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The effectiveness of galangal *Alpinia galanga* extract for the treatment of sangkuriang catfish *Clarias gariepinu*s infected *Aeromonas hydrophila*

Efektivitas ekstrak lengkuas *Alpinia galanga* untuk pengobatan ikan lele sangkuriang *Clarias gariepinus* yang diinfeksi *Aeromonas hydrophila*

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ABSTRACT

The purpose of this research is to find out the optimal dose of galangal extract to treatment of Sangkuriang catfish infected with *Aeromonas hydrophila*, the fish fry used are 5-7 cm in size. October-November 2022 was the research was carried out by an experimental method using a completely randomized design (CRD) with four treatments and three replications. The treatment used was immersing with galangal extract for 24 hours with doses of 0 mg/L (A), 250 mg/L (B), 500 mg/L (C) and 750 mg/L (D). Maintenance of test fish for 14 days. A*eromonas hydrophila* bacteria are used to infect fish with a density of 108 CFU/mL by immersing 10 ml in 10 liters of water. Clinical symptoms of infection, clinical symptoms of recovery period, survival rate and water quality were used as parameters in this research. The results showed that the use of galangal extract for treatment of Sangkuriang catfish fry infected with *Aeromonas hydrophila* by immersion method for 24 hours at a concentration of 500 mg/L was the best treatment with the survival rate of 90%.

Keywords: *Aeromonas hydrophila*, Sangkuriang catfish fry, galangal extract

ABSTRAK

Riset ini bertujuan untuk menentukan dosis optimum ekstrak lengkuas untuk mengobati ikan lele sangkuriang yang terinfeksi *Aeromonas hydrophila*, benih ikan yang digunakan berukuran 5-7 cm. Oktober-November 2022 adalah waktu penelitian dilaksanakan. Metode eksperimen yang digunakan dalam penelitian ini adalah Rancangan Acak Lengkap (RAL) yang menggunakan empat perlakuan serta tiga ulangan. Perlakuan yang digunakan adalah perendaman dengan ekstrak lengkuas selama 24 jam dengan dosis masing-masing 0 mg/L (A), 250 mg/L (B), 500 mg/L (C) dan 750 mg/L (D). Pemeliharaan ikan uji selama 14 hari. Bakteri *Aeromonas hydrophila* digunakan untuk menginfeksi ikan dengan kepadatan 108 CFU/mL dengan cara merendam 10 ml dalam 10 liter air. Parameter yang diamati meliputi gejala klinis penginfeksian, gejala klinis masa pemulihan, kelangsungan hidup dan kualitas air. Hasil riset menunjukkan bahwa penggunaan ekstrak lengkuas untuk mengobati benih ikan lele sangkuriang yang terinfeksi *Aeromonas hydrophila* dengan metode perendaman selama 24 jam pada konsentrasi 500 mg/L merupakan perlakuan terbaik dengan kelangsungan hidup sebesar 90%

Kata kunci: *Aeromonas hydrophila*, benih ikan lele sangkuriang, ekstrak lengkuas

INTRODUCTION

Fish farming activities or aquaculture sectors is expected to bring prosperity to coastal and fisheries communities. The aquaculture sector has become a reliable sector for fostering and advancing economic well-being in Indonesia and its local populations. Fish farming or aquaculture is the maintenance and breeding of fish or other aquatic organisms (Mulyono & Ritonga, 2019). Catfish is one of the fisheries commodities that is extensively cultivated by fish farmers, and even by the general public.

Catfish has been commercially cultivated by the Indonesian society. Catfish strains consists of dumbo catfish (*Clarias gariepinus*), sangkuriang catfish (*Clarias gariepinus* var. sangkuriang), local catfish (*Clarias batrachus*), phyton catfish (*Clarias* sp.), limbat catfish (*Clarias nieuhofii*), masamo catfish (*Clarias* sp.), and mutiara catfish (*Clarias* sp.). Among these, two catfish species that are well-known and extensively cultivated by the Indonesian society, especially in Java, are dumbo catfish (*Clarias gariepinus*) and local catfish (*Clarias batrachus*). In West Java, the sangkuriang catfish, such as in Depok, Bogor, Sumedang, and Sukabumi areas, is also widely cultivated (Kiagus, 2018). Indonesian national catfish production has been steadily increasing over the years. For instance, in 2010, catfish production in Indonesia reached 242 tons, and this values continued to rise, reaching 679 tons in 2014. By the year 2020, Indonesian national catfish production had reached a total of 993,768.29 tons (KKP, 2020).

