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Efficacy of Teak (Tectona grandis) Leaf Extract Against Aeromonas hydrophila in Catfish

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ABSTRACT

Catfish is a leading commodity in the fisheries cultivation sector, one of the main problems in catfish cultivation activities is infection with Motile Aeromonas Septicemia (MAS) disease caused by the bacteria Aeromonas hydrophilla. This study aims to determine the therapeutic activity of young teak leaf (*Tectona grandis*) solution against *Aeromonas hydrophila* bacteria and to find the most effective dose of young teak leaf (*Tectona grandis*) solution in inhibiting the performance of *Aeromonas hydrophila* bacteria. using a completely randomized experimental design with 4 treatments and 3 replicates conducted over 19 days. The fish were injected intramuscularly with a concentration of 10^6 cfu/ml (0.1 ml) and subjected to treatments A (Control), B (10 g/L), C (15 g/L), and D (20 g/L). The fish were immersed for 20 minutes. The results obtained from the study were analyzed using analysis of variance (ANOVA). The results of the study showed that herbal therapy by soaking catfish in a solution of young teak leaves was effective in treating catfish infected with A. hydrophila at the optimal dose, namely in treatment B (10 g/L), which was characterized by a decrease in the number of bacteria calculated using the ALT method of 0.06 x 10^7 in the kidneys and 0.02 x 10^7 in the liver, as well as a survival rate and a cure rate of 100%. The results of this study are expected to be easily implemented and beneficial for fish farmers.

INTRODUCTION

Catfish is one of the freshwater fish species that is a leading commodity in Indonesia. This can be seen from the potential for increased national catfish production based on data from the Ministry of Maritime Affairs and Fisheries in 2023, which reached 1.2 million tons with an average annual growth of 5%. However, aquaculture production activities are still often hampered by disease problems along with the increase in aquaculture intensification (Pasaribu, 2023).

Diseases in aquaculture consist of non-infectious and infectious diseases. Non-infectious diseases include those caused by the environment, feed, and genetics, while pathogens such as viruses, bacteria, fungi, protozoa, and parasites are classified as infectious diseases. One of the most dangerous diseases is bacterial infection or bacterial disease. Bacterial diseases that may attack fish include *Edwardsiella ictaluri*, *Aeromonas salmonicida*, *Pseudomonas*, *Streptococcus agalactiae*, *Vibrio*, *Edwardsiella tarda*, *Aeromonas hydrophila* (Rahmawati *et al.*, 2021).

Aeromonas hydrophila bacteria are pathogenic bacteria that cause *Motile Aeromonas Septicemia* (MAS), especially in freshwater fish. Fish infected with these bacteria are characterized by the appearance of bleeding or wounds on the skin or other parts of the body.

In severe cases, the wounds will become red ulcers, the abdomen will swell, and the fish's internal organs, such as the kidneys and liver, will bleed. (Susandi & Rosmawati, 2017).

Prevention and treatment of fish diseases using chemicals and antibiotics are considered practical, effective, and inexpensive, but they have the negative effects of leaving residues and causing antibiotic resistance. Therefore, one alternative is to use herbal ingredients, which are environmentally friendly and have minimal side effects on fish. (Pasaribu & Djonu, 2021).

One herbal ingredient that can be used is young teak leaves (*Tectona grandis*), which contain active compounds and can be used as a test ingredient for herbal therapy. According to Febriana & Chaidir, (2023) The antibacterial compounds found in young teak leaves are saponins, tannins, alkaloids, and flavonoids. This indicates the potential of young teak leaves as a herbal therapeutic agent in the treatment of catfish (*Clarias* sp.) infected with *Aeromonas hydrophila* bacteria. However, there has been no research on its use in fish treatment. Based on the explanation and several reasons above, it is necessary to conduct research on the effectiveness of soaking young teak leaves in solution on catfish infected with *Aeromonas hydrophila* bacteria, with several parameters observed, namely the calculation of the number of bacteria using, the survival rate of fish, the recovery rate of fish, and observation of clinical symptoms.

This study aims to determine the therapeutic activity of young teak leaf (*Tectona grandis*) solution against *Aeromonas hydrophila* bacteria and to find the most effective dose of young teak leaf (*Tectona grandis*) solution in inhibiting the growth of *Aeromonas hydrophila* bacteria.

METHOD

Time and Place

This research was conducted over a period of 19 days from November to December 2025 at the Pharmaceutical Microbiology Laboratory, Faculty of Sports and Health, Gorontalo State University.

Preparation of Containers

The container used in this study was an aquarium. 12 aquariums were used, each measuring 30x30x30 cm^3 with a water volume of 13 liters per aquarium, and the placement of the containers was randomized using a lottery method. Before use, the aquariums were sterilized first to minimize contaminants so that the research conditions were truly controlled. The aquariums were washed with clean water and sterilized using 0.1 ml of methylene blue dissolved in 1 liter of water, left for 24 hours, then rinsed with running water. After cleaning, the aquariums were filled with water and aerated.

