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EFEKTIVITAS EKSTRAK DAUN MANGROVE (*Rhizophora apiculata*) TERHADAP PENCEGAHAN INFEKSI VIBRIO ALGINOLYTICUS PADA KAKAP PUTIH (*Lates calcarifer*)

Effectiveness of Mangrove Leaf Extract (Rhizophora apiculata) in Preventing Vibrio alginolyticus Infection in White Snapper (Lates calcarifer)

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ABSTRAK

Kakap putih (*Lates calcarifer*) merupakan salah satu ikan laut yang mempunyai nilai ekonomis penting serta mengandung nilai gizi yang tinggi sebagai ikankonsumsi. Pada sistem budidaya ikan kakap putih munculnya wabah penyakit menjadi salah satu ancaman utama. Salah satu penyakit yang sering timbul dalam budidaya ikan kakap putih adalah penyakit bakterial. Jenis bakteri yang menyebabkan penyakit serius dan mengancam ekonomi dan keberlanjutan produksi kakap putih adalah *Vibrio alginolyticus*. Penelitian ini bertujuan untuk menganalisis efektivitas perendaman ekstrak daun mangrove (*Rizophora apiculata*) pada kakap putih untuk mencegah *Vibrio alginolyticus* serta mengetahui dosis ekstrak daun bakau minyak yang tepat untuk mencegah penyakit bakteri *Vibrio alginolyticus.* Metode penelitian yang digunakan yaitu Rancangan Acak Lengkap (RAL) dengan 4 perlakuan dan 3 ulangan. Perlakuan tersebut yaitu: P1= 0 ml ekstrak mangrove/L, P2= 0,5 ml ekstrak mangrove/L, P3= 1,5 ml ekstrak mangrove/L dan P4= 2,5 ml ekstrak mangrove/L. Hasil lama waktu penyembuhan terbaik berada pada P4 dengan lama waktu 23 jam dan kelangsungan hidup terbaik juga terdapat pada P4 dengan persentasi 96,67%, sedangkan kelangsungan hidup terendah berada pada P1 yakni 56,67%. Kisaran parameter perairan selama penelitian yaitu suhu berada pada kisaran 28^{o}C-29^{o}C, pH 8, DO 4-5 mg/L air, salinitas 31 ppt, ammonia 0,4, nitrat <0,01 dan nitrit <0,1. Kesimpulan dari penelitian ini yaitu perendaman ekstrak daun baku minyak efektif pada pencegahan bakteri *Vibrio alginolyticus* pada kakap putih.

ABSTRACT

White snapper (Lates calcarifer) is a marine fish that has important economic value and contains high nutritional value as a consumption fish. In the white snapper cultivation system, the emergence of disease outbreaks is one of the main threats. One of the diseases that often occurs in white snapper cultivation is bacterial disease. The type of bacteria that causes serious disease and threatens the economy and sustainability of white snapper production is Vibrio alginolyticus.This study aims to analyze the effectiveness of soaking mangrove leaf extract (Rhizophora apiculata) in white snapper to prevent Vibrio alginolyticus and to determine the appropriate dose of mangrove leaf oil extract to prevent Vibrio alginolyticus bacterial disease.The research method used is a Completely Randomized Design (CRD) with 4 treatments and 3 replications. The treatments were: P1 = 0 ml mangrove extract/L, P2 = 0.5 ml mangrove extract/L, P3 = 1.5 ml mangrove extract/L and P4 = 2.5 ml mangrove extract/L Results: The best healing time was found in P4 with a duration of 23 hours and the best survival was also found in P4 with a percentage of 96.67%, while the lowest survival was found in P1 at 56.67%. The range of water parameters during the study were temperature in the range of 28oC-29oC, pH 8, DO 4-5 mg/L water, salinity 31 ppt, ammonia 0.4, nitrate <0.01 and nitrite <0.1. The conclusion of this study is that soaking in standard oil leaf extract is effective in preventing Vibrio alginolyticus bacteria in white snapper.

Kata Kunci	*Bakteri, Kakap Putih, Ekstrak Daun Mangrove, Vibrio Alginolyticus*
Keywords	*Bacteria, White Snapper, Mangrove Leaf Extract, Vibrio Alginolyticus*
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INTRODUCTION

Asian seabass (*Lates calcarifer*) is one of the marine fish species that has important economic value and contains high nutritional value as a food fish (Nurmasyitah *et al.*, 2018). Asian seabass has become a commercial aquaculture enterprise to be developed, due to its relatively fast growth, ease of culture, and high tolerance to environmental changes, making Asian seabass suitable for both small- and large-scale aquaculture businesses (Jaya *et al.*, 2013).

