50} value.

Work Procedures

The bark samples of *R. mucronata* were collected from Pangandaran Beach, Bojong Salaweh Village, Ciamis Regency. Sampling was performed by purposive random sampling where samples were collected from locations where the desired sample commodity exist. *R. mucronata* barks were collected as much as 1 kg in wet weight. Harvesting was only done on plants that have become trees. They were washed with clean water and placed into plastic bags, then kept in clean cardboard to be brought to the laboratory.

The preparation of *R. mucronata* dry powder was carried out before the identification and extraction of class compounds. Dry powder preparation has two stages, i.e., drying and refining. The barks were cut into small pieces and dried for 7 days to reduce evaporation, which includes the compounds contained inside them. This drying process aims to reduce the water content to make sure that the samples are not quickly grown with mold and bacteria and to eliminate enzyme activities that can further decompose the materials of active substances found in the plant's bark (Gunawan and Marina, 2004). The dried barks were then mashed with a blender to form a powder. The refinement intention of *R. mucronata*'s bark is to speed up the extraction process.

The extraction of *R. mucronata* was carried out by the maceration method using methanol solvent. *R. mucronata* powder was soaked with methanol (MeOH) solvent in a glass bottle until all parts of the sample were submerged. The glass bottle containing the marinade was then closed for 1 x 24 hours while occasionally stirring to speed up contact between the sample and the solvent. After 1x24 hours, the marinade was filtered using Whatman No. 1 filter paper, its filtrate collected in a beaker glass, and its volume measured. The separated filtrate was then evaporated with a Rotary Evaporator (Rotavapour) at a temperature of 40°C to form a concentrated extract in the form of paste on the flask wall. The obtained concentrated extract (paste) was then weighed and placed in a vial.

Tryptic Soy Broth (TSB) media is used for bacterial growth media in liquid

microdilution test. This media was made by dissolving 3 g of TSB into 100 ml of distilled water. The solution was then stirred until homogenized on a hot plate and sterilized by autoclave for 2 hours at 121°C (Merck). Test bacteria that had been rejuvenated for 24 hours were inoculated into a test tube containing 5 ml of TSB. The solution was then homogenized with vortex, and absorbance was measured with a UV-Vis spectrophotometer at a 600 nm wavelength. Absorbance values in the range 0.08-0.12 are equivalent to 0.5 McFarland (1-2 x 108 cfu/ml) as standard.

The turbidity solution is equivalent to the standard, which is used as a test bacteria. The microdilution method is an antibacterial activity testing method that can determine the value of MIC. This test was carried out using 96-well microplate, which has 96 micro-plate wells (8 rows and 12 columns). The test was performed twice, and in each test, extract control test, solvent control, and growth control are included. Test extract control only contained test extract, solvent control contained 10% DMSO, and growth control contained test extract, DMSO, and bacteria test suspension.

Tryptic Soy Agar (TSA) media was used for bacterial growth media to determine the Minimum Bacteriocidal Concentration (MBC) value. Briefly, 3 grams of TSA was dissolved into 100 ml of distilled water (Merck). The solution was then stirred until it's homogeneous on a hot plate and sterilized by autoclave for 2 hours at 121°C. The sterile solution was poured into a petri dish in laminar airflow and incubated for 24 hours at 37oC. Determining the MBC value or minimum concentration of antibiotics that can kill bacteria was performed by plating the bacteria in the liquid incubator, which is used for MIC into the agar. MBC determination can be observed by observing the presence or absence of bacterial colony growth at specific concentrations on TSA media. Briefly, the MIC solution results were transferred from the microplate into a petri dish using a micropipette in laminar airflow. The solution was then flattened using L-glass so that it spreads evenly on the TSA media's surface before incubation at 37°C for 24 hours.

