Original article DOI: 10.19027/jai.17.2.120-129

The effectivity of pandanus leaf extract for the treatment of Sangkuriang catfish juvenile *Clarias gariepinus* infected by *Aeromonas hydrophila*

Efektivitas ekstrak daun pandan wangi *Pandanus amaryllifolius* untuk pengobatan benih lele Sangkuriang *Clarias gariepinus* yang terinfeksi bakteri *Aeromonas hydrophila*

Siti Sopiah[1*], Rosidah[1], Walim Lili[1], Iskandar[1], Ibnu Bangkit Suryadi[1]

[1]Study Program of Fisheries, Faculty of Fisheries and Marine Science, Padjadjaran University
Jalan Raya Bandung–Sumedang, Jatinangor 40600, Sumedang, West Java
*E-mail: sitisopiah345@yahoo.co.id

(Received September 22, 2017; Accepted July 21, 2018)

ABSTRACT

The aim of this study was to determine the effective concentration of pandanus *Pandanus amaryllifolius* leaf extract for the treatment of Sangkuriang catfish juvenile infected by *Aeromonas hydrophila*. The method used in this study was an experimental method with completely randomized design consisted of five treatments and three replications. The treatments were A (control), B (400 mg/L), C (600 mg/L), D (800 mg/L), and E (1000 mg/L) trough immersion for 24 hours through immersion for 24 hours. The fish has infected with *A. hydrophila* bacteria as much as 20 mL of NaCl/L in 10 L of water medium with a density of 108 CFU/mL through immersion. The observed parameters were clinical symptoms, recovery process, survival rate, and water quality. The results showed that pandanus leaf extract at a concentration of 800 mg/L for 24 hours was effective for treating Sangkuriang catfish juvenile that infected by *A. hydrophila* with a survival rate of 86.67%. Based on the regression analysis, it discovered that the optimum concentration of pandanus leaf extract through immersion for 24 hours was 774.39 mg/L and the predicted survival was 92.16%.

Keywords: *Aeromonas hydrophila*, catfish fingerlings, effective concentration, infection, pandanus leaf extract.

ABSTRAK

Penelitian ini bertujuan untuk menentukan konsentrasi yang efektif dari ekstrak daun pandan wangi *Pandanus amaryllifolius* untuk pengobatan benih lele Sangkuriang *Clarias gariepinus* yang terinfeksi bakteri *A. hydrophila.* Metode yang digunakan dalam penelitian ini adalah metode eksperimental rancangan acak lengkap dengan lima perlakuan dan tiga ulangan. Perlakuan penelitian adalah A (kontrol), B (400 mg/L), C (600 mg/L), D (800 mg/L), dan E (1000 mg/L). Ikan uji diinfeksi bakteri *A. hydrophila* sebanyak 20 mL NaCl/L dalam 10 L media air dengan kepadatan 108 CFU/mL melalui perendaman selama 24 jam. Parameter yang diamati meliputi gejala klinis, waktu pulih, kelangsungan hidup, dan kualitas air. Hasil penelitian menunjukkan bahwa penggunaan ekstrak daun pandan wangi pada konsentrasi 800 mg/L selama 24 jam efektif untuk pengobatan benih lele Sangkuriang yang terinfeksi bakteri *A. hydrophila* dengan tingkat kelangsungan hidup sebesar 86,67%. Berdasarkan analisis regresi diketahui bahwa konsentrasi optimum penggunaan ekstrak daun pandan wangi melalui perendaman selama 24 jam yaitu 774,39 mg/L dan prediksi kelangsungan hidup ikan adalah 92,16%.

Kata kunci : *Aeromonas hydrophila*, benih lele Sangkuriang, ekstrak daun pandan, infeksi, efektifitas konsentrasi

INTRODUCTION

According to Marine Affairs and Fisheries (DKP) of West Java, the demand of Sangkuriang catfish experienced an increase from year to year. In 2016, the average production of catfish increased to 532.410 tons (KKP, 2016). The Sangkuriang catfish owns a rapid growth, capable of adapting in an unfavorable environment, has good taste, and contains 17.7% of protein, therefore lots of aquaculturist and consumers are interested in this fish (Sunarma, 2004). The obstacle that faced in Sangkuriang fingerlings supply was the attack of bacterial disease, especially *Aeromonas hydrophila* bacteria.

A. hydrophila is a dangerous pathogenic bacteria that affect the freshwater aquaculture because it can infect all stadia of fish. *A. hydrophila* can cause an epidemic with a high mortality rate reached 80–100% in a short period (1–2 weeks) (Lukistyowati & Kurniasih, 2012). The specific clinical symptom was lesions on the surface of the fish body. The transmission of *A. hydrophila* is rapid through water, direct contact with the lesions, and the contaminated equipment. Mangunwardoyo *et al*. (2010) stated that the pathogenic bacteria infection starts with the bacteria attached to the surface of the skin by utilizing its pili, flagella, and hook to move and strongly attach to the outer layer of the fish body (scales covered with chitin). During the infection process, *A. hydrophila* produces chitinase enzymes to degrade the chitin layers thereby the bacteria can easily penetrate.