Fish under stress conditions are more susceptible to disease attacks. One type of disease that frequently affects marine fish is bacterial infection. Bacterial infections can cause fish mortality. However, not all bacteria are pathogenic; there are also groups of bacteria that provide beneficial effects to their hosts, namely Gram-positive bacteria. These bacteria are usually found in the digestive tract and provide positive impacts on fish growth. The role of beneficial bacteria in nutritional aspects can assist fish in the digestion process by producing various types of extracellular hydrolytic enzymes, in which enzymes in the digestive tract also play a role in host metabolism (Gangguly & Prasad, 2012; Ray *et al.*, 2012; Wang *et al.*, 2018).

In Asian seabass aquaculture systems, the emergence of disease outbreaks is one of the main threats (Novriadi *et al.*, 2014). One disease that often arises in Asian seabass culture is bacterial disease. A bacterial species that causes serious disease and threatens the economic value and sustainability of Asian seabass production is *Vibrio alginolyticus* (Sharma *et al.*, 2013).

Several bacterial species from the genus *Bacillus* are known to inhibit the growth of *Vibrio harveyi* that attacks larvae in hatcheries and causes production losses, including *Paenibacillus* spp., *Bacillus cereus*, and *Paenibacillus polymyxa*, which are directly mixed into the water and can inhibit the growth of *Vibrio harveyi* in tiger shrimp larvae (Ravi *et al.*, 2007), while *Bacillus subtilis* has the ability to inhibit *Vibrio harveyi* bacteria. Efforts to enhance the immune system or disease resistance of fish without adverse side effects have recently begun to be developed (Hastuti and Karoror, 2007). The application of natural materials with the aim of improving immune resistance has started to be developed. Mangrove leaves of oil mangrove (*Rhizophora apiculata*) are one of the natural materials that are beneficial for health.

Vibrio alginolyticus is a Gram-negative bacterium with a curved rod shape, oxidase- and catalase-positive, ferments glucose without gas production, and possesses polar flagella. This bacterium is commonly found in brackish and marine waters. Some are saprobic, but several species cause vibriosis in aquatic animals, including fish (Bauman *et al.*, 1994; Barrow and Feltham, 1993).

Antiseptic measures that can be applied to prevent vibriosis include the use of natural materials as alternative disease prevention strategies in fisheries, which have been developed, including the utilization of oil mangrove plants and their associations. Laith and Najiah (2014) and Mouafi *et al.* (2014) reported the potential of oil mangrove plants and their associations for fish diseases. According to Shamsuddin *et al.* (2013) and Maulida *et al.* (2014), several types of oil mangrove are effective against *Vibrio alginolyticus* in fish.

MATERIALS AND METHODS

This study was conducted for 40 days from June to July 2023 at the Brackish Water Aquaculture Center (BPBAP) Ujung Batee, Aceh Besar. The research method used was an experimental method with a Completely Randomized Design (CRD) consisting of 4 treatments and 3 replications. The treatments applied were P1 = without mangrove leaf extract application, P2 = mangrove leaf extract application at 0.5 ml/L, P3 = mangrove leaf extract application at 1.5 ml/L, and P4 = mangrove leaf extract application at 2.5 ml/L.

Research Procedures

A. Preparation of Containers and Equipment

The containers used were 12 jars with a volume of 25 liters. The jars were washed thoroughly using soap and dried under sunlight until completely dry. The jars were filled with 10 liters of water measured from the height of the jar and equipped with aerators to supply oxygen.

B. Biota Selection

The Asian seabass used in this study were healthy and disease-free fish measuring 2–3 cm, totaling 120 individuals, with a stocking density of 10 fish per jar.

C. Acclimatization

Asian seabass intended for the study were acclimatized for 1 week or until the fish showed normal conditions, with the aim of adapting to the new environment. On the first day of acclimatization, the fish were fasted for 24 hours.

D. Preparation of Mangrove Leaf Extract

The preparation of mangrove leaf extract (*Rhizophora apiculata*) was carried out by collecting mangrove leaves, cleaning them, and then drying them. The dried leaves were oven-dried at 50°C for 24 hours. After drying, the leaves were ground into powder using a blender and sieved to obtain a fine powder. The next step involved soaking the mangrove leaf powder in 70% ethanol (Suciati, 2012). The material was then filtered to obtain an extract solution without residue, and this process was repeated three times.

