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## **Effectivity of Solution Mangosteen Rind (*Garciana mangostana*) as Medicine for Black Tilapia Juvenile (*Oreochromis niloticus* Bleeker) when Infected by *Aeromonas hydrophila***

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### **ABSTRACT**

Many tropical plants have interesting biological activities with potential therapeutic applications. *Garcinia mangostana* Linn. (GML) belongs to the family of Guttiferae and is named “the queen of fruits”. It is cultivated in the tropical rainforest of some Southeast Asian nations like Indonesia, Malaysia, Vietnam, Philippines, and Thailand. This research aims to determine the concentration of solution mangosteen rind (*Garciana mangostana*) which is the most effective to healing of black tilapia juvenile (*Oreochromis niloticus* Bleeker) when infected by *A. hydrophila* bacteria to produce the highest survival of black tilapia juvenile. This research used experimental method and experimental design by including a complete randomized design (CRD) with five treatments and three replications. The used treatment was soaking black tilapia juvenile for 24 hours in mangosteen rind. The sample infected by *A. hydrophila* bacteria as much as 0.1 mL/fish at concentration of  $10^8$  cfu/mL with intramuscular injection method was used. The concentrations of mangosteen rind solution were 0, 500, 600, 700, and 800 ppm. The parameters observed were clinical symptoms, fish response to feed, fish response to shock, recovery process, and water quality. Data of the clinical symptoms, response fish to feed, response fish to shock, recovery process and water quality were analysed descriptively, while the survival rate data of black tilapia juvenile were analysed using F test at 95% confidence level and continued with Duncan test. The results showed, the use of mangosteen rind solution with 600 ppm concentration was effective to treat tilapia juvenile which infected by *A. hydrophila* bacteria resulted in the fastest recovery on the third day and the highest survival rate of 91.76.

**Keywords:** Solution mangosteen rind, *Aeromonas hydrophila*, black tilapia, medicine, *Garciana mangostana*, *Oreochromis niloticus*

## 1. INTRODUCTION

Black tilapia (*Oreochromis niloticus* Bleeker) is one type of freshwater consumption fish that has high economic value and a potential market prospect because of its superiority, which has a specific taste, solid meat, not many thorns, and easy to get. In 2011 Indonesia was ranked as the third largest producer of tilapia by providing production of around 20.3% of the total world tilapia production of 3,197,330 tons (DKP 2013) (Fig. 1) [1-8].



**Figure 1.** Black Tilapia Juvenile (*Oreochromis niloticus* Bleeker)



**Figure 2.** Mangosteen (*Garciana mangostana* L.)

One of the diseases causing bacteria that often attacks black tilapia juveniles is *A. hydrophila*. This disease often attacks black tilapia in the juvenile stage where in this juvenile stage this fish has a weaker immunity than the adult stage. One alternative to the prevention of this disease is to use medicinal plants containing antimicrobials as a substitute for synthetic antibiotics. Indonesia has a lot of diversity of plants that contain antimicrobial compounds in them. Some of these plants contain antimicrobial compounds that are antimicrobial (bacterial killers), and bactericidal (inhibitors of bacterial growth). One of the plants that is antimicrobial is the mangosteen (Fig. 2) [9-16].

Mangosteen rind is one of the natural ingredients that has the potential to act against *A. hydrophila* bacterial infections. It is known that the active compounds of the mangosteen rind can inhibit the growth of *A. hydrophilla* bacteria. Based on the research it is known that mangosteen rind contains alkaloid compounds, flavonoids, quinones and terpenoids. Up-to date studies show, the mangosteen rind after investigations turned out to contain several compounds with pharmacological activities such as hypo-allergenic, anti-inflammatory, antioxidant, anticancer, antimicrobial, and anti-atherosclerosis.

The aim of this study was to determine concentration of the solution of mangosteen rind (*Garciana mangostana*) which is the most effective for the healing of black tilapia juvenile (*Oreochromis niloticus* Bleeker) when infected by *A. hydrophila* [17-23].

## 2. MATERIALS AND METHODS

The materials used in this study include tilapia juveniles black (*Oreochromis niloticus* Bleeker) originating from BBI Cibiru as many as 360 tails with a size of 7-9 cm. Mangosteen rind flour as an herbal ingredient for the medicine of fish juveniles infected by *Aeromonas hydrophila* comes from the Herbal Babah Kuya Store.