The treatments through administrating the antibiotics have done often, yet the use of antibiotics for a prolonged period of time will generate the negative effects, both in fish and the environment. One of the herbal ingredients that can be used for treating the infected catfish was pandanus leaf *Pandanus amaryllifolius*. According to phytochemical tested, pandanus leaf contains an antibacterial compound (tannin, saponins, flavonoids, triterpenoids, monoterpenoids, sesquiterpenoids, quinone, alkaloids).

The active ingredients in pandanus leaf extract have a different working mechanism. Flavonoids as antimicrobe agent work to inhibit nucleic acid synthesis, to inhibit the function of the cell membrane, and to inhibit the energy metabolism (Hendra *et al.*, 2011). Saponins works as an antibacterial and antimicrobe agent. This is according to the cytotoxic characteristic of saponins and its capability to affect the permeability of the cytoplasm membrane, therefore, the cell microbe become lysis. Tannins have an antibacterial activity to inhibit the reverse transcriptase enzymes and topoisomerase DNA (Nuria *et al*., 2009). The accumulation of triterpenoid compounds inhibits the bacteria grow through protein synthesis inhibition that can cause changes in the compiler components of bacteria (Siregar *et al*., 2012). This study aimed to determine the effective concentration of pandanus leaf extract for the treatment of Sangkuriang catfish juvenile infected by *A. hydrophila.*

MATERIALS AND METHODS

The preparation of experimental fish

The experimental catfish used in this study originated from Center for Research and Development of Freshwater Aquaculture (BBPBAT) in Sukabumi, West Java, sizing of 5–7 cm in total length, as much as 250 fishes for the initial study, 300 fishes for the further study, and as much as 150 fishes for rearing stock. The experimental fishes were acclimatized for seven days to find out the healthy fish.

The preparation of aquarium

As much as 15 aquariums sizing of 40×30×30 cm^3 were used in this study. The aquariums were cleaned, then the water was added fully and as much as 30 mg/L of chlorine was added to sterilize the containers for 24 hours. The aquariums were dried before being used. After that, the aqariums were filled with 10 L of water and were aerated.

The preparation of pandanus leaf extract

Pandanus leaf extract was prepared by maceration method. As much as 4000 g of wet pandanus leaves were dried for 14 days, and obtained as much as 2000 g of dry weight. The dried pandanus leaves were performed by using 96% ethanol solution as much as 20 L for three days. The filtrate was filtered by using Whatman paper filter number 42. The maceration filtrate was evaporated and concentrated by using rotatory evaporator at 60°C to obtain the pandanus leaf thick extract as much as 153.21 g.

Fish rearing

The fish fed with commercial feed 781-1 with 33% of protein content twice a day in ad libitum. In order to keep the water quality in every aquarium, the excess feed and fish feces were siphon once a

day during the fish rearing to avoid the high-stress level in fish because of excess feed in the bottom of the aquarium.

The challenge test

The experimental method used in this study was complete randomized design with five treatments and three replications, consisted A (control), B (400 mg/L), C (600 mg/L), D (800 mg/L), and E (1000 mg/L). The fish that infected by *A. hydrophila* was soaked with pandanus leaf extract for 24 hours appropriate to the treatments.

The method to obtain the bacteria density was serial dilution. The bacteria culture of *A. hydrophila* derived from the petri dish was added into 20 ml of NaCl 0.9% then was homogenized using vortex. The homogenized bacteria culture was put into a cuvette as much as 2 ml and was calculated by using spectrophotometer in 540 nm of wavelength and 0.235 of absorbance value. The challenge test was done through immersion. The fish was soaked in 10 L of water that has already added with 20 mL of *A. hydrophila* (the bacteria density was 10^8 CFU/mL). White (1989) stated that water might be the intermediary transmission of disease, therefore the smaller the size of fish, the disease will transmit easier.

The initial study

Disk diffusion test

The zone of inhibition used to test the effectiveness of pandanus leaf extract as an antibacterial to inhibit the growth of *A. hydrophila.* The zone of inhibition was used disk diffusion test with five variances of pandanus leaf extract concentration, consisted negative control (aquades), 600 mg/L, 800 mg/L, 1.000 mg/L, and 1.200 mg/L for 24 hours. During the test, it only used the negative control. The material and equipment were sterilized using an autoclave. The disc paper was put to a petri dish with NA medium and 1 mL of *A. hydrophila* inoculation (the bacteria density was 10^8 CFU/mL). The petri dish then was incubated for 24 hours at 30°C. The zone of inhibition was measured by using a caliper. The disk diffusion test results showed in Table 1.