The filtrates from the first to third filtration were combined and concentrated using a rotary vacuum evaporator at 50°C to obtain the extract through evaporation (Putri, 2015).

E. Culture of *Vibrio alginolyticus*

Bacterial propagation was conducted on a laboratory scale by initially collecting *Vibrio alginolyticus* bacteria using a sterilized inoculation loop heated with a Bunsen burner. The bacteria were then streaked in a zig-zag pattern on the surface of TSB (Tryptone Soya Broth) agar medium, covered, and incubated in an inverted position at room temperature of 30–31°C for 24 hours. After incubation, a single colony was taken using an inoculation loop, vortexed until homogeneous, and incubated on TCBS (Thiosulfate Citrate Bile Salt) medium for 24 hours. Subsequently, 1 ml of the cultured bacteria was diluted in a test tube containing 9 ml of distilled water to obtain a total volume of 10 ml, then vortexed. The vortexed suspension was then transferred at 1 ml into each test tube, and the bacteria were ready for use. This procedure followed the method described by Ayini and Dewi (2014).

F. Challenge Test

The challenge test was conducted using large bottle aquaria with a volume of 1.5 liters. The challenge test used a dose of 10^9 CFU, as Asian seabass have relatively high resistance, thus a high dose was selected to infect the fish. Asian seabass measuring 2–3 cm were transferred and placed into each experimental aquarium. Subsequently, each experimental unit was infected with *Vibrio alginolyticus* at a dose of 10^9 CFU with a volume of 1 ml. The challenge test was conducted for 24 hours with feeding and aeration. During the challenge test, feed residues and waste were siphoned from the rearing containers to prevent mortality of the test animals due to water quality deterioration. Observations were carried out every 4 hours. Survival calculation at the end of the challenge test was performed after 24 hours of observation.

G. Immersion with Mangrove Leaf Extract

After the Asian seabass were subjected to the challenge test and exhibited clinical symptoms of disease, preventive treatment was carried out using mangrove leaf extract through the immersion method. Mangrove leaf extract was added to containers containing 1.5 liters of water and allowed to dissolve completely. Asian seabass were immersed in the medium for 4 hours. Subsequently, the immersed fish were returned to the rearing jars, and clinical symptoms were observed.

H. Rearing and Feeding

Rearing was conducted for 40 days, and fish were fed ad libitum with a feeding frequency of three times daily at 08:00, 12:00, and 16:00 WIB. The feed used was pellet feed with a protein content of 45%.

I. Water Quality Management

To maintain water quality, siphoning was carried out daily after feeding, while water replacement was conducted every three days at a rate of 30%. Siphoning aimed to remove fecal residues to prevent the accumulation of waste and an increase in ammonia concentration.

Research Parameters

A. Clinical Symptoms

Observation of clinical symptoms was conducted by observing the behavior and physical changes of Asian seabass. Clinical symptom observations were carried out before the challenge test, after the challenge test, and after treatment with mangrove leaf extract solution. The observed clinical symptoms are presented in Table 1.

Table 1. Observation of clinical symptoms in the study

No	Parameter	Observation of Clinical Symptoms
1	Behavior	Passive movement and decreased appetite
2	Physical Changes	Reddening of the external body surface, reddened eyes, and scale detachment

B. Healing Time

The purpose of the healing time was to observe which treatment was most effective in curing Vibrio alginolyticus bacteria in white snapper (Lates calcarifer). The observation of healing time was conducted to determine the effect of mangrove leaf extract on curing Vibrio alginolyticus infections. This was done after the test animals were soaked in mangrove leaf extract until the end of the 40-day maintenance period.

C. Survival Rate

The survival rate of white snapper can be calculated using the formula (Sihombing *et al*, 2023).

$$SR = Nt / No \times 100$$

Note: SR= Survival (%); Nt= Final number of fish (tails); No= Initial number of fish (tails)

D. Water Quality Measurement

Water quality observations included dissolved oxygen, salinity, temperature, ammonia, and pH. Water quality measurements were conducted every 10 days throughout the study period.

E. Data analysis

Data on clinical symptoms, healing time, survival rate, and water quality were presented in tables and graphs and analyzed using SPSS. If significant differences were found, where F count > F table, Duncan's further test was performed. Data analysis was performed at a 95% confidence interval.