The Median Lethal Concentration (LC_{50}) test describes a concentration that causes death as many as 50% of test organisms that can be estimated by graphs and calculations at a particular observation time, ca. LC_{50} 24 hours, LC_{50} 48 hours, LC_{50} 96 hours until the lifetime of tested animals (Koesoemadinata, 1983). Percentage of mortality was calculated by: the initial number of fish minus the final number of fish at the end of cultivation, then compared to the number of fish at the beginning of culture (Effendie, 1997):

$$M = \left[\frac{(No - Nt)}{No}\right] x100\%$$

Where:
M = Percentage of mortality (%)
Nt = Fish quantity at the end of cultivation
No = Fish quantity at the beginning of cultivation

The LC_{50} test method refers to a procedure carried out by Agung (2003). Nile tilapia with lengths of 3-5 cm from the Cibiru Fish Seed Center were acclimatized for 5 days before the experiment. A total of 10 fish were added into each aquarium containing 10 l of freshwater with different concentrations of *R. mucronata* extract (0, 10, 25, 50, 75, and 100 mg/l). Afterward, mortality observation was carried out for 24 hours with intervals; 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 16 hours, and 24 hours. The LC_{50} value was determined using the EPA Probit program. The acclimated tilapia (total length lengths of 8-10 cm) was infected by injecting 0.1 ml of *V. harveyi* with a density of 10^8 CFU/ml. This injection was conducted intramuscularly (Tendencia *et al*., 2004). After 48 hours of infection incubation period and the clinical symptoms of vibriosis, attack occurs, infected fish were submerged with the best extract solution of LC_{50} test results, with a concentration of 0% (control), 25%, 50%, 75% and 100% from

the fish tolerance LC_{50} values towards extract concentration.

Observation of clinical symptoms was carried out by looking at abnormal changes that occur. Clinical symptoms of tilapia were observed every day with intervals of 6 hours by observing the presence or absence of symptoms that arise after being treated with the extract of *R. mucronata*. After being tested with *V. harveyi*, observation of changes in fish behavior was performed, including physical damage, feed response, and reflex tests. The survival rate (SR) is a comparison of the number of living fishes with the total ones that were stocked at the beginning of cultivation. The SR was calculated according to the formula from Effendi (2004) as follows:

$$SR = (\frac{Nt}{No})x100\%$$

Where SR is the Survival rate (%), Nt is the number of fish seeds that lived at the end of the observation, and No is the total number of initial fish seeds. Survival rate observation of Nile tilapia infected by *V. harveyi* bacteria was carried out every day until the end of the inspection, which lasted for 14 days.

Water quality parameters, which include temperature, pH, and DO (dissolved oxygen), are measured during the experiment. Temperature and pH were measured daily using a thermometer in the morning and evening, whereas dissolved oxygen (DO) was measured daily by using a DO meter.

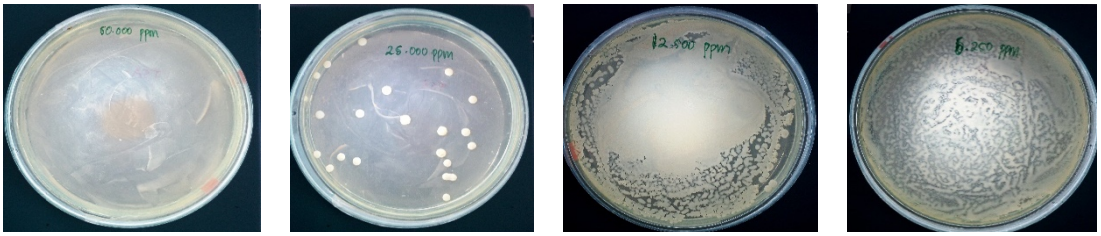
Data Analysis

The obtained SR data of Nile Tilapia were analyzed using the one-way ANOVA test at a 95% confidence level. Duncan post-test was performed when significant differences were observed between treatments at a 95% trust level (Gasperz, 1991). Observation data of the tested fish's clinical healing symptoms were analyzed descriptively.