Table 1 showed that due to the increase of pandanus leaf extract concentration, the diameter of inhibition zone was increased. The concentration of 600–1200 mg/L had a diameter more than 6 mm. Some categories of the diameter of inhibition zone are the diameter less than 5 mm (weak inhibition), the diameter between 5–10 mm (adequate inhibition), the diameter between

Table 1. The observation result of inhibition zone

Concentration (mg/L)	Inhibition zone in I, II, and III of replication (mm)			The average of the measured inhibition zone (mm)
	I	II	III	
Control	-	-	-	-
600	8.88	8.94	6.73	8.18
800	7.28	7.81	11.54	8.87
1000	13.72	11.55	9.74	11.67
1200	14.22	11.85	16.82	14.30

Table 2. The results of LC_{50}-24-hour test

Estimated LC/EC values and confidence limits			
Point	Exposure concentration	95% of confidence limits	
		Lower	Upper
LC/ EC 1.00	649.72	186.43	996.60
LC/ EC 5.00	866.71	341.65	1239.87
LC/ EC 10.00	1010.64	467.12	1407.91
LC/ EC 15.00	1121.07	573.06	1543.03
LC/ EC 50.00	1737.71	1205.65	2568.79
LC/ EC 85.00	2693.54	1945.74	5575.00
LC/ EC 90.00	2987.84	2126.23	6862.95
LC/ EC 95.00	3484.02	2404.86	9415.84
LC/ EC 99.00	4647.58	2981.39	17315.71

10–20 mm (strong inhibition), the diameter more than 20 mm (powerful inhibition) (Susanto *et al*., 2012).

The determination of LC_{50}-24-hour test

The LC_{50} test of pandanus leaf extract was done to measure the short-term poisoning potential which causes 50% of mortality. The concentration for LC_{50} consisted six treatments (0 mg/L, 10 mg/L, 100 mg/L, 500 mg/L, 1.000 mg/L, and 3.000 mg/L) with three replications. The experimental fish was acclimatized in fiber container with 100 L of water for seven days. Fish with the same weight and size transferred into an aquarium with 5 L of water and stocking density of 10 fishes/aquarium. The pandanus leaf extract added into each aquarium according to the treatments. The survival rate of fish has measured with EPA probit analysis software. T he result of the LC_{50} test showed in Table 2. It showed that as much as 1737.71 mg/L of pandanus leaf extract concentration caused more than 50% of mortality in Sangkuriang catfish juvenile in 24 hours, respectively. The concentration that used in the initial study had been coming from in vitro test and LC_{50} 24 hour test, amounting to 649.73 mg/L because it counted more than inhibition value and less than LC_{50} 24 hour test value.

The observed parameters

Clinical symtomps of Sangkuriang catfish juvenile after A. hydrophila infection

The clinical symtomps in Sangkuriang catfish juvenile were observed after *A. hydrophila* infection. Haryani *et al*. (2012) stated that the observation of clinical symtomps was done through observation in the lesion, response to feeding and the behavior of Sangkuriang catfish juvenile after *A. hydrophila* infection.

The recovery time and daily mortality

The observation of recovery time was done for 14 days, consecutively. It referred to the previous study by Pratama *et al*. (2017). The recovery time consisted response test towards feed and shock. The treatment was done after the clinical symptoms appeared. The daily mortality rate was calculated to find out the recovery time.

The survival rate

The observation of the survival rate of the experimental fish was done after 14 days of recovery time. The percentage of survival rate was calculated with this following formula:

$$SR\ (\%) = \frac{Nt}{No} \times 100$$

Notes:
SR = Survival rate (%)
Nt = The total fish at the end of the study
No = The total fish in the initial study

Water quality parameters

The water quality parameter consisted of temperature, pH, and dissolved oxygen (DO). The observation of water quality parameter was done in the initiation of treatment, after challenge test, in treatment time, and after treatment time (Wahjuningrum *et al*., 2013).

Data analysis

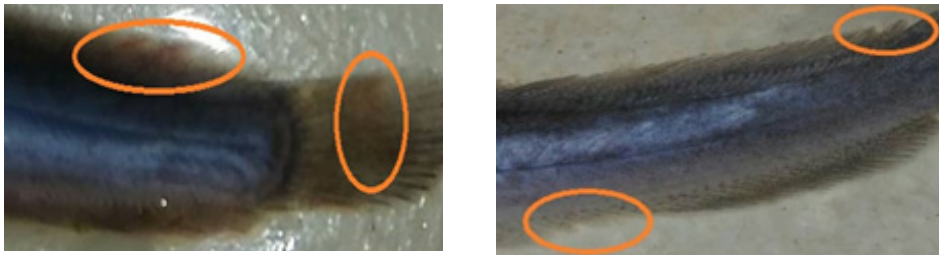
The survival rate data were analyzed using F-test of ANOVA, if it was significantly different among treatments, it had continued to Duncan test with 5% of standard error and regression test to determine the optimum concentration from treatments. The descriptive analysis was done for clinical symptoms, response toward feed and shock, recovery time, and water quality parameters.