RESULTS AND DISCUSSION

Clinical Symptoms

Clinical symptoms in barramundi were observed by visual inspection using the sense of sight. Clinical examination was divided into two observations, namely observation of fish behavior and morphological changes in barramundi. The observations were conducted at four stages, namely before the challenge test, after the challenge test, during the immersion period, and after the immersion of the fish using mangrove leaf extract. The results of the clinical examination of fish behavior and the clinical examination of barramundi morphology are presented in Table 2 and Table 3.

Table 2. Clinical symptoms of barramundi behavior

Information	Behavior category	Treatment			
		P1	P2	P3	P4
Before the challenge test	Movement/Swimming	+	+	+	+
	Appetite	+	+	+	+
	Response to Stimulus	+	+	+	+

After the challenge test	Movement/Swimming	++	++	++	++
	Appetite	++	++	++	++
	Response to Stimulus	++	++	++	++
During soaking	Movement/Swimming	++	++	++	+++
	Appetite	++++	++++	++++	++++
	Response to Stimulus	++	++	++	+++
After soaking	Movement/Swimming	++	+++	+++	+
	Appetite	++	+++	++	+
	Response to Stimulus	++	+++	+++	+++

Description:
+ : Active movement, high appetite, and good response to stimuli
++ : Passive movement, decreased appetite, and slow response to stimuli
+++ : Movement, appetite, and response to stimuli are starting to return to normal
++++ : Unknown due to not being fed

Table 3. Clinical symptoms of white snapper morphology

Information	Behavioral Category	Treatment			
		P1	P2	P3	P4
Before the challenge test	Body color	+	+	+	+
	Wounds/ulcers	+	+	+	+
	Scales	+	+	+	+
After the challenge test	Body color	++	++	++	++
	Wounds/ulcers	++	++	++	++
	Scales	++	++	++	++
During immersion	Body color	++	+++	+++	+++
	Wounds/ulcers	++	+++	+++	+++
	Scales	++	+++	+++	+++
After immersion	Body color	++	++++	+++	++++
	Wounds/ulcers	++	++++	++++	++++
	Scales	++	++++	++++	++++

+ : Normal body color, no wounds/ulcers, normal and complete scales
++ : Reddish exterior, wounds/ulcers appearing, scales peeling off
+++ : Reddish body color, wounds still present, scales no longer peeling off
++++ : Body color returning to normal, wounds starting to improve, scales no longer peeling off

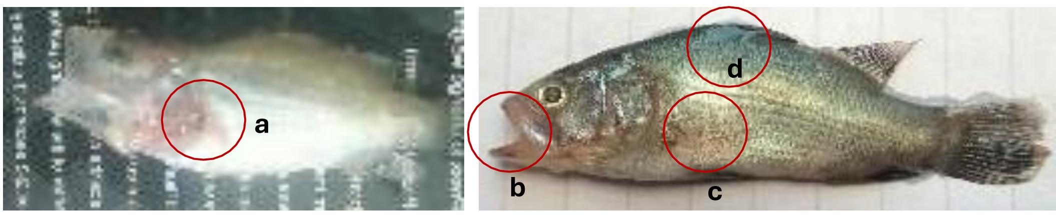

Figure 1. Clinical Symptoms of Morphological Changes in Snapper

Description: a (Gills injured); b (Mouth part reddened); c (Body part reddened); d (scales peeled off).

Healing Time

Observations were conducted to determine the dose at which the mangrove leaf extract could react more quickly and efficiently. The healing time for each phase is presented in Table 4.

Table 4. Healing Time for White Snapper

Treatment	Healing Time (Hours)
P1 (0 ml/L)	∞[a]
P2 (0,5 ml/L)	67,67±1,16[d]
P3 (1,5 ml/L)	45,67±0,58[c]
P4 (2,5 ml/L)	23,00±0,00[b]

Description: Numbers followed by the same letter are not significantly different according to the Duncan's Multiple Range Test (DMRT) at α = 5%

Survival Rate

The survival rate in this study was conducted to determine the percentage of snapper fish surviving after the treatment period. The results of the fish survival rate can be seen in Figure 2.