RESULTS AND DISCUSSION

The antibacterial activity test, which was based on inhibition zones produced by methanol extracts of roots, leaves, and barks of *R. mucronata* at a concentration of 50,000 ppm against *V. harveyi* bacteria were 2.1, 1.7, and 4.6 mm respectively. The results showed that the methanol extract from *R. mucronata* bark had the best inhibition against *V. harveyi* bacteria (Pertiwi, 2018). Therefore, bark extract testing was continued with a liquid microdilution method to determine the MIC value of *V. harveyi* bacteria. This result indicates that the analyzed mangroves have deficient antibacterial activities of *V. harveyi* in which their MIC values ranged from 6,250 to 50,000 ppm (6,250 to 50,000 mg/l). The lower the MIC value of an active ingredient, the higher the effectiveness is, and vice versa. According to Abed *et al.* (2013), an antibacterial compound is said to have a strong inhibition if its MIC value is 500 mg/l, medium inhibition if its MIC value is 600-1,500 mg/l, and a low inhibition if its MIC value is greater than 1,600 mg/l.

The observation results of the MIC value of *R. mucronata* bark extract are related in determining the MBC value. Extracts' concentrations that had low turbidity levels were inoculated on TSAmedia to observe bacterial colonies. The inoculation results on TSA extract concentration of 50,000 ppm did not have any growing bacterial colony, whereas, at a concentration of 25,000 ppm, bacterial colonies appear rare but still present (Figure 1). At a concentration of 12,500 ppm, bacterial colonies almost filled the TSA media. However, there were still some areas that were not grown with bacteria, while at a concentration of 6,250 ppm, bacterial growth covered the whole TSA media without any clear areas (Figure 1). This result suggests that the MBC value is at a concentration of 50,000 ppm because there is not a single bacterial colony that grows on the TSA media.



50,000 ppm 25,000 ppm 12,500 ppm 6,250 ppm

Figure 1. Minimum bacteriocidal concentration test results.

Arumugam *et al*. (2014) reported that the higher the concentration used, the greater the bacterial activity inhibition. Damage to cell membranes causes impediments of activities and biosynthesis of specific enzymes needed in metabolic reactions, and this condition can lead to bacterial death (Naiborhu, 2002).

The metabolite compounds mechanism contained in the extract of *Rhizophora* sp. such as alkaloid is estimated to work by disrupting the constituent components of peptidoglycan so that the bacterial cell wall is not intact, which causes the bacteria's death (Robinson, 1995). The flavonoids effects contained in the extract of *Rhizophora* sp. as an antibacterial is by inhibiting the synthesis of nucleic acids, cell membrane functions, and energy metabolism. These flavonoid compounds react with DNA, RNA, and proteins that cause functional disruption of these substances and lead to total damage in cells (Soemiati and Elfita, 2015).

In the present study, the preliminary toxicity test found that the concentrations used are; 0, 10, 25, 50, 75, and 100 ppm. A total of 50% mortality of tilapia seeds that were used, occurred between submersion treatment at concentrations of 50 and 100 ppm. The LC_{50} analysis results using EPA Probit 1.5 give a value of 64 ppm as a reference in taking the extract estimated concentration in testing the survival rate of tilapia seeds.

The clinical symptoms observed in tilapia seeds as test subjects that were infected with *V. harveyi* bacteria showed morphological and behavior changes. Injections infected tilapia seedlings at a dose of 0.1 ml with 10^8 cfu/ml density. Based on observations, clinical symptoms that appeared were red spots on the fins' base, flatulence, protruding eyes, erosion of tail shaft's foldings, bleeding (hemorrhage) in gills, mouth, and body, where the severe inflammation and bleeding occurred on day 2 of the experiment. Other changes in behavior were weakened movement, reduced balance, and decreased appetite. Our findings are in coherence with the previous study from Kamiso (1996), who found that the characteristics of fish that are affected by vibriosis are blackish backs, red spots on the fins' base, upright scales, slow movement, disturbed balance and lack of appetite. Symptoms that often occur are exophthalmos (protruding eyes), flatulence filled with light yellow fluid, hemorrhage in the gills, mouth, body, intestines, and internal organs.