RESULTS AND DISCUSSION

Clinical symptoms of Sangkuriang catfish juvenile infected by *A. hydrophila* bacteria

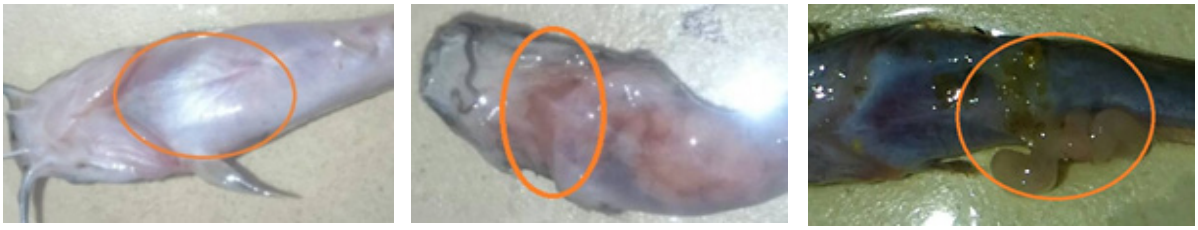
According to the observation results in the first to the third day after *A. hydrophila* infection, the Sangkuriang catfish juvenile had not shown the clinical symptoms yet. This was caused by the bacteria had not penetrated yet into the fish body or into the blood circulation. At the second day after bacteria infection, even the Sangkuriang catfish juvenile had not shown the clinical symptoms yet, but the swimming pattern was slower compared to the first day, the mucus was increased, and the response toward feed was decreased. The condition of catfish juvenile was weakened in the third day, the swimming pattern was slower, the fish swam close to the water surface, and the appetite was very low.

The fourth day after infection (Figure 1), the experimental fish started to experience some clinical symptoms, they were hemorrhage in caudal and dorsal (Figure 1a), and a damage in fins (Figure 1b). Hemorrhage is an escape of blood from a ruptured blood vessel, especially when profuse. The bacteria that entering the fish



(a) Hemorrhage (b) The damage of fins

Figure 1. The clinical symptoms of Sangkuriang catfish in the fourth day after infection with *Aeromonas hydrophila*



(a) Dropsy (b) Damage in operculum (c) Intestinal secretion spilled out

Figure 2. Clinical symptoms after infection at fifth day with *Aeromonas hydrophila*

body was activated the immune response through producing the polymorphonuclear leukocytes (melanophages, monocytes, and neutrophil) as phagocytic cells. Leukocytes triggered the bacteria to issue a hemolysin toxin that causing ulcer and hemorrhage in the surface of the fish body (Mangunwardoyo *et al.,* 2010).

The observation on the fifth day after *A. hydrophila* infection (Figure 2), the clinical symptoms in Sangkuriang catfish juvenile was getting worse, it experienced dropsy (Figure 2a), a damage in operculum (Figure 2b), and intestinal secretion spilled out (Figure 2c). Mangunwardoyo *et al.* (2010) stated that *A. hyrophila* infection in fish causes hemorrhagic septicemia and ulcer in membranes. Mulia (2012) stated that clinical symptoms in fish infected with *A. hydrophila* are swelling of the kidney, red spot on muscle and flesh, and dropsy in the intestines.

Clinical symptoms in Sangkuriang catfish juvenile was getting worse at the fifth day of infection, therefore, the Sangkuriang catfish juvenile treated with pandanus leaf extract through immersion for 24 hours according to the treatments. Haryani *et al*. (2012) stated that treating the infected juvenile fish is easier through immersion in a large scale.

The recovery time of Sangkuriang catfish juvenile after administrating pandanus leaf extract

According to the observation results on recovery time of infected Sangkuriang catfish juvenile after treatment showed that the recovery and clinical symptoms were different on each treatment.

In control (A) treatment on first day to 14th day, the catfish juvenile did not experience any recovery, instead, the mortality rate reached 86.66%. This was because the catfish juvenile had not treated yet, therefore the *A. hydrophila* infection would spread out and ruin the fish body. In treatment B (400 mg/L), the recovery process was happening at the seventh day, whereas in the other treatment, the recovery started on the fifth day. The recovery process in treatment B (400 mg/L) clearly marked with no damage in intestines, remained hemorrhage, and dropsy was worst than other treatments. The recovery time in treatment B was in 13th day to 14th day and the mortality rate was 11.67% during 14 days of rearing.