Preparation of Test Fish

The test fish used in this study were 10-12 cm catfish, with 10 fish in each aquarium, for a total of 120 fish in the 12 aquariums. Prior to the distribution and injection of Aeromonas hydrophila bacteria, the fish were acclimatized and their external clinical symptoms were observed.

Aeromonas hydrophila Bacterial Injection Process

The process of injecting Aeromonas hydrophila bacteria into catfish (Clarias sp.) using pure isolates of Aeromonas hydrophila bacteria obtained from Indilab Scientific Samarinda, with Aeromonas hydrophila culture concentration of 10^6 cfu/ml, injection was carried out on all fish with a total of 120 fish using a 1 cc dispo of 0.1 ml of bacterial culture. (Andrianti *et al*., 2023) by intramuscular injection (Ardulanisa *et al*., 2017).

Solution Preparation Process

The process of making the solution uses fresh young teak leaves (*Tectona grandis*) with surfaces that are not torn, and the procedure for making the solution refers to the research. (Kasiati *et al*., 2016) with the following manufacturing method:

1. Wash the young teak leaves that will be used, then air dry them until there is no water left on the leaves.
2. Separate the young teak leaf veins from the leaves and cut them into smaller pieces.
3. Put the weighed young teak leaves into a blender, add 1 liter of water, and blend.
4. The blended mixture is then left to settle for 3 minutes, filtered using a sieve and filter paper, and poured into the soaking container.
5. The test fish are placed in a soaking container for 20 minutes.

Soaking Process

The fish that had been injected with *Aeromonas hydrophila* bacteria were immersed in three containers. Each container was filled with a solution of young teak leaves at different doses, namely 10 g/L, 15 g/L, and 20 g/L. The immersion lasted for 20 minutes. (Ardulanisa *et al.*, 2017). After the soaking process, the fish were moved to the aquarium for a further maintenance period of 19 days for the recovery period and during that period clinical symptoms were observed in the fish.

Observation Variables

Calculation of Bacteria Count

The calculation of the number of bacteria was carried out to obtain observation results from each treatment performed twice, namely on day 0 after infection and on the last day after infection and immersion. The calculation of the number of bacteria used the following formula (Buda *et al.*, 2023) :

Number of cells = V x n x 1/f

Where:

V : Volume of sample added
N : Number of colonies in the dish
F : Dilution factor

Clinical Symptom Observation

Clinical observations were conducted twice during the study period, with the first observation conducted after the incubation period (48 hours) and the second observation conducted after the treatment period. These observations were made by taking the fish's medical history, including signs of slow swimming, pale skin color, excessive mucus, wounds, swollen abdomen, eye area wounds, broken whiskers, frayed tail fin tips, and ulcers (Damayanti *et al.*, 2024).

Fish Recovery Rate

The recovery rate of fish can be measured using the following formula:

$$\text{FH} = \frac{\text{Number of fish that recovered}}{\text{Number of infected fish}} \text{ x } 100\%$$

Survival Rate

Survival rate (SR) is the ratio of the number of fish alive at the beginning to the end of the study. Fish survival is calculated using the formula Zonneveld *et al* (1991) in Baring *et al.*, 2022

$$SR\ (\%) = \frac{\text{Nt}}{\text{N0}} X\ 100$$

Keterangan

SR = Survival rate (%)
Nt = Total number of live seeds at the end of the experiment (fishes)
N0 = Total number of seeds at the beginning of the experiment (fishes)

Water Quality

Water quality measurements were conducted to determine the quality of water as a maintenance medium during the study. Water quality is the medium in which fish live and is one of the most important factors to consider in order to provide support for organisms. The physical and chemical parameters observed in this study included measurements of temperature, pH, and DO.

Data Analysis

To determine the effect of immersion using young teak leaf solution at different doses on the treatment of Aeromonas hydrophila bacteria, the results obtained from the study will be analyzed using analysis of variance (ANOVA) at a 95% confidence level.