Additionally, De Vita *et al.* (2017) also found that there are head injuries, excessive mucus, swimming alone, ulcers on the body surface, reddish mouth, flatulence, flaky fins accompanied by reddish sores on the pectoral fins, dorsal fins, caudal fins, as well as pale liver and kidneys. Similar clinical symptoms have been reported by Kamiso *et al.* (1994); Rad and Shahsavani (2010) and Sarjito *et al.* (2014) in catfish infected by *Vibrio*. The observations showed that there were similarities in clinical symptoms such as ulcers on the injection site, reddish wounds on the body, reddish splints on the fins, flushed tips of the antenula and ulcers on its body.

The submersion of infected fish with mangrove bark extract produced inhibition of bacterial growth on the fish body. This was indicated by the severe

symptoms in treatment A where no mangrove extract added, such as bleeding in each fins' base, dark skin color, unresponsive or passive movement, reduced response to feeding, and even death. Whereas in treatment B, C, D, and E, clinical symptoms that occurred were less severe than treatment A (Figure 2).

The bacterial infection is initiated by pathogenic interaction that holds attachments or adhesions to the host's surface, followed by bacteria's entry into the host cell, and colonization process in the host's target tissue in a specific part (Rambukkana *et al.*, 1998).

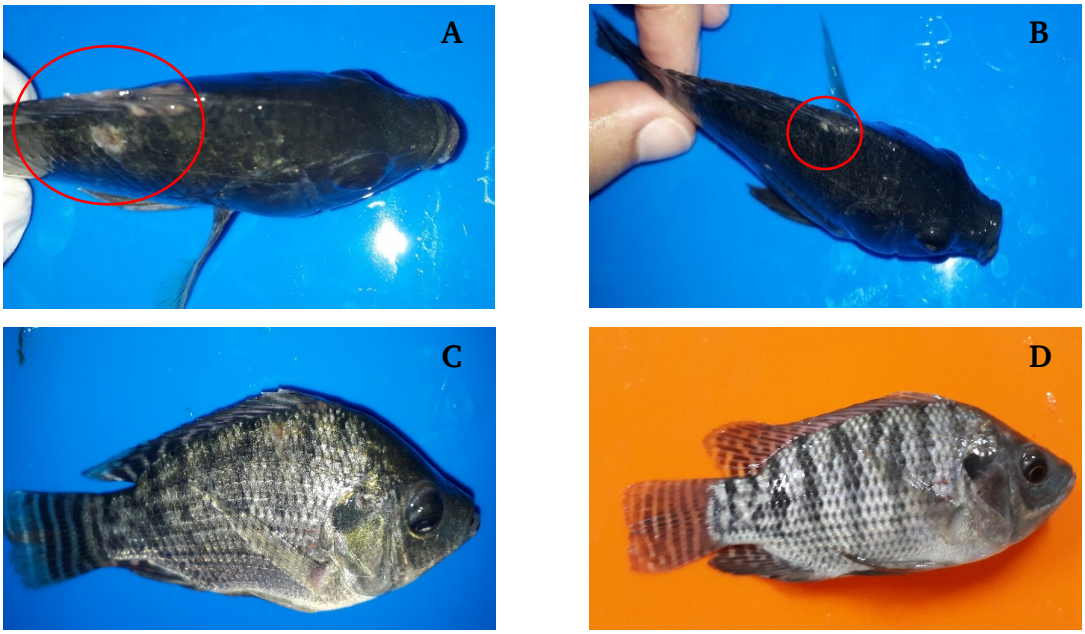


Figure 2. Test fish condition; (A and C) before submerged in mangrove bark extract, ulcer in the injection site and fin's base; (B and D) after immersed in mangrove bark extract, ulcers from injection shrink, and its body color returned to normal.

Table 1. Tilapia seed's response to feed during cultivation period.

Day	Treatment														
	A Control			B 16 ppm			C 32 ppm			D 48 ppm			E 64 ppm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2	-	-	-	-	-	-	-	-	-	-	-	-	+	-	++
3	-	-	-	-	-	-	-	-	-	+	+	+	++	++	++
4	-	-	-	-	-	-	+	+	+	+	+	+	++	++	++
5	-	-	-	+	+	-	+	+	+	+	+	+	++	++	++
6	+	+	+	+	+	+	+	+	+	+	+	++	++	++	++
7-14	+	+	+	+	+	+	++	++	++	++	++	++	++	++	++

Note: (++) feed response is normal; (+) low feed response; (-) feed response is missing.