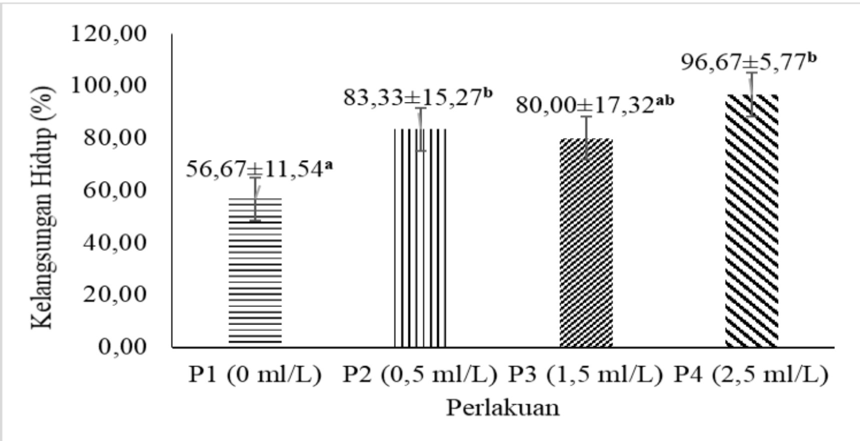

Figure 2. Survival rate of white snapper

Water Quality

Water quality measurements in this study were conducted every 10 days, with physical and chemical parameters observed. The water quality results are presented in Table 5.

Table 5. Water quality parameters during the study

Water Quality Parameters	Treatment				SNI Quality Standards
	P1 (0 ml/L)	P2 (0,5 ml/L)	P3 (1,5 ml/L)	P4 (2,5 ml/L)	
Temperature (oC)	28,7-29,1	27,5-29,0	28,9-29,0	28,6-28,8	25-35
Salinity (ppt)	31,2-31,7	31,2-31,7	31,2-31,7	31,0-31,2	20-30
pH	8,37-8,47	8,47-8,50	8,42-8,47	8,32-8,50	7,22-8,00
DO (mg/l)	4,9-5,2	5,4-5,5	4,9-5,5	5,4-6,0	3 – 6
Ammonia (mg/l)	0,3	0,3	0,4	0,4	< 0,1
Nitrate	<0,1	<0,1	<0,1	<0,1	0,06
Nitrite	<0,1	<0,1	<0,1	<0,1	0,1 – 2,0

DISCUSSION

Based on Table 2, the observed clinical symptoms in barramundi juveniles prior to the challenge test exhibited normal behavioral and morphological characteristics in all treatments, namely active movement, high appetite, responsiveness to stimuli, bright body coloration, and the absence of external injuries. This is supported by the statement of Aulia (2018), that the criteria of healthy fish include active movement, balanced and agile body condition, responsiveness to stimuli and provided feed, complete fins, and brightly colored gills.

Meanwhile, the clinical symptoms observed after the challenge test showed changes in fish behavior and morphology starting from the first day of the challenge test until the third day (Table 3). The clinical symptoms of fish behavior indicated passive movement, decreased appetite, and slower response to external stimuli. The clinical symptoms observed in fish body morphology included discoloration of several external body organs turning reddish, such as the mouth, body, dorsal area, and tail. In addition, scale detachment on the fish body and the appearance of wounds or ulcers on the fish body were observed. This is in accordance with the study by Tukan *et al*. (2023), which reported more severe clinical symptoms such as darkened body coloration, enlarged infection wounds, scale loss, lesions on the eyes and mouth, increased mucus production, reddish fins causing fin damage, and the presence of reddish nodules.

In Table 4, the best healing duration was observed in P4, which was 23.00 hours, while the longest healing duration occurred in P3 with a duration of 67.67 hours. The Duncan test results indicated that each treatment showed a significant effect compared to the other treatments. Specifically, P1 showed a significant effect on P2, P3, and P4, and similar patterns were observed among the other treatments.

The differences in healing duration were caused by the dosage of mangrove leaf extract used in each treatment. The fastest healing duration in this study occurred at the highest dosage, namely 2.5 ml of mangrove leaf extract. This is because mangrove leaves contain antibacterial compounds such as saponins, flavonoids, tannins, phenols, and alkaloids, which can maintain the immune resistance of cultured organisms against disease attacks as antibacterial agents. It is known that flavonoid compounds can prevent oxidation and inhibit the rapid spread of wounds (Putri *et al*., 2015).

Furthermore, Saifudin (2006) also explained that one of the active compounds capable of inhibiting bacterial growth is alkaloids. These compounds can inhibit protein synthesis, thereby preventing bacterial replication, which ultimately leads to bacterial death. Meanwhile, tannin compounds present in mangrove leaves can cause bacterial cell contraction due to the presence of tannic acid, which can inhibit bacterial growth.