RESULTS AND DISCUSSION

Calculation of Bacteria Count

Based on the results of testing *Aeromonas hydrophila* bacteria in catfish (*Clarias* sp.) conducted at the Pharmaceutical Microbiology Laboratory, Faculty of Sports and Health, Gorontalo State University from the beginning to the end of the study on treatments A (Control), B (10 g/L), C (15 g/L), D (20 g/L), the results can be seen in the table below:

Table 1. Calculation of Bacteria Count at the Beginning and End of the Study

Treatment	Kidney Before ($\times 10^7$ CFU)	Kidney After ($\times 10^7$ CFU)	Reduction (%)	Liver Before ($\times 10^7$ CFU)	Liver After ($\times 10^7$ CFU)	Reduction (%)
Control (A)	3.67	1.61	56.1	2.71	1.78	34.3
10 g/L (B)	3.67	0.06	98.4	2.71	0.02	99.3
15 g/L (C)	3.67	0.05	98.6	2.71	0.01	99.6
20 g/L (D)	3.67	0.01	99.7	2.71	0.03	98.9

Based on Table 1, the initial bacterial count results before treatment for treatments A (Control), B (10 g/L), C (15 g/L), and D (20 g/L) were 5.03 x 10^7 for wounds, 3.67 x 10^7 for kidneys, and 2.71 x 10^7 for livers. These bacterial counts were obtained by taking one infected fish with clinical symptoms that met the observation guidelines and were interpreted as 100% infected with A. hydrophila bacteria. The results of bacterial counts in the kidneys at the end of the study for treatment A (Control) were 1.61 x 10^7, a reduction in bacteria of 56.1%, B (10 g/L) was 0.06 x 10^7, a reduction in bacteria of 98.4%, C (15 g/L) was 0.05 x 10^7, a reduction in bacteria of 98.6%, D (20 g/L) was 0.01 x 10^7, a reduction in bacteria of 99.7%. Furthermore, for the liver at the end of the study, treatment A (Control) obtained 1.78 x 10^7, a reduction in bacteria of 34.3%, B (10 g/L) obtained 0.02 x 10^7, a reduction in bacteria of 99.3%, C (15 g/L) obtained 0.01 x 10^7, a reduction in bacteria of 99.6%, D (20 g/L) obtained 0.03 x 10^7, a reduction in bacteria of 98.9%. The calculation of the number of bacteria was carried out by taking one fish from each aquarium to obtain the results of each treatment.

Febriana & Chaidir, 2023 states that ethanol extract of teak leaves contains tannins, flavonoids, and alkaloids. The mechanism of action of flavonoids is that the OH compounds found in flavonoids are thought to interfere with the components that make up peptidoglycan in bacterial cells, preventing the cell wall from forming completely and causing the death of the bacterial cells (Kurama *et al.*, 2020), The mechanism of action of tannins is to inactivate bacterial cell adhesion and enzymes, as well as disrupt protein transport in the inner layer of cells. Tannins damage cell wall polypeptides, resulting in incomplete formation of bacterial cell walls, which causes bacterial cells to lyse (Nurjannah *et al.*, 2022).

Clinical Symptom Observation

Observations of catfish infected with *A. hydrophila* bacteria include slow swimming, frayed dorsal and tail fins, wounds on the body, swelling of the abdominal area, and decreased appetite. According to (Rochani *et al*., 2021), Infected fish exhibit behavioral changes such as weak swimming and slow response to feed. A decrease in feed response may also be caused by low hemoglobin levels.

Clinical symptoms of catfish infected with *A. hydrophila* bacteria were observed visually by noting the clinical symptoms seen each day after the catfish were infected until the end of the 19-day maintenance period. The observation process was carried out by defining the symptoms that appeared in each treatment and categorizing them in a table, where fish that showed symptoms on the first day with a description of (-1) and on the second day changed to a description of (-2) meant that the clinical symptoms of the fish increased without reducing or eliminating the initial clinical symptoms. Clinical symptoms are considered to have changed if the notation changes from (-) to (+). The clinical symptoms that appeared after infection and during the rearing period can be seen in Table 2 below:

Table 2. Clinical Symptom Observation

Day	Treatment											
	0 g/L			10 g/L			15 g/L			20 g/L		
	A1	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	D3
After Injection of *Aeromonas Hydrophila*												
1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
2	-2	-3	-2	-2	-2	-2	-2	-2	-2	-2	-3	-2
After Soaking Young Teak Leaves (*Tectona grandis*)												
3	-3	-3	-3	+1	+1	+1	+1	+1	+1	+1	+1	+1
4	-3	-3	-3	+1	+2	+2	+2	+1	+1	+1	+1	+1
5	-3	-3	-3	+1	+2	+2	+2	+2	+2	+1	+1	+1
6	-3	-3	-3	+2	+2	+2	+2	+2	+2	+1	++	+1
7	-3	-3	-3	+2	+2	+2	+2	+2	+2	+2	+2	+2
8	-3	-3	-3	+2	+2	+2	+2	+2	+2	+2	+2	+2
9	-3	-3	-3	+2	+2	+2	+2	+2	+2	+2	+2	+2
10	-3	-3	-3	+2	+2	+2	+2	+2	+2	+2	+2	+2
11	-3	-3	-3	+2	+2	+2	+2	+2	+2	+2	+2	+2
12	-3	-3	-3	+2	+2	+2	+2	+2	+2	+2	+2	+2
13	-3	-3	-3	+2	+2	+2	+2	+2	+2	+2	+2	+2
14	-3	-3	-3	+2	+2	+2	+2	+2	+2	+2	+2	+2
15	-3	-3	-3	+2	+2	+2	+2	+2	+2	+2	+2	+2
16	-3	-3	-3	+2	+2	+2	+2	+2	+2	+2	+2	+2
17	-3	-3	-3	+2	+2	+2	+2	+2	+2	+2	+2	+2
18	-3	-3	-3	+2	+2	+2	+2	+2	+2	+2	+2	+2
19	-3	-3	-3	+2	+2	+2	+2	+2	+2	+2	+2	+2