Observations of behavioral changes were made towards the response to feed and shock. Control fish were less responsive to feed compared to that of submerged with mangrove extract (Table 1). This was indicated by the amount uneaten feed at the bottom of the aquarium when it was siphoned. This result was probably due to bacteria's distribution and activities throughout the tilapia seeds' bodies that were infected with *V. harveyi* bacteria. According to Austin and Austin (2012), bacteria's penetration into body organs, especially

organs of the digestive system, through body fluids and blood flow can cause digestive disorders in fish infected with bacteria.

Also, to feed response, other observed changes in behavior were the ability of tilapia seeds' response to shocks (reflex tests) by hitting the aquarium wall (Table 1). Generally, tilapia seeds' reflex reactions occurred in all treatments except control. This fish reaction was by moving away from the source of pats.

Table 2. Reflex testing of tilapia seed during cultivation period.

Day	Treatment														
	A			B			C			D			E		
	Control			16 ppm			32 ppm			48 ppm			64 ppm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
4	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
5	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7-14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Note: (+) presence of reflexes; (-) absence of reflexes.

Table 2 shows the response of tilapia seeds to different shocks in each treatment. In Treatment B, C, D, and E, starting from the 3rd day to the last observation day on the 14th, tilapia seeds' reflex reactions occurred by moving away from the source of pats. Unlike the case with treatment A, a new response appeared on the 6th cultivation day. This proves that the active compounds contained in *R. mucronata* bark extract with different concentrations provide faster healing for tilapia seeds compared to the ones which were not submerged in *R. mucronata* bark extract. According to Heath *et al.* (1993), the entry of mangrove bark extract into fish bodies can be through gills, food, water and skin.

The highest survival rate of tilapia seed was obtained at an extract concentration of 64 ppm while the lowest survival rate occurred in control fish. During those 14 cultivation days, it can be deduced that treatment A (Control) has the lowest survival rate, 50% (Figure 3). This significant difference in SR is because treatment A did not give mangrove bark extract, which contained active ingredients that behave as antibacterial agents. *V. harveyi* bacteria that have infected the fish will continue to grow, thereby affecting the fish's survival.

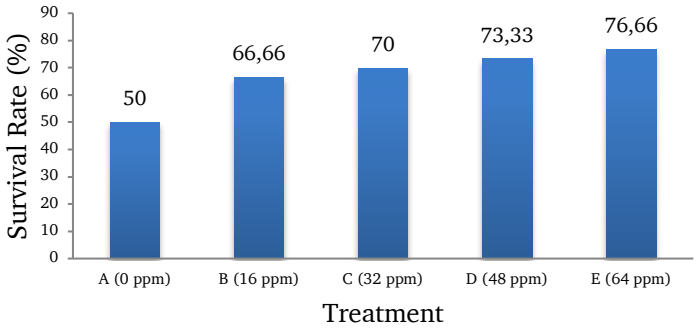


Figure 3. Average survival rate graph of nile tilapia seeds after submerged in *R. mucronata* bark extract.

According to Sukenda and Wakabayashi (1999), the body surface is a medium for bacteria entering into the host body, and this area can be the main gate to cause infection. When the host cuticle or other body surface is injured, pathogenic bacteria can infiltrate. *Vibrio* attacks by damaging the cuticle layer containing chitin because *Vibrio* has the enzymes chitinase, lipase, and protease.

According to Ajizah (2004), tannins have antibacterial activities by wrinkling cell walls or cell membranes, thereby disrupting cell permeability, which can result in disruption of metabolic activities and leads to an inhibition of bacterial growth and death. Monalisa *et al.* (2011) also stated that flavonoid compounds could coagulate proteins, flavonoid compounds are also lipophilic so they can damage the lipid layer on bacterial cell membranes.

Saponins are glycosides and sterols, which, when fully hydrolyzed, produce sugars and a non-sugar fraction called sapogenin or genin. Saponins are active surface compounds and are like soap; their abilities to form foam and blood hemolysis can be detected (Handayani, 2013).