Limitations in living space and competition for oxygen consumption among fish in aquaculture activities are causative factors leading to several diseases in fish. According to Sumpeno (2005), higher stocking density leads to poorer water quality compared to lower stocking density, especially concerning decreased dissolved oxygen and increased ammonia concentration. One of the common diseases affecting cultured fish, including catfish, is MAS (motile aeromonas septicemia), caused by the bacterial infection of *Aeromonas hydrophila*.

According to Agustini (2014), the outbreak of MAS disease can result in the death of fish larvae up to 90%. Due to the high mortality rate of fish larvae caused by *A. hydrophila*, it is necessary to implement mitigation efforts. Typically, fish farmers combat bacterial infections in fish using antibiotics such as oxytetracycline and ampicillin (Arifin *et al*., 2017). According to Octaviana *et al*. (2015), the use of antibiotics can have negative effects, including the potential for residues in fish and the potential to harm human health if consumed. Therefore, the use of safer alternative treatments for fish and the environment is necessary.

Natural materials sourced from plants have been widely used to treat fish infected with *Aeromonas hydrophila*, including garlic and binahong leaves (Fitriyanti *et al*., 2020). Another natural material that can be used to treat fish infected with this disease is galangal (Sari *et al*., 2017). Galangal contains essential oils, phenols, and terpenoids that have bactericidal properties. Furthermore, according to Kurniawati (2015), galangal also contains other compounds such as tannins, flavonoids, and saponins. However, there has been no study on the use of galangal rhizomes to treat catfish infected with *A. hydrophila*. Therefore, it is necessary to conduct a study on the use of galangal extract for the treatment of catfish infected with *A. hydrophila*.

MATERIALS AND METHODS

Methods

The method used in this research is experimental study with a Completely Randomized Design (CRD) consisting of four treatments and three replications. The treatments used involve the treatment of catfish infected with *A. hydrophila* using galangal rhizome extract through immersion for 24 hours, with various concentrations based on the results of *in vivo* and *in vitro* analysis, namely:

A = Without immersion of the extract (0 mg/L)
B = Immersion of galangal extract with a concentration of 250 mg/L
C = Immersion of galangal extract with a concentration of 500 mg/L
D = Immersion of galangal extract with a concentration of 750 mg/L

The catfish used in this study were Sangkuriang catfish, with sizes ranging from 6 cm and weights of 2.5 g, 120 fish obtained from fish farmers in Cileunyi, Bandung. They were acclimatized for three days before the experiment. The stocking density was 10 fish per aquarium (40×30×30 cm^3). Feeding was carried out *ad libitum* twice a day at 08:00 in the morning and 16:00 in the afternoon. Aquarium siphoning was performed every morning to remove wastes and leftover feed at the bottom of the aquarium before feeding. Throughout the study, water quality parameters including temperature, dissolved oxygen, and pH were also monitored.

Research procedure

A. hydrophila isolates were obtained from the Research and Disease Control Development Installation for Fish in Depok, West Java. Galangal extracts were administered at concentrations of 0, 250, 500, and 750 mg/L. Treated fish were infected with *A. hydrophila* at a density of 10^8 CFU/mL. The infected fish were then immersed in galangal extract for 24 hours, and their recovery process was observed over a 14-day maintenance period. Water quality observations were also conducted every seven days until the end of the study.

Data collection

Sangkuriang catfish infected with *A. hydrophila* were then treated by immersion in galangal extract for 24 hours. Clinical symptom observations during the recovery period were conducted after the infected fish had been immersed in galangal extract for 24 hours. This observation involved monitoring the recovery of body damage, such as bleeding (hemorrhagic), abdominal swelling (dropsy), ulceration, fin damage, and changes in fish behavior, including responses to food and stimuli. Water quality observations were also carried out, including temperature, dissolved oxygen, and pH. Survival rate observations for catfish began after treatment and continued until the end of the study (for 14 days of maintenance) by counting the number of surviving catfish at the beginning and end of the study. This calculation used the formula according to Effendi (1979).

Data analysis

Survival data were analyzed using ANOVA with an F-test. If there were differences between treatments, it was followed by the Duncan multiple range test with a 95% confidence level. Data on macroscopic clinical symptoms, including surface body damage, food response, stimuli response, and water quality, were analyzed descriptively.

RESULT AND DISCUSSION

Result

Clinical Symptoms

Observations were conducted following the infection of catfish with *A. hydrophila* at a density of 10^8 CFU/mL. Based on the observations, 24 hours post-injection with the bacteria, initial clinical symptoms that emerged included ulceration, hemorrhage, fin damage, and abdominal distension (dropsy). However, these clinical symptoms were not uniformly present in all fish. Hemorrhage occurred due to the leakage of blood from blood vessels within the tissues. This is in accordance with Rahman *et al*. (2002), that hemorrhage commonly occurs in the operculum, tail fin, and base of the dorsal fin.