Pasar Baru Bandung, bacterial isolates were *Aeromonas hydrophila* obtained from BBPBAT Sukabumi, commercial fish feed, nutrient broth for making a solution of bacteria *A. hydrophila*, 70% alcohol and sterile distilled water for tool sterilization and dilution, and nutrient agar as bacterial culture media of *A. hydrophila*.

This study used an experimental method with Completely Randomized Design (CRD) consisting of 5 (five) treatments and 3 (three) replications. Each treatment uses 20 black tilapia juveniles. The treatment given was soaking the juveniles of black tilapia infected by *A. hydrophila* in a solution of mangosteen rind for 24 hours with a concentration of 0 ppm, 500 ppm, 600 ppm, 700 ppm, and 800 ppm.

The concentration used is based on the results of preliminary research that has been done. The LC<sub>50</sub> 24-hour analysis using software EPA Probit Analysis was applied. It appears that LC<sub>50</sub> 24-hour of 1000 ppm indicates that at a that concentration of the solution of mangosteen rind can result in 50% black tilapia juvenile mortality within 24 hours.

From the *in vitro* test it is known that the concentration of 800 ppm has the largest average inhibitory zone, but high concentrations will have an impact on the survival of the fish. Therefore the use of a concentration of 600 ppm is thought to be effective because it approaches a concentration of 700 ppm with an average inhibition zone almost the same as at 800 ppm. Thus with a concentration of 600 ppm away from the LC<sub>50</sub> value it is expected to reduce the mortality of the fish tested.

## 2. 1. Culture Preparation

Aquaria, hoses, and aeration stones are cleaned with chlorine, then washed again with fresh water, with installation of aeration equipment in each aquarium. There was 15 L of water in each aquarium, treatment was randomly used and marked according to its layout.

## 2. 2. Research Implementation

This research was conducted for 50 days. Preparation of tools and materials was carried out for 3 days, then acclimatization of the test fish was done for 7 days. Next, followed by a preliminary test that was to make the mangosteen rind solution, *in vitro* test and LC<sub>50</sub> for 10 days were done. Then *Aeromonas hydrophila* bacterial culture and infecting fish test was continued with packing clinical symptoms for 30 days.

The first research procedure carried out was a preliminary study which included making mangosteen rind solution, *in vitro* test and 24-hour LC<sub>50</sub> test, and then the main research was carried out which included observing clinical symptoms in response to feed, response to shock, body damage, and water quality. Preliminary research was conducted on 5-22 November 2018, including LC<sub>50</sub> test conducted at Building 4 Aquaculture Laboratory, making solution of mangosteen rind carried out at the Building 4 Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University, *in vitro* tests conducted at the Biotechnology Laboratory Padjadjaran University. The main research was conducted in December 2018 until January 2019 at the 4th Laboratory of Aquaculture, Faculty of Fisheries and Marine Sciences, Padjadjaran University. The *in vitro* test aims to determine the bacterial inhibition caused by immersion using mangosteen rind while the 24-hour LC<sub>50</sub> was done to determine the toxicity level of mangosteen rind solution against black tilapia seeds. Feeding was done twice a day in the morning and evening. Checking water quality was done twice, namely during the first week of observation and the second week of observation.

## 2. 3. Data Analysis

Analysis of the data used, namely analysis of variance (ANOVA) with F test at 95% confidence interval, was used to determine whether the treatment had an effect on healing black tilapia juvenile who were infected by *A. hydrophila* and resulted in the highest survival rate. If the treatment has a significant effect, then further testing is done with Duncan's multiple range test, and the results of the data obtained from the studies are to be compared with the data in the catfishes case study to find out whether the results have a significant effect or not.