The recovery process in treatment C (600 mg/L) and treatment D (800 mg/L) nearly looked the same at fifth to the ninth day of rearing. The experimental fish did not experience a damage in intestines. On the 10th day, the experimental fish started to recover, it only experienced a damage in fins and hemorrhage in 11th day, then it fully recovered at 12th day. Tannin compound can accelerate the wound recovery through the cellular mechanism, it will cleanse the free radical and reactive oxygen, increase the formation of capillary blood vessels and fibroblast (Sheikh *et al*., 2011).

In treatment D (800 mg/L) at 10th day to 11th day, the clinical symptoms in experimental fish were only damage in fins because of the

Table 3. The recovery time of Sangkuriang catfish juvenile after soaking with pandanus leaf extract

Observation day	The pandanus leaf extract concentration (mg/L)				
	A (control)	B (400)	C (600)	D (800)	E (1000)
1	DGHU	DGHU	DGHU	DGHU	DGHU
2	DGHU	DGHU	DGHU	DGHU	DGHU
3	DGHU	DGHU	DGHU	DGHU	DGHU
4	DGHU	DGHU	DGHU	DGHU	DGHU
5	DGHU	DGHU	DGH	DGH	DGH
6	DGHU	DGHU	DGH	DGH	DGH
7	DGHU	DGH	DGH	DGH	DGH
8	DGHU	DGH	DGH	DGH	DGH
9	DGHU	DGH	DGH	DGH	DGH
10	DGHU	GH	GH	G	GH
11	DGHU	GH	G	G	G
12	DGHU	G	S	S	S
13	DGHU	S	S	S	S
14	DGHU	S	S	S	S

Notes : (D) : *Dropsy* (U) : Intestinal secretion spilled out
(G) : A damage fin (S) : Recovered
(H) : Hemorrhage

active compound of pandanus leaf extract at a concentration of 800 mg/L can cure the infected fish with 5% of low mortality. In treatment E (1000 mg/L), recovery process in first to 14th day was nearly look the same with treatment C (600 mg/L).

Although in the treatment E (1000 mg/L), the Sangkuriang catfish juvenile experienced a recovery, the dosage of pandanus leaf extract was too high, thereby the mortality rate became 10% higher and the recovery time was longer compared to treatment D (800 mg/L). Some of the active compound in pandanus leaf extract will be toxic if the concentration is too high, especially for saponin. Saponin is completely cytotoxic. This was in line with Septriarusli *et al*. (2012), saponin is a toxin that will ruin the blood cell (hemolysis).

The observation during 14 days after the immersion showed a recovery process in Sangkuriang catfish juvenile. The recovery process showed that some compounds in pandanus leaf extract can inhibit the *A. hydrophila* growth. The active compounds that induce the recovery process are flavonoid, tannin, quinone, and saponin. Kurniawan and Wayan (2015) stated that flavonoid works as an anti-inflammatory compound that cause a damage in membrane permeability of bacteria. The anti-inflammatory mechanism occurs through inhibitory effect in arachidonic acid metabolism, prostaglandin formation, and the release of histamine. The other benefit of flavonoid is protecting the body cells. Flavonoid or carbolic acid contains phenol compound, known as kind of alcohol. Phenol can denature the protein and ruin the bacteria cell's membrane.

Quinone compound can inhibit the bacteria growth through irreversible complex compound formation with nucleophilic amino acid residue in the transmembrane protein of plasma membrane, membrane cell polypeptide, and enzymes in the surface of cell membrane thereby it ruins the bacteria (Sapara *et al*., 2016). As an anti-bacterial compound, saponin interacts with cholesterol of cell membrane, therefore, the cell membrane can experience lipid modification to ruin the ability of bacteria to interact with the modified membrane (Karlina *et al*., 2013). The interrupted interaction between the bacteria and its membrane will ruin the ability of bacteria to destruct the host. While the membrane cell is interrupted, the anti-bacterial compound can easily enter into the cell and ruin the metabolism to kill the bacteria.

The response toward the feed

The observation on response toward feed was done for 14 days through observation the catfish juvenile reaction by the time of feeding. According to the first to second day of observation after infection, in all treatments, the Sangkuriang catfish juvenile did not show any response to the feed. Austin and Austin (1993) stated that the

bacteria that enters into the organ, especially digest organ, through the body fluid and blood circulation can cause indigestion in infected fish. The observation results on Sangkuriang catfish juvenile response toward feed after the immersion showed in Table 4 below.

According to Table 4, the Sangkuriang catfish juvenile in the treatment A (control), until the sixth day of rearing, had no response on given feed shown by the excess of feed at the bottom of the aquarium. On the seventh day, the survived fish tried to maintain their life by giving weak response toward the feed. It was suspected that in treatment A, the bacteria was infected the digest organ through the blood circulation, therefore the fish might digest the feed slower.