Hemorrhage occurs due to the rupture of red blood cells by hemolysin toxins, resulting in a red discoloration on the fish's skin surface. Ulceration or sores develop as a consequence of the high bacterial density in the area under attack by the bacteria, thereby increasing the volume and intensity of toxins released in that specific area (Mangunwardoyo *et al*., 2010). On the other hand, dropsy, as explained by Austin and Austin (1999), manifests in fish as a condition where the abdomen appears bloated due to the release of Aerolysin Cytotoxic Enterotoxin (ACT-gene), leading to tissue damage. After 48 hours, catfish began to exhibit nearly uniform clinical symptoms, including ulceration (Figure 1a), hemorrhage (Figure 1b), fin damage (Figure 1c), and dropsy (Figure 1d).

Clinical symptoms of catfish after treatment with galangal extract (recovery period)

Observations of clinical symptoms in catfish after treatment with galangal extract were conducted for 14 days. The catfish that were infected with *A. hydrophila* and subsequently treated showed varying degrees of recovery (Table 1). It can be observed that from the first day to the second day after immersion in galangal extract, clinical symptoms such as dropsy, hemorrhage, ulcers, and frayed fins were still evident in each treatment group. The fish appeared weak, had reduced appetite, exhibited slow movement, and congregated around the aeration stones. On the third and fourth days, hemorrhagic symptoms in treatments C and D began to disappear (recovery) (Figure 2), but in treatments B and treatment A, there were no signs of recovery in the fish's bodies.

On the third and fourth days as well, the fish's appetite remained relatively low, and their response to stimuli was weak. Although the fish in treatment D showed recovery, on the third day, there were fish deaths in all replicates, totaling

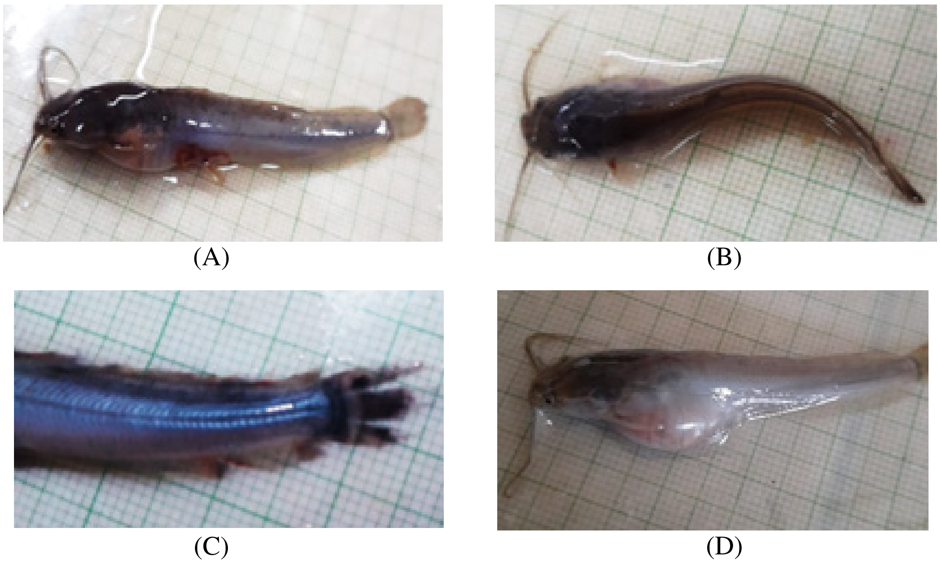


Figure 1. Clinical symptoms of catfish during the study.
Note: (A) Ulcer; (B) Hemorrhagic; (C) Fin damage; (D) Dropsy.

Table 1. Clinical symptom observations post-treatment with galangal extract.

Days-	Galangal extract concentration (mg/L)			
	A (0)	B (250 mg/L)	C (500 mg/L)	D (750 mg/L)
1	abcd	abcd	abcd	abcd
2	abcd	abcd	abcd	abcd
3	abcd	abcd	acd	acd
4	abcd	abcd	acd	acd
5	abcd	acd	cd	cd
6	abcd	acd	cd	cd
7	abcd	cd	cd	cd
8	abcd	cd	cd	cd
9	abcd	cd	d	d
10	abcd	d	d	d
11	acd	d	d	d
12	acd	d	+	+
13	acd	+	+	+
14	acd	+	+	+

Note: (a) Dropsy; (b) Hemorrhagic; (c) Fin damage; (d) Dropsy.