## 3. RESULT AND DISCUSSION

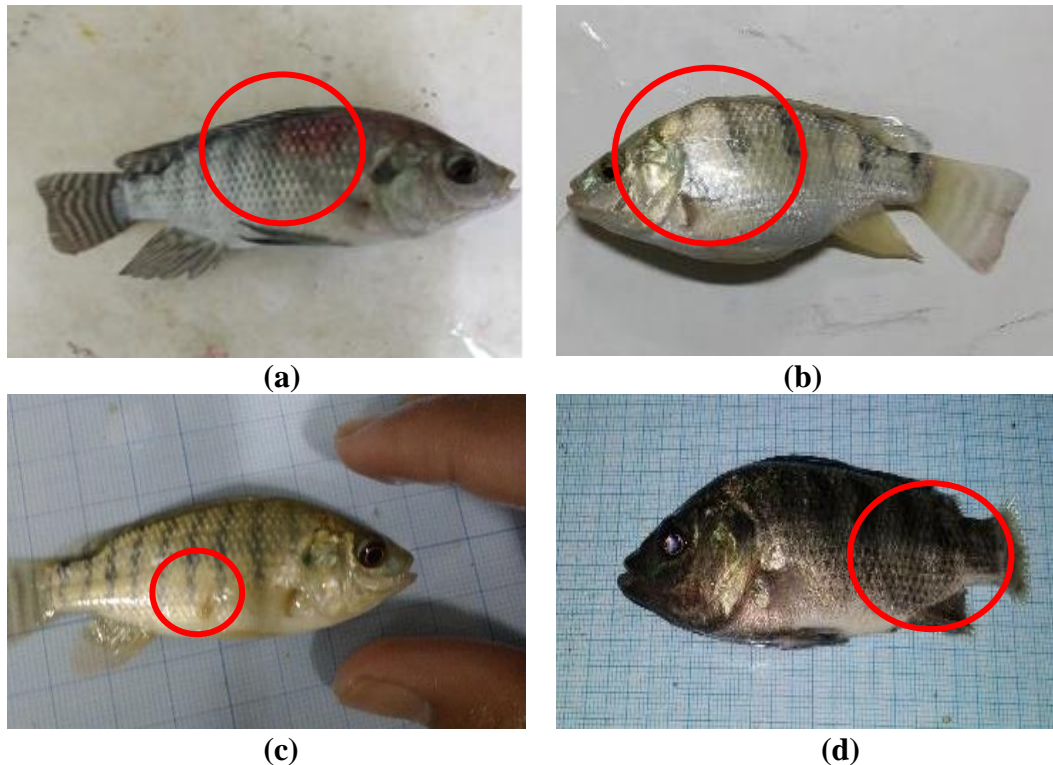
### 3. 1. Clinical Symptoms of Black Tilapia Juvenile as Infected by *Aeromonas hydrophilla*

Juveniles of black tilapia infected with *A. hydrophila* bacteria are characterized by the appearance of clinical symptoms in the form of damage to the surface of the fish body, response to feed and response to shock. After infection with bacteria *A. hydrophila* less than 50% of tilapia juveniles began to show clinical symptoms of being attacked by these bacteria.

Clinical symptoms usually seen are damage to the surface of the body of the fish in the form of red spots (hemorrhagic) (Figure 3a), the color of the fish's body becomes dark, the stomach bulges (dropsy) (Figure 3b). Samples of fish attacked by bacteria *A. hydrophila* have

clinical symptoms of dark body color, bleeding occurs on the skin to wounds and ulcers, flatulence (dropsy), damaged or protruding eyes, flaky fins and slow moving.

Other clinical symptoms are bleeding in the liver, kidneys and spleen during surgery. Fish that experience dropsy are caused by the release of Aerolysin Cytotoxic Enterotoxyn from *A. hydrophila* which causes damage to fish tissue.



**Figure 3.** Clinical symptoms Black Tilapia Juvenile Infected *Aeromonas hydrophila*  
(a) hemorrhagic (b) dropsy (c) wound (ulcer) (d) flaked fins

Within 72 hours after infection with *A. hydrophila* the sample showed clinical symptoms of bacterial infection evenly. Then the test fish was immediately performed with mangosteen rind solution through 24-hour immersion with extract concentrations according to the treatment, namely A (0 ppm /control group), B (500 ppm), C (600 ppm), D (700 ppm), and E (800 ppm). Clinical symptoms observed after immersion of fish in mangosteen rind solution include damage to the body surface, response of fish to feed, response of fish to shock, and aquarium water quality in each treatment.

### 3. 2. Recovery of Body Surface Damage

After test fish infected with *A. hydrophila* show clinical symptoms, the curative treatments were carried out by soaking mangosteen rind in solution for 24 hours, then maintenance and observation carried out for 14 days to see whether there was a recovery that occurred from clinical symptoms that appeared after treatment. The studies were carried out to

make observations of hemorrhages (H), flaky fins (F), inflammatory (I), ulcers (U), and dropsy (D) (see Table 1).