In treatment B (400 mg/L), from the third day to the eighth day, there was a weak response toward feed and from ninth day to 14th day, the fish response toward feed was started to normal. The Sangkuriang catfish juvenile in treatment C (600 mg/L) was on a recovery from the fifth day to the seventh day, but there was still an ulcer thereby the fish response toward feed was weak.

The fish response toward feed in treatment D (800 mg/L) from the second day to the fourth day showed a weak response, nonetheless, from the fifth day to 14th, the fish response toward feed started to normal. It was because from the recovery process, the treatment D experienced faster recovery than others. Whereas the fish response toward feed in treatment E (1000 mg/L) from the first day to the second day had not shown the response yet because during the fish was soaked in pandanus leaf extract, it got stress, showed by the fish that swam near the water surface and had a passive movement. The fish started to show a weak response toward feed in the third to fifth day, while at sixth to the 14th day, the response started to normal (no excess feed was left) .

The response toward shock

The response test toward shock in experimental fish was done by plumping the aquarium for every treatment. At the first to the fifth day, the experimental fish was showed a weak response toward shock because the fish had been infected by *A. hydrohila*, thereby the response toward shock was weak. Table 5 below showed the observation result on the response toward shock.

In the treatment A (control), at seventh day from the first day of replication, the response of Sangkuriang catfish juvenile was normal,

Table 4. The Sangkuriang catfish juvenile response toward feed after the immersion of pandanus leaf extract

Observation day	The concentration of pandanus leaf extract (mg/L)														
	A (control)			B (400)			C (600)			D (800)			E (1.000)		
	Replication														
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
4	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
5	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
6	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
7	+	-	+	+	+	+	+	++	++	++	++	++	+	+	++
8	+	-	+	+	+	+	++	++	++	++	++	++	+	++	++
9	+	+	+	+	++	++	++	++	++	++	++	++	++	++	++
10	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++
11	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++
12	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++
13	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++
14	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++

Notes: (-) No response on feed
(+) Weak response on feed
(++) Normal response on feed (no excess feed)

Table 5. The observation result on the response toward shock after the immersion of pandanus leaf extract

Observation day	The concentration of pandanus leaf extract (mg/L)														
	A (control)			B (400)			C (600)			D (800)			E (1.000)		
	Replications														
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
5	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
6	-	-	-	+	+	+	++	++	++	++	++	++	++	++	++
7	-	-	-	+	+	+	++	++	++	++	++	++	++	++	++
8	+	-	-	+	+	+	++	++	++	++	++	++	++	++	++
9	+	-	-	++	++	++	++	++	++	++	++	++	++	++	++
10	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++
11	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++
12	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++
13	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++
14	+	+	+	++	++	++	++	++	++	++	++	++	++	++	++

Notes: (-) No response on shock
(+) Weak response on shock
(++) Normal response on shock

meanwhile at the second to third day of replication, the experimental fish showed an abnormal response, however at 10th day, the fish started to show a response on the shock. In treatment B (400 mg/L), the experimental fish started giving a response on shock at the fifth to the eighth day, yet at ninth to the 14th day, the experimental fish started to normal on given response on the shock.

In the treatment C (600 mg/L), D (800 mg/L), and E (1000 mg/L), a sixth day of treatment, the experimental fish started giving a normal response on the shock. This was because the fish started to recovery after administering the pandanus leaf extract contained an anti-bacterial compound, thereby the anti-bacterial compound could inhibit the *A. hydrophila* growth. The wound in fish body started to recover because the saponin compound in pandanus leaf extract. The saponin accelerated the formation of collagen, a protein structure to recover the wound (Astuti *et al*., 2011). Saponin is triterpene glycosides and sterol complex that has been detected in more than 90 of plants genus.

The survival rate of Sangkuriang catfish juvenile

According to the observation result, the infected Sangkuriang catfish juvenile that has been given a pandanus leaf extract through immersion method in different concentration for 24 hours showed the different survival rate in each treatment (Figure 3).

Figure 3 showed that Sangkuriang catfish juvenile that has been soaked in pandanus leaf extract provided a higher survival rate compared to treatment A (no soaking treatment). This was showed that the active compound in pandanus leaf extract is potential to inhibit *A. hydrophila* growth that could infect the Sangkuriang catfish juvenile.