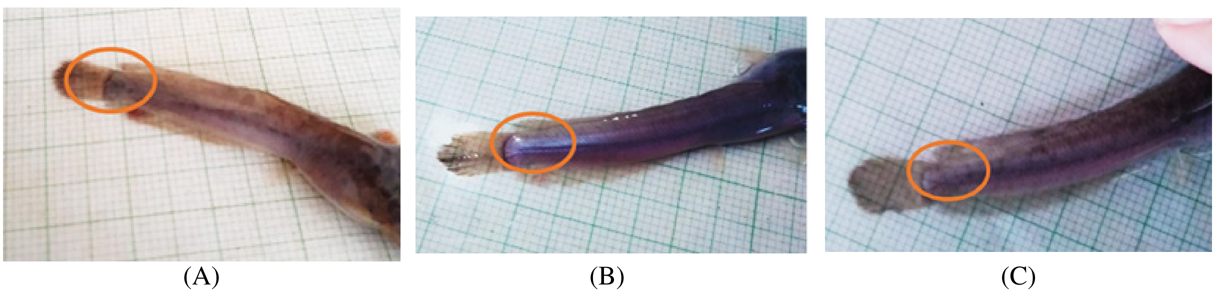
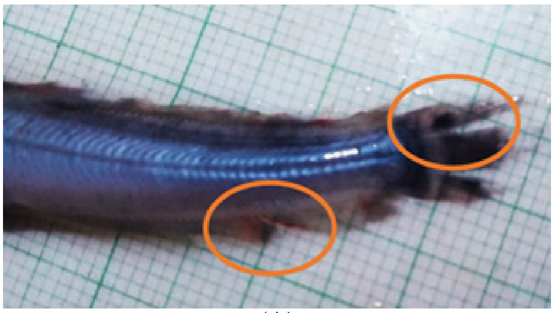


Figure 2. Hemorrhagic recovery in the anal fin of catfish.

nine individuals. Meanwhile, on the fourth day, there were three fish deaths in treatment D. Hemorrhagic symptoms were present in the anal fin and dorsal fin (Figure 3a), along with ulceration and fin fraying (Figure 3b).

On the fifth and sixth days, hemorrhagic symptoms in treatment B began to recover. Dropsy in catfish in treatments C and D also disappeared (recovery) (Figure 4), but in treatment A, the recovery process was not yet apparent. On the fifth day, there were seven fish deaths in treatment A, followed by five fish deaths in treatment D. On the sixth day, five fish deaths occurred in treatment A, one fish death in treatment B and C each, and two fish deaths in treatment D. From the 7th to the 8th day, dropsy in treatment B had disappeared (recovery).

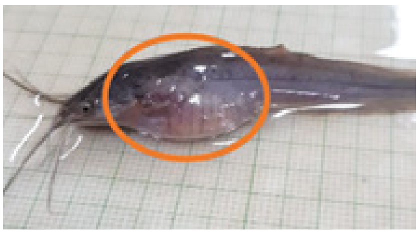
However, on the 7th day, three fish deaths occurred in treatment A, and fish deaths were also observed in other treatments, all replicates in treatment B, as well as one fish each in treatments C and D. On the 8th day, one fish died in treatment A. Ulcers in catfish in treatments C and D disappeared on the 9th day, but in treatment A, two fish deaths occurred. On the 10th and 11th days, ulcers in treatment B had also disappeared (Figure 5), leaving only the clinical symptom of fin fraying. On the 10th day, one fish died in treatment A.


(A)


(B)

Figure 3. Damage on the bodies of deceased fish.
Note: (A) Hemorrhagic in anal fin and tail fin; (B) Ulcer & fin damage.


(A)


(B)


(C)

Figure 4. Dropsy recovery of catfish.


(A)


(B)


(C)

Figure 5. Ulcer recovery of catfish.

On the 11th day, for the surviving catfish in treatment A, hemorrhagic symptoms were no longer visible (recovey). On the 12th day, catfish in treatments C and D had already recovered, but in treatment B, fin fraying was still noticeable. On the 13th and 14th days, treatments B, C, and D were seen to have recovered, whereas for treatment A, hemorrhage, ulcers, and fin fraying were still evident. The final count of surviving fish at the end of the observation period was 11 individuals in treatment A and 10 individuals in treatment D.