**Table 1.** Clinical Symptoms of Post-Treatment Observation with Mangosteen Rind Solution

Observations to-Day	Mangosteen Rind Solution Concentration (ppm)				
	A (0)	B (500)	C (600)	D (700)	E (800)
1	BIFUD	BIFUD	BIFUD	BIFUD	BIFUD
2	BIFUD	BIFUD	BIFUD	BIFUD	BIFUD
3	BIFUD	BIFUD	BIFU	BIFUD	BIFUD
4	BIUD	BIFUD	BIFU	BIFU	BIFU
5	BIUD	BIFU	BIFU	BIFU	BIFU
6	BIUD	BIFU	BIFU	BIFU	BIFU
7	BIUD	BIFU	BF	FIU	BF
8	BIUD	BFU	BF	FU	BF
9	BIUD	BU	F	FU	BF
10	BIUD	BU	+	FU	F
11	BIUD	B	+	F	F
12	BIU	+	+	F	+
13	BI	+	+	+	+
14	BI	+	+	+	+

Description:

- H = hemorrhagic
- F = flaked fins
- I = inflammatory
- U = ulcer
- D = dropsy
- + = healed

At Treatment A (0 ppm) there was no healing observed and the death of black tilapia increased every day. The remaining fish that survived until day 14 were fish that still showed red spots. This is because in treatment A (0 ppm) no change was seen by soaking the mangosteen rind in the solution so that the bacteria in the fish body developed and damaged the

body parts of the fish. The process of invasion of pathogenic bacteria *A. hydrophila* into the body of the fish is preceded by the attachment of bacteria to the surface of the skin using pili, flagella, and hooks to move and attach tightly to the outermost layer of the fish's body, with the scales protected by chitin substances. During the process, *A. hydrophila* bacteria produce chitinase enzymes which play a role in degrading chitin layers so that bacteria can easily enter the fish's body. In addition to using bacterial chitinase, *A. hydrophila* also secretes other enzymes such as lecithinase in an effort to enter the blood vessels of the fish.

In treatment B (500 ppm) healing began on the 5<sup>th</sup> day with loss of dropsy. On the 11<sup>th</sup> day, the clinical symptoms that were still visible, were red spots. It is suspected that treatment B (500 ppm) of compounds contained in the solution of mangosteen rind began to react in inhibiting bacterial growth, but it was not optimal because the concentration was still low.

In treatment D (700 ppm), black tilapia juveniles began to heal on the 4<sup>th</sup> day seen with loss of dropsy. Test fish experienced full recovery on the 13<sup>th</sup> day.

At treatment E (800 ppm), the black tilapia began to heal on day 4 with a loss of dropsy. Test fish in treatment E experienced complete healing on day 12, but the number of dead black tilapia juveniles was relatively large. This is presumably because the mangosteen rind solution provided was too high, even though it could inhibit bacterial growth but the antibacterial compounds contained in the solution of mangosteen rind produced toxics for black tilapia juveniles.

One of the compounds contained in the solution of mangosteen rind is saponin. Saponins enter the body through an osmoregulation process, then enter the bloodstream which results in damage to the gill tissue which is characterized by the gills of the fish becoming red and swollen. Swollen gills interfere with the respiratory process which results in reduced oxygen supply to the body.

In the treatment C (600 ppm), the test fish experienced the fastest healing process that occurred on day 3 where the distended stomach (dropsy) began to disappear and return to normal shape. On the 7<sup>th</sup> day, the ulcer began to disappear. The test fish in treatment C experienced the fastest healing and the solution of the mangosteen rind given did not cause toxicity to the black tilapia. This could be seen from the number of dead black tilapia juveniles compared to the number after other treatments and produced the highest survival rate.

It is suspected that the compounds contained in these concentrations were able to inhibit the growth of *A. hydrophila* bacteria and did not cause toxicity to black tilapia juveniles at 24-hour immersion. Injected scars (inflammation) on the surface of the body that appeared, began to close, and some fish began to experience healing (Figure 4).