Steroids inhibit bacterial growth by mechanism, inhibit protein synthesis, and cause changes in the constituent components of bacterial cells. Steroid compounds can be linked to proteins and lipids found in cell membranes and cause lysis of bacterial cells (Dewi, 2010). Steroids can increase cell membranes permeability, which leads to cell leakage, followed by the release of intracellular material (Tarman *et al.*, 2013).

The water quality parameters measured in this study include temperature are temperature, dissolved oxygen (DO), and pH. In general, water quality during the experiment was in an ethical and controlled condition. The temperature range from the beginning until the end of the study was 23-25°C, DO level was 5.39-5.60, and pH was between 8.10-8.28. This proves that during the investigation, media and water quality were in a controlled state and a safe tolerance range for Nile tilapia fish cultivation starting from temperature, DO, and pH. Thus it can be concluded that the water quality met the optimum standards, and fish mortality was not caused by low water quality, but due to *V. harveyi* bacterial infection.

CONCLUSION

The present study demonstrated that Mangrove extract from *R. mucronata* displays antibacterial activities against the pathogenic bacterium *V. harveyi*. The MIC of this mangrove extract is 6,250 ppm, while the MBC is 50,000 ppm. The submersion of infected tilapia seeds by the extract of *R. mucronata* can improve the healing process. The use of mangrove extract at 64 ppm (treatment E) produced the highest survival rate for Nile tilapia fish seeds as high as 76.66%. This study, perhaps the pioneering work demonstrating the antibacterial activity of mangrove *R. mucronata* bark extract, deserves further research to support sustainable aquaculture practice.

ACKNOWLEDGEMENT

We thank to Padjadjaran University, Brawijaya University, and all parties who have aided in the completion of this research.

REFERENCES

Abed, S.A., Sirat, H.M. and Taher, M., 2013. Total phenolic, antioxidant, antimicrobial activities and toxicity study of Gynotroches axillaris Blume (Rhizophoraceae). *EXCLI journal*, *12*, pp.404-412. https://doi.org/10.17877/DE290R-10755

Agung, M.U.K. and Pringgenies, D., 2007. Isolasi Fraksi Gabungan Aktif Agen Antibakteri dari Ekstrak Klorofom Bakteri Photobacterium phosphoreum yang Bersimbiosis pada Organ Cahaya Cumi-cumi Loligo duvauceli. Prosiding Seminar Nasional Moluska. BRKP-DKP-Jurusan Ilmu Kelautan FPIK.

Universitas Diponegoro: Semarang. pp.309-337.

Ajizah, A., 2004. Sensitivitas Salmonella typhymurium Terhadap Ekstrak Daun Jambu Biji (Psidium guajava L.). *Jurnal Bioscientiae*, 1(1), pp.31–38. https://ppjp.ulm.ac.id/journals/index.php/bioscientiae/article/download/130/113

Andrews, J.M., 2001. Standardized disc susceptibility testing methods. *J. Antimicrob. Chemother., P.:(48-57)*.

Arumugam, S., Palanisamy, D. and Sambandam, R.T., 2014. Identification of bioactive compounds of Rhizophora mucronata poir. leaves using supercritical fluid extraction and GC-MS. *World Journal of Pharmacy and Pharmaceutical Sciences*, *3*(10), pp.1621-1631. https://www.wjpps.com/Wjpps_controller/abstract_id/2192.

Austin, B. and Austin, D.A., 2012. *Bacterial fish pathogens* (Vol. 481, p. 482). Dordrecht, The Netherlands: Springer. 383p.