Feed response

Another clinical symptom that was observed is the change in fish behavior, one of which is the response of catfish to the provided feed. Based on the observation results, catfish infected with *A. hydrophila* exhibited varying responses to the feed given after treatment (Table 2). According to Ridwantara *et al*. (2019), the response to feed is considered low when the fish do not consume all of the provided feed, and it is considered normal when the fish are highly responsive to the feed, eat eagerly, and leave no remaining food at the bottom of the aquarium. Based on the Table 2 above, the observation results indicate that all fish in each treatment and replicate from day one to day three exhibited a similar low response to the feed. From day four to day six, fish in treatments B, C, and D showed a change in their feed response to normal.

This suggests that the active compounds present in the galangal extract, such as flavonoids, began to work in the recovery process. However, complete recovery had not occurred in all fish, as fish in treatments B3, C2, D1, and D3 still exhibited a low response to the feed. From day seven to day nine, the feed response of fish in all treatments changed to normal, except for the majority of fish in treatment A (A1 and A3) and a small portion in treatment C (C2). From day 10 to day 14, the feed response in treatment C2 had already shifted to a normal response. This was evident during feeding, as the catfish in treatment C2 quickly consumed the provided feed, leaving only a small amount of leftover food at the bottom of the aquarium. However, fish in treatment A still exhibited a low response to the feed.

Startle Response

Behavioral changes observed during the recovery process, in addition to the response to feed, include the startle response. Observations of catfish's startle response were conducted by tapping the aquarium walls in each treatment and its replicate. A normal reaction is considered when, upon tapping the aquarium wall, the catfish will move away from the wall. Nevertheless, if tapping the aquarium wall causes the catfish to only make a slight movement away from the point of contact, it indicates a mild response from the fish.

On the other hand, if the catfish remains completely motionless when the aquarium wall is tapped, this suggests a lack of any discernible reaction. Based on the observation results, catfish infected with *A. hydrophila* and treated with galangal extract exhibited varying responses to startle (Table 3). In Table 3, it can be seen that catfish from day one to day three still exhibited a low response to the startle. This was evident from the minimal movement of the catfish away from the aquarium wall when tapped, and in some cases, the catfish appeared lethargic and hardly showed any response at all.

This indicates that the fish were still in a diseased condition due to the *A. hydrophila* infection. From the 4th to the 6th day, the startle response became normal in treatments C1 and C3, but in the other treatments, the startle response remained low. From the 7th to the 9th day, all treatments had shifted to a normal response

Table 2. Observation of feed responses during the study.

Day	Feed Response											
	A (0 mg/L)			B (250 mg/L)			C (500 mg/L)			D (750 mg/L)		
	Replication			Replication			Replication			Replication		
	1	2	3	1	2	3	1	2	3	1	2	3
1-3	+	+	+	+	+	+	+	+	+	+	+	+
4-6	+	+	+	++	++	+	++	+	++	+	++	+
7-9	+	++	+	++	++	++	++	+	++	++	++	++
10-14	+	++	+	++	++	++	++	++	++	++	++	++

Note: (+) Low response; (++) Normal response.

except for treatment A1, B3, C2, and D1. From the 10th to the 14th day, the catfish's startle response in all treatments and replicates became normal.

Survival

Based on the observations conducted over 14 days, catfish infected with *A. hydrophila* and subsequently treated through immersion in galangal extract exhibited varying mortality rates and survival rates in each treatment (Figure 6). In Figure 6, it can be seen that low survival rates occurred in treatments A and D. High survival rates were observed in treatments B and C. The low survival of catfish in treatment A was due to the fact that after infection, these fish experienced a high mortality rate as they did not receive treatment with galangal extract.

Meanwhile, the low survival of catfish seeds in treatment D was because, following infection with *A. hydrophila*, the seeds were treated with a relatively high concentration of galangal extract. Based on the analysis of variance results, the administration of galangal extract had a statistically significant effect because the calculated F-value (Fhit) was greater than the tabulated F-value (Ftab) at the 0.05 significance level. Duncan's test results indicate that there is a significant difference between treatment A (control) and treatments B (250 mg/L) and C (500 mg/L), but there is no significant difference with treatment D (750 mg/L). Treatment B (250 mg/L) is not significantly different from treatment C (500 mg/L) but is significantly different from treatment A (control) and treatment D (750 mg/L) (Table 4).

Table 3. Startle response observations during the study.

Day	Startle response											
	A (0)			B (250 mg/L)			C (500 mg/L)			D (750 mg/L)		
	Replication			Replication			Replication			Replication		
	1	2	3	1	2	3	1	2	3	1	2	3
1-3	+	+	+	+	+	+	+	+	+	+	+	+
4-6	+	+	+	+	+	+	++	+	++	+	+	+
7-9	+	++	++	++	++	+	++	+	++	+	++	++
10-14	+	++	+	++	++	++	++	++	++	++	++	++

Note: (+) Low response; (++) Normal response.