Observations carried out for 14 days after treatment with soaking mangosteen rind solution presented healing in fish morphology. The immersion system can facilitate the treatment process especially for small-scale fish on a large scale. Healing of black tilapia showed that the content of antibacterial compounds in the solution of mangosteen fruit skin was able to inhibit the growth of *A. hydrophila* bacteria. This is in accordance with the opinion [9-16] which stated that mangosteen rind contains compounds such as alkaloids, tanins, phenolics, flavonoids, and triterpenoids. The compound proved to be able to inhibit the growth of *Staphylococcus aureus* and *Staphylococcus epidermis*, and with a concentration of 50% can produce an inhibition zone of 11 mm, mangosteen rind also contains active compounds belonging to the xanthones. Xanthone compounds that have been identified include 1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methyl-2-butenyl)-9H-xanten-9-on and 1,3,6,7-tetrahydroxy-2,8-bis(3-methyl-2-butenyl)-9Hxanten-9-on. Both are better known as alpha-mangosteen and

gamma-mangosteen which have antibacterial properties. The alkaloid compounds, tanins, phenolics, flavonoids, and triterpenoids are known to have antibacterial properties. Saponins are active substances that can increase membrane permeability resulting in cell hemolysis. Flavonoids are a group of phenol compounds that have a tendency to bind to proteins, thus disrupting bacterial metabolic processes. The tannin contained in the mangosteen rind works as an antimicrobial by coagulating or agglomerating bacterial cell protoplasts. The mechanism of action of antibacterial compounds against microorganisms can be done in several ways, including disturbances in the constituents of cell walls, increased permeability of cell membranes which can cause loss of cell constituent components, activation of enzymes, and destruction or damage to the function of genetic material.



**Figure 4.** Recovery of Inflammation

The mechanism of action of flavonoids is thought to be able to denature bacterial cell proteins, which can inhibit bacterial growth by damaging cell walls, deactivating enzyme action and damaging cell membranes. It should be added that the ability of flavonoids to provide antibacterial effects includes inhibiting the function of cytoplasmic membranes. Inhibiting nucleic acid synthesis, and antibacterial activity by inhibiting energy metabolism, flavonoids inhibit oxygen consumption by disrupting the transport chain of respiration electrons.

Tannin compounds can interfere with cell permeability because of its ability to shrink cell walls or cell membranes. Tannin is able to activate microbial adhesion, enzymes, and protein transport to cell membranes. One may state that some of the enzymes produced by microbes are capable of being inhibited by astringent possessed by tannins. Saponins are included in the antibacterial group which can disrupt the permeability of the microbial cell membrane. It results



in damage which causes various important components to emerge from microbial cells, namely proteins, nucleic acids, nucleotides and others.

### 3. 3. Response to Feed

Indicators, as said to be healthy fish, can be seen by observing the response to feed and response to surprises. Observation of the response to black tilapia juvenile feed was carried out for 14 days by looking at how fast the reaction of black tilapia occurs during feeding. Based on the observations of black tilapia after treatment with soaking in mangosteen rind solution, one my notice a different response to the feed (Table 2).

**Table 2.** Response of Black Tilapia Juvenile to Mangosteen Rind Solution

Concentration (ppm)															
Observation Day	A (Control)			B (500)			C (600)			D (700)			E (800)		
	Repeat														
	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	-	+	+	++	++	++	++	++	++	++	++	++	++	++	++

Description:

- (-) Feed response does not exist
- (+) Low feed response
- (++) Normal feed response

In Table 2 it can be seen that day 1 after treatment with soaking mangosteen rind solution, black tilapia juveniles in all treatments do not provide a response to feed. This can be seen by the presence of feed remaining on the bottom of the aquarium. Black tilapia juvenile in

treatment A (Control) gave a less responsive feed response from day 5. It is thought that the bacteria had infected the digestive tract through the bloodstream, so the fish was slow in digesting the food provided. This is in accordance with the statement which shows that fish attacked by *A. hydrophila* bacteria will experience a decrease in appetite.

On days 2 to 5, black tilapia in all treatments other than treatment A (Control) showed a response to feed but still relatively low. Starting on the 5<sup>th</sup> day of the black tilapia in treatment B (500 ppm) it was seen that the response returned to normal for feed. This is presumably because the concentration of mangosteen rind solution given in treatment B (500 ppm) is not maximum in inhibiting *A. hydrophila* bacteria.