From Figure 2, the survival rate of barramundi during the maintenance period was obtained. The highest survival rate was observed in P4 (2.5 ml mangrove leaf extract/L water) with a barramundi survival percentage of 96.67%. Meanwhile, the lowest survival rate was observed in P1 (without mangrove leaf extract) with a percentage of 56.67%.

The high survival rate in treatment P4 with the highest dose of mangrove leaf extract, namely 2.5 ml, was due to the effective healing process occurring in this treatment. This indicates that a high dose of mangrove leaf extract and high flavonoid content have a positive effect on the healing process of barramundi infected with *Vibrio* bacteria. This is reinforced by the study of Putri *et al*. (2015), which reported that increasing survival rates along with higher dosages were associated with the active compounds present in mangrove leaf extract.

The presence of antibacterial compounds in mangrove leaf extract was evidenced by the formation of inhibition zones in the preliminary test. According to Maryani *et al*.

(2002), the increase in survival rate in test crabs was due to mangrove leaves containing active compounds that function as antibacterial agents capable of inhibiting and killing bacteria.

Table 5 shows the water quality results during the maintenance period, where the temperature ranged from 27–29°C, salinity was 31 ppt, pH ranged from 8.3–8.5, dissolved oxygen (DO) ranged from 4.9 mg/L to 5.5 mg/L, ammonia was 0.3 mg/L, nitrate was <0.1, and nitrite was <0.1. These water quality parameters were still within the normal quality standards according to the Decree of the Minister of Forestry and Environment No. 51 of 2004 concerning seawater quality standards for aquaculture.

Water quality is a supporting parameter, including dissolved oxygen, temperature, pH, salinity, ammonia, nitrate, and nitrite, where deviations from standard quality thresholds can disrupt other factors (Mahenda, 2021). The water quality values in this study were obtained from weekly measurements. Dissolved oxygen levels in this study were influenced by the aerator valve regulating the amount of oxygen supplied to each container. Aeration was adjusted to avoid excessive or insufficient oxygen supply. Excessive aeration can cause stress and mortality in fish, while insufficient aeration can lead to oxygen deficiency and result in mortality (Adithiya *et al*., 2023). In this study, good water quality management was achieved by siphoning to remove waste accumulated at the bottom of the container. If water conditions do not meet the required standards, they can become a major source of disease and cause mortality in brackish water fish. Temperature is one of the important controlling factors that can influence the physiological and chemical activities of aquatic organisms. Optimal water temperature depends on species and parameters such as growth, development, feed conversion, and disease resistance. The temperature during maintenance ranged around 26°C, which is still within a favorable range for barramundi culture. This is consistent with the findings of Effendi *et al*. (2015), who stated that the optimal temperature for fish growth ranges from 25–32°C. The pH during the study was 7 and remained within the tolerable range for barramundi culture, in accordance with Effendi (2003), who stated that the optimal pH range for barramundi culture is 6–8.5. Dissolved oxygen (DO) in water is very important for the survival of all organisms. Oxygen requirements depend on fish species, age, and activity level (Fitriadi *et al*., 2014). In waters with oxygen concentrations below 4 mg/L, some fish species can still survive, but their appetite begins to decline. Therefore, a suitable oxygen concentration for aquaculture is between 5 ± 7 mg/L (Monalisa and Minggawati, 2010). Ammonia is one of the water quality parameters that poses a major problem for fish in aquaculture activities. Several factors can cause increased ammonia concentrations, including the decomposition of uneaten feed. High ammonia levels can be toxic in aquaculture activities and may cause gill irritation and respiratory disorders. According to Tatangindatu *et al*. (2013), ammonia levels suitable for fish life are below 1 ppm. If ammonia levels exceed 1.5 ppm, the waters are considered polluted.

CONCLUSIONS AND RECOMMENDATIONS

Immersion using mangrove leaf extract is effective in preventing *Vibrio alginolyticus* infection in barramundi. The effective dose of mangrove leaf extract for preventing *Vibrio alginolyticus* bacterial disease was found in treatment P4 at a dose of 2.5 ml/L, where the best survival rate and healing duration were observed in treatment P4.

RECOMMENDATIONS

The recommendation of this study is that further research should be conducted using challenge tests through injection methods and treatment by increasing the dosage of mangrove oil leaf extract.

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