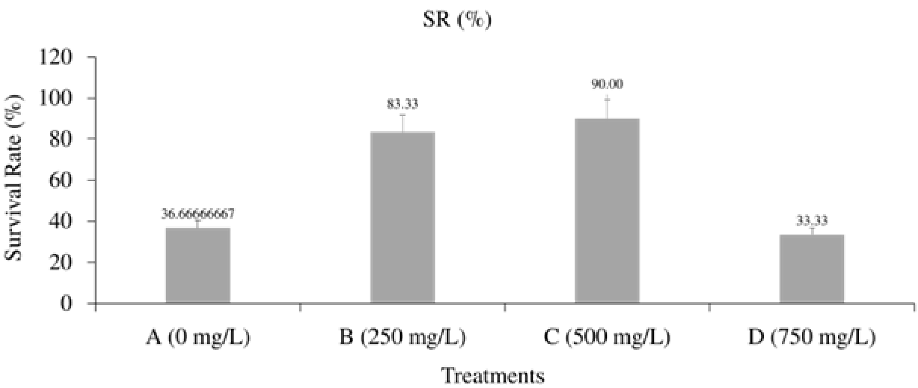


Figure 6. Survival of catfish.

Table 4. Average survival of catfish.

Treatment	Survival rate (%)	Note
A (0 mg/L)	36.67 ± 5.77	a
B (250 mg/L)	83.33 ± 15.28	b
C (500 mg/L)	90.00 ± 10.00	b
D (750 mg/L)	33.33 ± 5.77	a

Note: Values followed by the same letter are not significantly different based on Duncan's test at a 95% confidence level.

Water Quality

The results of water quality observations can be seen in Table 5, and are used as supporting data that provide information that the catfish seed rearing medium is in good and controlled condition. Water quality observations were conducted three times during the study, namely at the beginning of the rearing, in the middle of the rearing, and at the end of the rearing. The average temperature during the study ranged from 25.2 to 26.4°C, which is within the reference range specified in the Indonesian National Standard (SNI). The temperature requirement for catfish seed rearing typically falls between 25–30°C.

Additionally, the pH levels remained within the optimal range for catfish seed rearing, with pH values during the study ranging from 6.5 to 7.1, aligning with the pH reference in the SNI, which is 6.5–8. The dissolved oxygen (DO) levels during the study also met the minimum oxygen requirements for catfish seed rearing, with DO values ranging from 4.2 to 6.9 mg/L.

Discussion

After 48 hours, Sangkuriang catfish began to exhibit clinical symptoms that were almost uniform, such as ulceration (Figure 1a), hemorrhagic (Figure 1b), fin damage (Figure 1c), and dropsy (Figure 1d). Hemorrhagic symptoms occurred due to the presence of blood leaking from blood vessels within the tissue. This is in line with Rahman *et al*. (2002), findings, where hemorrhagic symptoms typically occur in the operculum, tail fin, and the base of the dorsal fin. Hemorrhaging results from the rupture of red blood cells by hemolysin toxins, leading to a red discoloration on the fish's skin surface. The initial clinical symptoms that appeared included not only damage to the fish's body but also changes in behavior, such as decreased appetite and responses to stimuli.

In treatment D, the treated fish showed signs of recovery; however, on the third day, some fish died in all replicates, totaling nine fish. Meanwhile, on the fourth day, three fish in treatment D died. The mortality in treatment D is suspected to be due to the high toxic effects of saponin compounds in the galangal extract. According to Purbosari *et al*. (2022), saponin can be toxic to fish and amphibians, and it can also kill protozoa and mollusks. This aligns with Pasaribu (2019), statement that saponin can be toxic or referred to as sapotoxin to cold-blooded animals like fish by destroying red blood cells through a hemolysis reaction.

On the sixth day, deaths occurred among the fish in treatment A, totaling five fish. Fish mortality also occurred in treatments B and C, with one fish each, and in treatment D, with two fish. The mortality of fish in treatment A is suspected to be due to the absence of treatment with galangal extract. Meanwhile, the mortality in treatment B is suspected to be because the concentration of 250 mg/L of extract was not effectively inhibiting the activity of *A. hydrophila*. Consequently, the *Aeromonas hydrophila* continued to infect the catfish.