On days 2 to 5, black tilapia in treatment D (700 ppm) and E (800 ppm) showed a response to low feed, but on the 6<sup>th</sup> day the response of black tilapia to feed began to improve. This is presumably because the concentration of the mangosteen rind solution given was too high. The high concentrations cause high levels of active compounds. The saponin content that is too high can disrupt the function of the digestive tract. Saponins are components that are bitter in nature, and can cause functional disorders of the digestive tract as a result of the inhibition of peristaltic muscle activity. Black tilapia juveniles in treatment C (600 ppm) showed a response to normal feed from day 3 and was the response to the fastest feed compared to other treatments. This is presumably because the black tilapia has been cured after treatment by soaking in the solution of the mangosteen rind with the right concentration.

### 3. 4. Reflex Test

Observation of the reflex test on black tilapia juveniles was carried out by tapping the aquarium glass in each treatment for 14 days (Table 3).

**Table 3.** Reflex Test

		Concentration of Mangosteen Rind Solution (ppm)														
Observation Day		A (Control)			B (500)			C (600)			D (700)			E (800)		
		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	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++

Description:

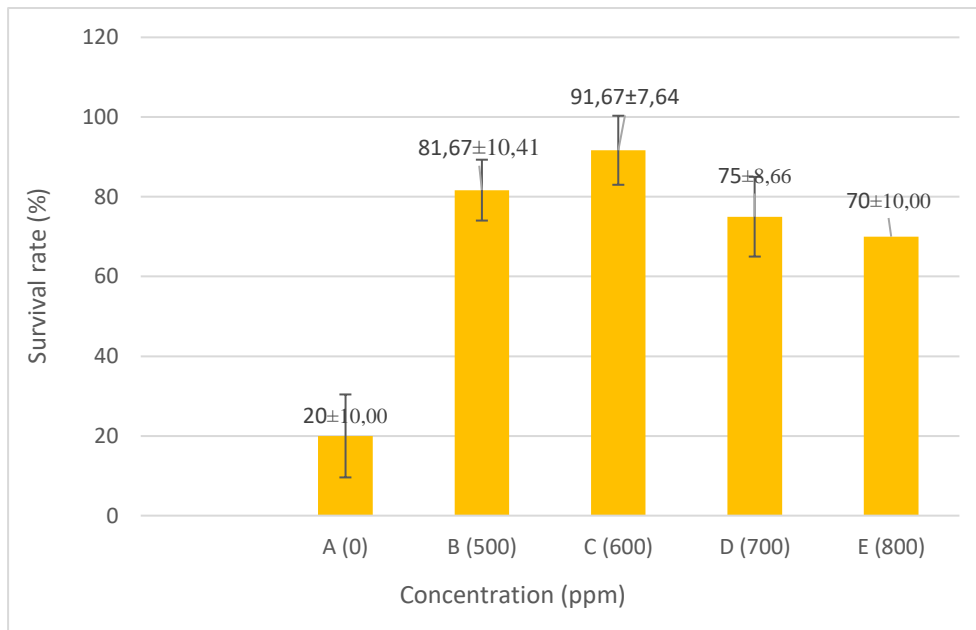
- (-) No response
- (+) Low response
- (++) Normal response

In Table 3, it can be seen that black tilapia juveniles after treatment using mangosteen rind solution on day 1 did not respond to surprises. It is suspected that the black tilapia juveniles experienced stress due to the attack of *A. hydrophila* bacteria and the effect of soaking in the solution of mangosteen rind which has toxic properties to bacteria. Black tilapia juveniles undergo behavioral changes after being infected by *A. hydrophila* bacteria, namely slow movements of fish and irregular position of fish, and always go to surface to look for oxygen.

This is consistent with the statement that sick fish look like slow fish movements, some fish swim close to the surface or around aeration and swim with sloping body position due to reduced body balance and damage to fish fins due to bacterial attack *A. hydrophila*. In treatment A (control), the surviving black tilapia juveniles did not show a normal response to shock; it was assumed that treatment A (control) was not operated with a treatment of mangosteen rind solution so that the black tilapia juveniles did not change their behavior and died. On the 5<sup>th</sup> day, black tilapia in treatment B (500 ppm) began to give a normal response to the surprise. It is suspected that black tilapia juveniles have not experienced stress due to soaking in the solution of the mangosteen rind. In treatments D (700 ppm) and E (800 ppm), they began to respond to surprises normally on day 4. This is presumably because the mangosteen rind solution is too high. High flavonoid content in the body is thought to cause hemolytic anemia, namely the death of red blood cells causes fish to become stressed due to lack of oxygen and can even cause fish death. Of all the concentrations given, treatment C (600 ppm) was the fastest in responding to shocks normally, i.e. on day 3. It is assumed that saponin compounds in the solution of mangosteen rind can inhibit *A. hydrophila* bacteria and stimulate erythrocytes so that the fish quickly recover.