Cabello, F.C., 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental microbiology*, *8*(7), pp.1137-1144. https://doi.org/10.1111/j.1462-2920.2006.01054.x

Darwisito, S., Junior, M.Z., Sjafei, D.S., Manalu, W. and Sudrajat, A.O., 2008. Feeding Broodstock on a Diet Containing Vitamin E and Fish Oil Improve Eggs and Larval Quality of Nile tilapia (Oreochromis niloticus). *Jurnal Akuakultur Indonesia*, *7*(1), pp.1-10. https://doi.org/10.19027/jai.7.1-10

De Vita, D., Angeli, A., Pandolfi, F., Bortolami, M., Costi, R., Di Santo, R., Suffredini, E., Ceruso, M., Del Prete, S., Capasso, C. and Scipione, L., 2017. Inhibition of the α-carbonic anhydrase from Vibrio cholerae with amides and sulfonamides incorporating imidazole moieties. *Journal of enzyme inhibition and medicinal chemistry*, *32*(1), pp.798-804. https://doi.org/10.1080/14756366.2017.1327522.

Dewi, F.K., 2010. Aktivitas Antibakteri Ekstrak Etanol Buah Mengkudu (Morinda citrifolia L.) Terhadap Bakteri Pembusuk Daging Segar. *Jurusan Biologi. Fakultas Matematika dan Ilmu Pengetahuan Alam. Universitas Sebelas Maret. Surakarta*, 38p. http://eprints.uns.ac.id/388/1/169682309201001141.pdf

Effendie, M.I., 1997. Biologi perikanan. *Yayasan Pustaka Nusatama. Yogyakarta*, p.*163*.

Effendi, I., 2004. Pengantar akuakultur. *Penebar Swadaya. Jakarta*, p.*188*.

Feliatra, 2000. Studi Awal Tumbuhan Mangrove sebagai Antimikroba, Lembaga Penelitian Universitas Riau. 22p.

Gaspersz, V., 1991. Metode perancangan percobaan. *Armico. Bandung*, p.*384*.

Gunawan, D. and Mulyani, S., 2004. Ilmu Obat Alam (Farmakognosi) Jilid 1. *Penebar Swadaya, Jakarta*. http://kin.perpusnas.go.id/DisplayData.aspx?pId=4408&pRegionCode=MANADO&pClientId=626

Handayani, S., 2013. Kandungan flavonoid kulit batang dan daun pohon api-api (Avicennia marina (Forks.) Vierh.) sebagai senyawa aktif antioksidan. *Bogor. Fakultas Perikanan dan Ilmu Kelautan Institut Pertanian Bogor. Skripsi*, 51p. https://repository.ipb.ac.id/bitstream/handle/123456789/61383/C13sha.pdf?sequence=6&isAllowed=y

Heath, A.G., Turner, B.J. and Davis, W.P., 1993. Temperature preferences and tolerances of three fish species inhabiting hyperthermal ponds on mangrove islands. *Hydrobiologia*, *259*(1), pp.47-55.

Kamiso H.N., Triyanto and Hartati, S., 1994. Karakteristik Aeromonas hydophila pada Ikan Lele (Clarias sp.) Di Daerah Istimewa Yogyakarta Dan Jawa Tengah Selatan, Agric.

Sci., V(4), pp.741-750. http://i-lib.ugm.ac.id/jurnal/download.php?dataId=5701

Kamiso, H.N., 1996. Vibriosis Pada Ikan dan Alternatif Cara Penanggulangannya, *Jurnal Perikanan UGM (GMU J. Fish. Sci) I* (1), pp.78-86. https://doi.org/10.22146/jfs.8859

Koesoemadinata, S., 1983. Pedoman Umum Pengujian Laboratorium Toksisitas Lethal Pestisida pada Ikan untuk Keperluan Pendaftaran Komisi Pestisida. *Departemen Pertanian, Jakarta*.

Monalisa, D., Handayani, T. and Sukmawati, D., 2011. Uji Daya Antibakteri Ekstrak daun Tapak Liman (Elephantopus scaber L.) Terhadap Staphylococcus aureus dan Salmonella typhi. *Jurnal Bioma*, *9*(2), pp.13-20.