According to the observation result of the survival rate, as the increase of the concentration of pandanus leaf extract up to 800 mg/L, the survival rate of Sangkuriang catfish juvenile increased. Otherwise, in the concentration of 1000 mg/L, the survival rate of Sangkuriang catfish juvenile was decreased. The survival rate of treatment B (400 mg/L) and E (1000 mg/L) were 65% and 68.33%, respectively. This was because in 400 mg/L of pandanus leaf extract have not been able to inhibit *A.hydrophila* growth, therefore the survival rate in this treatment was still low. Meanwhile, in treatment E with a concentration of 1000 mg/L (the highest concentration) could poison the fish. The anti-bacterial compound in high concentration that can poison the fish is tannin. Tannin works in iron compound (Fe) reduction, moreover, tannin can bind with others protein and

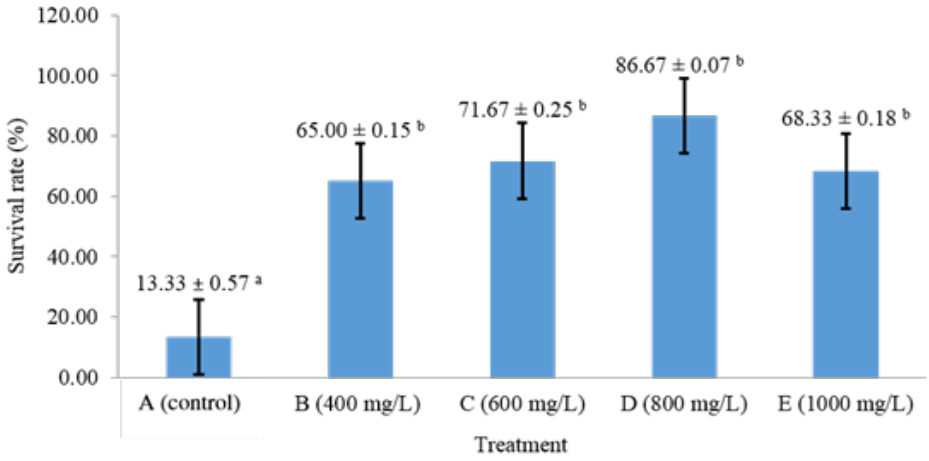


Figure 3. The survival rate of Sangkuriang catfish juvenile. The value followed by different superscript indicates significantly different (P<0.05), the fish has infected with *A. hydrophila* bacteria as much as 20 mL of NaCl/L in 10 L of water medium with a density of 108 CFU/mL through immersion, the treatments were A (control), B (400 mg/L), C (600 mg/L), D (800 mg/L), and E (1000 mg/L) trough immersion for 24 hours through immersion for 24 hours.

mineral, thereby the protein and mineral cannot be used by the body (Fajrina *et al*., 2017).

According to the Duncan test, the administration of pandanus leaf extract generated significantly different effects on Sangkuriang catfish juvenile that has been infected by *A. hydrophila* for 14 days of recovery time. The result of regression analysis showed that among the pandanus leaf extract concentration and the survival rate of infected Sangkuriang catfish juvenile showed quadratic equation ($Y = -0.0001x^2+0.1786x+12.806$). In addition, there was the coefficient of determination showed (R^2) = 0.8494, therefore the correlation coefficient was 0.9216 that indicated the use of pandanus leaf extract was provided 92.16% toward the recovery of infected Sangkuriang catfish juvenile.

Water quality

The observation on water quality was done to obtain the parameters in the aquarium. The water quality was very influential toward the recovery process of Sangkuriang catfish juvenile during this study. The observation result of water quality was shown in Table 6 with the optimal water quality according to Standar Nasional Indonesia (SNI, 2000) as a comparison.

The average temperature of each treatment was 25.0–26.3°C, the pH was 6.9–7.2, and the DO was 4.0–4.5 mg/L (Table 6). According to SNI (2000), the water quality condition during the study has fulfilled the optimum standard level for rearing the Sangkuriang catfish juvenile. Thus, the water quality analysis showed that the difference. Based on these data shows that the difference in survival of fish is due to treatment not because os water quality.

CONCLUSION

According to the study result, the pandanus leaf extract in a concentration of 800 mg/L is effective for Sangkuriang catfish juvenile treatment that has been infected by *Aeromonas hydrophila*, it provided the highest survival rate up to 86.67%.

Table 6. The range of water quality during the study

Treatments (mg/L)	Water quality parameters		
	Temperature (°C)	pH	DO (mg/L)
A (control)	25.31–26.00	7.00–7.02	4.00–4.26
B (400)	26.00–26.33	7.06–7.16	4.00–4.33
C (600)	26.00–26.42	7.00–7.24	4.17–4.42
D (800)	26.00–26.31	6.92–7.03	4.36–4.53
E (1.000)	25.14–26.00	6.95–7.06	4.00–4.31
The optimal level according to SNI	25–30	6.5–8.5	≥ 4

REFERENCES

Austin B, Austin DA. 1993. Bacterial Fish Pathogens. Desease in Farmed and Wild Fish. Chichester. England : Ellis Horwood Ltd, Publisher.

Astuti SM, Sakinah M, Andayani R, Risch A. 2011. Determination of saponin compound from *Anredera cordifolia* (Ten) steenis plant (Binahong) to potential treatment for several desease. Journal of Agricultural Science 3: 224–232.