Furthermore, on the tenth day, one fish in treatment A died. On the eleventh day, surviving catfish in treatment A no longer showed hemorrhagic symptoms (indicating recovery). Even without treatment, the disappearance of hemorrhagic symptoms in treatment A is suspected to be due to its stronger immune system compared to the other fish. In a study conducted by Sopiah *et al*. (2018), the treatment of Sangkuriang catfish infected with *A. hydrophila* using pandan wangi leaf extract resulted in the fastest clinical symptom recovery in five days of maintenance. On the other hand, when treated with galangal extract, the clinical symptoms recovered even faster, specifically on the third day.

Table 5. Water quality during treatment.

Treatment	Parameter		
	Temperature (°C)	pH	DO (mg/L)
A (0 mg/L)	25.2–26.3	6.5–6.8	4.9–6.8
B (250 mg/L)	25.3–26.2	6.6–6.7	4.3–6.7
C (500 mg/L)	25.3–26.2	6.6–7.1	4.9–6.7
D (750 mg/L)	25.3–26.4	6.5–6.9	4.2–6.9
Optimal (SNI 2014)	25–30	6.5–8	min.3

Fish feeding response from day one to day three showed a consistent pattern of low response, in line with the statement by Dewantoro *et al*. (2021), where fish experience changes in behavior after being infected with *A. hydrophila*, including reduced appetite, solitary swimming, and decreased swimming activity. On days seven to nine, fish responses to feed in all treatments returned to normal, except for most fish in treatment A (A1 and A3) and a small portion in treatment C (C2). The fish in treatment A that exhibited a normal feeding response may be due to their naturally higher immune resistance compared to other catfish. This aligns with Dadiono (2022), that the fish's natural defense mechanisms can fight bacteria from within the fish's body, referred to as the immune system.

Physical barriers like skin and mucous layers containing immune-reactive molecules (such as lysozyme, complement, and immunoglobulins), specialized cells like macrophages and NK cells, as well as specific soluble molecules like complement and interferon, are examples of the non-specific immune system (Baxter, 2007). The non-specific immune cells in the fish immune system include monocytes or tissue macrophages, granulocytes (neutrophils) and cytotoxic cells, as well as complement, while the specific immune system has the ability to recognize foreign agents in the body. This system can only destroy foreign agents that have been previously recognized. The specific immune system consists of the humoral system (B lymphocytes) and the cellular system (T lymphocytes) (Dharmayanti *et al*., 2020). In the study conducted by Aisiah *et al*. (2021), which used *Acanthus ilicifolius* leaf extract to treat catfish infected with *A. hydrophila*, it was found that the feeding response returned to normal within 5-7 days of the maintenance period.

The fish became active, consumed food eagerly, and did not leave any food residue at the bottom of the aquarium. The use of galangal extract was more effective because within 4-6 days of the maintenance period, the fish's feeding response had already returned to normal. The fish's stratle response in Table 3 shows that the catfish still had a relatively low response from day one to day three. This is evident from the catfish's limited movement away from the aquarium wall when the wall was tapped, and in some cases, the catfish appeared lethargic and hardly showed any response at all. This indicates that the fish were still in a diseased condition due to the *A. hydrophila* infection.

As suggested by Suryadi *et al*. (2022), changes in fish behavior after infection with *A. hydrophila* are characterized by slow swimming movements and a tilted body position due to the loss of balance. Azis (2019), also proposed a similar thought, stating that fish infected with *A. hydrophila* exhibit slow swimming behavior as a result of post-infection stress. From day four to day six, the startle response returned to normal in treatments C1 and C3, but in the other treatments, the startle response remained low. From day seven to day nine, all treatments had returned to normal except for treatments A1, B3, C2, and D1. It is suspected that the healing process of galangal extract on catfish infected with *A. hydrophila* had not worked optimally and uniformly in each treatment and replicate, except for treatment A, which did not receive any treatment.

Furthermore, from day 10 to day 14, the startle response of catfish in all treatments and replicates returned to normal. The change in the catfish's startle response to normal in treatment A, even without galangal extract treatment, is suspected to be due to the catfish in treatment A having a favorable living environment and strong body resistance. According to Rosidah *et al*. (2019), which used *Moringa oleifera* L. leaf extract on the resistance of Sangkuriang catfish infected with *A. hydrophila*, there was no observable response from the fish to the strartle from day one to day three. On the fourth day, the fish's startle response began to appear but was still low. However, by the sixth day, the fish's startle response started to become normal when compared to the use of galangal extract, which became more effective.