### 3. 5. Survival Rate

Based on observations on black tilapia infected with *A. hydrophila* bacteria after treatment by immersion using mangosteen rind solution for 24 hours, the average survival rate varied for each treatment (Figure 5). Figure 5 (A) shows black tilapia juveniles that were not given treatment by soaking the mangosteen rind solution (control) resulting in the lowest survival rate of 20%. This is because the control treatment was not carried out with soaking mangosteen rind solution (0 ppm) so that the juveniles suffered a lot of deaths. In fact, there was no attempt to inhibit bacterial growth.



**Figure 5.** Graph of Black Tilapia Juvenile Survival Rate

*Aeromonas hydrophila* causes an outbreak of disease with a high mortality rate (80-100%) in a short period of time (1-2 weeks).

Black tilapia juveniles treated with soaking mangosteen rind solution showed a higher survival rate compared to the control treatment. The higher concentration gives a higher survival rate to the limit of 600 ppm. Above 600 ppm the survival rate decreases despite the healing process. This is because in treatment B (500 ppm) the concentration of the solution given has not been able to inhibit the growth of *A. hydrophila* bacteria optimally resulting in a lower survival rate of treatment C (600 ppm). Treatment D (700 ppm) and treatment E (800 ppm) produced a survival rate that was also lower than treatment C (600 ppm) because the solution of the skin of the mangosteen fruit was given too high so that in addition to inhibiting bacterial growth, it could also cause toxic black tilapia juvenile.

Based on the results of variance analysis it was shown that the administration of mangosteen rind solution gave a significantly different effect on the survival of black tilapia infected with the bacterium *A. hydrophila*. The Duncan test results showed that treatment A (control) was different from the cases performed with treatment B (500 ppm), treatment C (600 ppm), treatment D (700 ppm) and treatment E (800 ppm) (Table 4). It could be found that treatment B (500 ppm), treatment C (600 ppm), treatment D (700 ppm), and treatment E (800 ppm) did not give a real difference.

### 3. 6. Water Quality

Water quality is one of the factors that influence the emergence of diseases in fish, because diseases arise from interactions between hosts, pathogens, and the environment. Water quality is measured at the beginning and the end of treatment. Measurement results of water quality is presented in Table 4.

**Table 4.** Observation Results of Water Quality

Treatment (ppm)	Water Quality Parameters		
	(°C)	pH	DO (mg/L)
A (0)	23,1-27,3	7,51-7,7	6,8-7,6
B (500)	23,5-24,1	6,9-7,2	7,8-7,1
C (600)	23,7-27,3	6,8-7,2	7,1-7,9
D (700)	23,9-24,2	7,1-7,5	7,4-8
E (800)	23,5-27,2	7,4-7,6	7,3-8,6
Optimal	23-30 °C	6,5-8,5	> 5 mg/l
	SNI	SNI	SNI

The results revealed that the oxygen content at the beginning of the treatment was in the range of 23.1- 27.3% and it could be stated to be in optimum condition according to SNI (2009), which is 23-30%. The pH measurements during the study showed to be in the range of 6.8-7.6. The range is still in the optimum, according to SNI (2009), and in accordance to the needs of black tilapia juveniles. Dissolved oxygen is a requirement that must be fulfilled in fish maintenance media. Based on Table 5, it is known that the dissolved oxygen range for the maintenance of black tilapia juveniles during the study was in the range of 9.8-12.6 mg /L, so that the condition of the media was able to support the healing process of fish. According to the Indonesian National Standards, dissolved oxygen content of more than >5 mg /L can be categorized as optimum.

Based on the above discussion, the condition of water quality during the study met the optimum standard for black tilapia juvenile media during maintenance.

#### 4. CONCLUSIONS

Based on the results of the studies carried out, it can be concluded that the mangosteen rind solution is effective for the healing of black tilapia juvenile when infected by *Aeromonas hydrophila*. The concentration of 600 ppm resulted in the fastest recovery, namely on the third day the highest survival rate of 91.67% was noted.

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