Fajrina A, Junuarty J, Stevani S. 2017. Determination of tannin content in tea bags the marketplace by UV–VIS spectrophotometry. Jurnal Sains dan teknologi Farmasi 19: 17−19.

Haryani A, Roffi G, Ibnu DB, Ayi S. 2012. Effectiveness of papaya leaf *Caricca papaya* for treatment of *Aeromonas hydrophila* infection in goldfish *Carassius auratus*. Journal Fisheries and Marine 3: 213–220.

Hendra R, Syahid A, Aspollah S, Muhammad YS, Ehsan O. 2011. Flavonoid analyses and antimicrobial activity of various parts of *Phaleria macrocarpa* (Scheff.) boerl fruit. International Journal of Molecular Science 12: 3422–3431.

Karlina CY, Ibrahim M, Trimulyono G. 2013. Antibacterial activity of krokot herb extract *Portulaca oleracea* L. toward *Staphylococcus aureus* and *Escherichia coli*. Lentera Bio 2: 87–93.

[KKP]. Marine and Fisheries Ministry. 2016. Performance report of directorate general of aquaculture. https://www.djpb.kkp.go.id/ [June 23, 2017].

Kurniawan B, Wayan FA. 2015. Binahong *Cassia alata* L as inhibitor of *Escherichia coli* growth. Majotiry Journal 4: 100–104.

Lukistyowati I, Kurniasih. 2012. Detection aerolysin gen from *Aeromonas hydrophila* in common carp fed with garlic extract. Jurnal Veteriner 13: 43–50.

Mangunwardoyo W, Ratih I, Etty R. 2010. Pathogencity and virulence test *Aeromonas hydrophila* stainer on tilapia *Oreochromis niloticus* Lin. through Koch postulates. Jurnal Riset Akuakultur 5: 245–255.

Mulia DS. 2012. Use of *Aeromonas hydrophila* cell debris vaccine with different booster time interval on immune response of dumbo catfish *Clarias gariepinus* Burchell. Sains Aquatic 10: 86–95.

Nuria MC, Arvin F, Sumantri. 2009. Test of antibacterial activity *Jatropha cuircas* L ethanol extract against *Staphylococcus aureus* ATCC 25923, *Escheria coli* ATCC 25922 and *Salmonella typhi* ATCC 1408. Jurnal Ilmu Pertanian 5: 26–37.

Pratama RC, Rosidah, Sriati, Ike R. 2017. Effectiveness of rambutan seed extract for treating carp fingerling that infected by *Aeromonas hydrophila* bacteria. Journal Fisheries and Marine 8: 130–136.

Sapara TU, Olivia W, Juliatri. 2016. Antibacterial effectiveness of garden balsam leaves extract in inhibiting *Porphyromonas*. Journal of Pharmaceutical Science Unsrat 5: 10–17.

Septriarusli IE, Kiki H, Yenny M, Danar. 2012. Potential secondary metabolite compounds from seed extract of keben fruit *Barringtonia asiatica* in anesthesia process of tiger grouper *Ephinephelus fuscoguttatus*. Journal Fisheries and Marine 3: 295−299.

Sheikh AA, Sayyed Z, Siddiqui AR, Pratapwar AS, Sheakh SS. 2011. Wound healing activity of *Sesbania grandiflora* Linn flower ethanolic extract using excision and incision woun model in wistar rats. International Journal of PharmTech Research 3: 895–898.

Siregar AF, Agus S, Delianis P. 2012. Antibacterial potential of seaweed extract againts skin disease bacteria *Psedomonas aeruginosa, Staphylococcus epidermis,* and *Micrococcus luteus*. Journal of Marine Research 1: 152–160.

[SNI]. Indonesian National Standard. 2000. Production of dumbo catfish seeds (*Clarias gariepinus* x *C. fuscus*) class of seeds. http://sisni.bsn.go.id/?/sni_main/sni/index_sniptspt/1285 [June 23, 2017].

Sunarma A. 2004. Increasing the productivity of the business of catfish Sangkuriang *Clarias* sp. Sukabumi: Sukabumi Freshwater Aquaculture.

Susanto, Sudrajat D, Ruga R. 2012. The study of active ingredients of red meranti plant *Shorea leprosula* Miq as a source of antibacterial compounds. Mulawarman Scientific 11: 181–190.

Wahjuningrum D, Retno A, Mia S. 2013. Prevention of *Aeomonas hydrophila* infection on 11−day−old catfish *Clarias* sp. juvenile using garlic *Allium sativum* and meniran *Phyllanthus niruri*. Indonesian Aquaculture Journal 12: 94–104.

White MR. 1989. Diagnosis and treatment of *Aeromonas hydrophila* infection of fish. Animal Desease Diagnotic Laboratory. Indiana, USA: Purdue University.