This is because, from day one to day three, the startle response appeared weak, but from day four to day six, the fish's startle response had already returned to normal in treatment C. In Figure 6, it can be observed that there was low survival in treatments A and D, while high survival occurred in treatments B and C. The low survival of catfish in treatment A was due to a high mortality rate after infection because no treatment with galangal extract was administered. On the other hand, the low survival of catfish seeds in treatment D was because they were infected with *A. hydrophila* and treated with galangal extract at a relatively high concentration.

At high concentrations, the active ingredients contained in the extract are also high. Among the active ingredients found in galangal extract is saponin. At high concentrations, saponin can be toxic to fish. As suggested by Yulistyana *et al*.

(2020), for cold-blooded animals like fish, saponin is toxic. Saponin produces foam when shaken with water and produces sugar and sapogenins when hydrolyzed.

In Table 4, treatments A and D show no significant difference and exhibit low values. The low survival of catfish in treatment A is because the fish were not treated after being infected with *A. hydrophila*. Red blood cells in catfish will exit the blood vessels, resulting in a reddish color. The rupture of red blood cells is caused by hemolysin toxins, leading to hemorrhage on the dorsal fin, tail fin, and anal fin of catfish. The appearance of ulcers is due to the high density in that location (Cahyani, 2020). According to Wahjuningrum *et al*. (2016), fish that have been severely infected with bacteria can lead to death.

Treatment D shows low survival due to treatment with a high concentration of the extract, as mentioned earlier, saponin is an active ingredient found in galangal that, in high concentrations, can cause death in fish. According to Sariri and Yakin (2019), saponin can hemolyze red blood cells and inhibit the chymotrypsin enzyme, resulting in impaired fish productivity and growth. The same is stated by Yudhistira *et al*. (2020), where fish experience nervous disorders and loss of balance because saponin metabolite compounds inhibit the binding of oxygen to gill filaments. Treatments B and C did not show significant differences and resulted in high survival rates.

The high survival rate of fish in these treatments is because the active ingredients at these concentrations work optimally as antibacterial agents to inhibit the growth of *A. hydrophila* that infect catfish. This is further supported by the faster wound healing process (Table 1) as well as the quicker feeding response (Table 2) and shock response (Table 3). According to Dewantoro *et al*. (2021), effective treatment occurs because polar compounds like flavonoids and tannins, which function as antimicrobials, damage bacterial cytoplasmic membranes and kill bacterial epidermis cells. The flavonoid content in galangal extract can prevent the release of toxins by *A. hydrophila*.

According to Rahmadona *et al*. (2020), the mechanism of action of flavonoids involves denaturing bacterial cell proteins and forming complex compounds with extracellular proteins, disrupting bacterial cell permeability and causing irreparable membrane damage. The same is stated by Haryani *et al*. (2012), where flavonoids have anti-inflammatory properties that can reduce inflammation and pain in wounds in case of bleeding. Furthermore, according to Mawardi (2016), tannin compounds function to inhibit the inflammatory process and act as antiseptics.

Tannins also inhibit reverse transcriptase and DNA topoisomerase enzymes, preventing bacterial cell formation. Based on the preliminary test conducted, it can be observed that the inhibitory activity of galangal extract against *A. hydrophila* falls into the moderate category. At a concentration of 250 mg/L, an average clear zone of 6.80 mm was obtained, while the highest average clear zone was at a concentration of 1250 mg/L, which measured 11.11 mm. According to Dindayani (2021), the classification of inhibitory response is weak when the diameter of the clear zone is ≤ 5 mm, moderate when the diameter is 5–10 mm, strong when the diameter is 10-20 mm, and very strong when the diameter is ≥ 20 mm.

Based on the LC50 24-hour test, a safe concentration for fish is below the LC50 value, which is below 760 mg/L. In the study by Sari *et al*. (2017), which used galangal extract to treat Nile tilapia infected with *A. hydrophila*, the optimal dose was 1000 mg/L. When compared to the treatment of catfish infected with *A. hydrophila*, it can be considered more effective and efficient because at a concentration of 500 mg/L, it already resulted in the best survival rate of 90%. In another study, the treatment of catfish infected with *A. hydrophila* using the extract of *Blumea balsamifera* L leaves at a concentration of 700 mg/L showed the highest survival rate of 60% (Zubaidah *et al*., 2021). The use of galangal extract is more effective because at a concentration of 500 mg/L, it achieved a survival rate of 90%.

CONCLUSION

Based on the results of the conducted study, it can be concluded that a galangal extract concentration of 500 mg/L is effective for the treatment of catfish (*C. gariepinus*) infected with *A. hydrophila* through 24-hour immersion, resulting in the highest survival rate of 90%.

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