Mulyani, Y., Bachtiar, E. and Agung, M.U.K., 2013. Peranan senyawa metabolit sekunder tumbuhan mangrove terhadap infeksi bakteri Aeromonas hydrophila pada ikan Mas (Cyprinus carpio L.). *Jurnal Akuatika*, *4*(1), pp.1-9. http://jurnal.unpad.ac.id/akuatika/article/download/2613/2351

Naiborhu, P.E., 2002. Ekstraksi dan manfaat ekstrak mangrove (Sonneratia alba dan Sonneratia caseolaris) sebagai bahan alami antibakterial pada patogen udang windu, Vibrio harveyi. *Scientific Journal of Bogor Agricultural University. Institut Pertanian Bogor. Bogor*. 63p. https://repository.ipb.ac.id/handle/123456789/20041

Pertiwi, H. 2018. Potensi Antibakteri Ektrak Mangrove Rhizophora mucronata Dari Pesisir Pangandaran Terhadap Bakteri Staphylococcus aureus dan Vibrio harveyi, FPIK, Universitas Padjadjaran.

Rad, M. and Shahsavani, D., 2010. Isolation and characterization of Vibrio (Listonella) anguillarum from catfish. *Turkish Journal of Veterinary and Animal Sciences*, *34*(4), pp.413-415. https://doi.org/10.3906/vet-0812-5

Rambukkana, A., Yamada, H., Zanazzi, G., Mathus, T., Salzer, J.L., Yurchenco, P.D., Campbell, K.P. and Fischetti, V.A., 1998. Role of α-dystroglycan as a Schwann cell receptor for Mycobacterium leprae. *Science*, *282*(5396), pp.2076-2079. https://doi.org/10.1126/science.282.5396.2076

Rinawati, N.D., 2011. Daya Antibakteri Tumbuhan Majapahit (Crescentia cujete L.) Terhadap Bakteri Vibrio alginolycticus. *Surabaya: Jurusan Biologi Fakultas Matematika Ilmu Pengetahuan Alam Institus Teknologi Sepuluh November*, *1*(1), pp.1-5. http://digilib.its.ac.id/public/ITS-Undergraduate-13710-Paper-370813.pdf

Robinson, T., 1995. Kandungan Organik Tumbuhan Tinggi Edisi 6. *Bandung: ITB*. http://kin.perpusnas.go.id/DisplayData.aspx?pId=1055&pRegionCode=JIUNMAL&pClientId=111

Sarjito, S., Radjasa, O.K., Haditomo, A.H.C. and Prayitno, S.B., 2014. Insidensi Bakteri genus Vibrio pada Lele Dumbo (Clarias gariepinus) dari Sentral Produksi Provinsi Jawa Tengah. Prosiding Seminar Nasional Tahunan ke-III Hasil-Hasil Penelitian Perikanan dan Kelautan. FPIK Universitas Diponegoro, Semarang, pp. 416-424. http://eprints.undip.ac.id/49883/1/Insidensi_Bakteri_Genus_Vibrio_pada_lele_dumbo.pdf

Soemiati, A. and Elfita, L., 2015. Uji aktivitas Antimikroba Ekstrak Daun Garcinia benthami Pierre dengan Metode Dilusi. Farmasi Fakultas Kedokteran dan Ilmu Kesehatan, UIN Syarif Hidayatullah Jakarta, 75p. http://repository.uinjkt.ac.id/dspace/handle/123456789/29264

Sukenda, S. and Wakabayashi, H., 1999. Immersion immunization of ayu (Plecoglossus altivelis) with Pseudomonas plecoglossicida bacterin. *Fish Pathology, 34*, pp.163-

164. https://www.jstage.jst.go.jp/article/jsfp1966/34/3/34_3_163/_pdf

Tarman, K., Purwaningsih, S. and Negara, A.A.A.P.P., 2013. Aktivitas Antibakteri Ekstrak Daun Bakau Hitam (Rhizophora Mucronata) Terhadap Bakteri Penyebab Diare. *JPHPI*, *16*(3), pp.249-258. https://doi.org/10.17844/jphpi.v16i3.8063

Tendencia, E.A., dela Peña, M.R., Fermin, A.C., Lio-Po, G., Choresca Jr, C.H. and Inui, Y., 2004. Antibacterial activity of tilapia Tilapia hornorum against Vibrio harveyi. *Aquaculture*, *232*(1-4), pp.145-152. https://doi.org/10.1016/S0044-8486(03)00531-3