Description:
-1: Passive swimming, pale skin color, fin clamping, decreased appetite
-2: Excess mucus, swollen belly, wounds, red gills, red fins, black spots appear
-3: Injection marks begin to turn black, more aggressive, more time at the surface, gills torn
+1: Active swimming, normal skin color, normal appetite
+2: Mucus decreases, abdomen begins to return to normal, whiskers begin to normalize, and redness on fins begins to normalize

Based on Table 2 above, clinical symptoms in catfish infected with *A. hydrophila* bacteria began to appear 24 hours after the fish were injected. The fish began to swim passively and their body color, which had previously been grayish, turned pale. Their feeding response began to decrease, redness appeared in the fin and barbels area, there was excess mucus, and the fish's stomach began to swell. The changes trigger the appearance of black marks in the injection site area, leading to the formation of wounds in that area. This is consistent with research conducted by Safitri *et al* (2021) that after 24 hours of infection with *A. hydrophila*, clinical symptoms change. Clinical symptoms include red spots and lesions on the back, a pale red color on the head, and redness on the skin. Jumina *et al* (2024) Adding that fish infected with *A. hydrophila* bacteria will experience a decrease in response to feed. A decrease in response to food is often experienced in unhealthy fish, swimming slowly and abnormally, where catfish are more often found vertically at the surface of the water, motionless and lethargic at the bottom of the aquarium. This is because the fish are stressed due to infection with *A. hydrophila* bacteria.

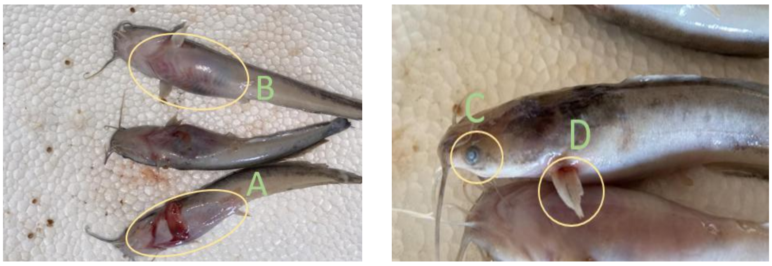

Figure 1. Clinical symptoms of sudden death in catfish

Description : (A). Bleeding in the abdomen and internal organs; (B) Swelling of the abdomen; (C). Exophthalmos; (D) Fin rot.

Fish Recovery Rate

The cure rate of fish is the percentage of infected fish that are successfully cured. Several factors that can be used as a reference in calculating the cure rate of fish are survival rate and recovery of internal and external symptoms to obtain the percentage. In this study, the recovery rate of fish was measured through the assessment of observed internal clinical symptoms. The following table shows the results of the recovery rate of fish:

Table 3. Fish recovery rate results

Fish Recovery Rate					
Treatment	**Test**	**HF**	**IF**	**FH**	**Average (%)**
	A1	2	10	20	
A	A2	1	10	10	13
	A3	1	10	10	
	B1	10	10	100	
B	B2	10	10	100	100
	B3	10	10	100	
	C1	10	10	100	
C	C2	9	10	90	97
	C3	10	10	100	
	D1	4	10	40	
D	D2	10	10	100	50
	D3	1	10	10	

Description: (HF) Healthy Fish, (IF) Infected Fish, and (FH) Fish Health

Based on the results in Table 3, treatment A (control) had the lowest cure rate, with a cure rate of 13% compared to treatments B, C, and D. This was because treatment A (control) involved injecting *Aeromonas hydrophila* bacteria but did not use young teak leaves (Tetcona grandis) for treatment. Treatment B (10 g/L) obtained a result of 100%, while treatment C (15 g/L) obtained a result of 97% and treatment D (20 g/L) obtained a result of 50%. From these results, it was found that treatments B, C, and D, which were treated with young teak leaves (Tetcona grandis) after being injected with *A. hydrophila* showed higher health levels compared to treatment A (control), which was injected with *A. hydrophila* bacteria but not treated. This aligns with the data graph shown in Table 4 below:

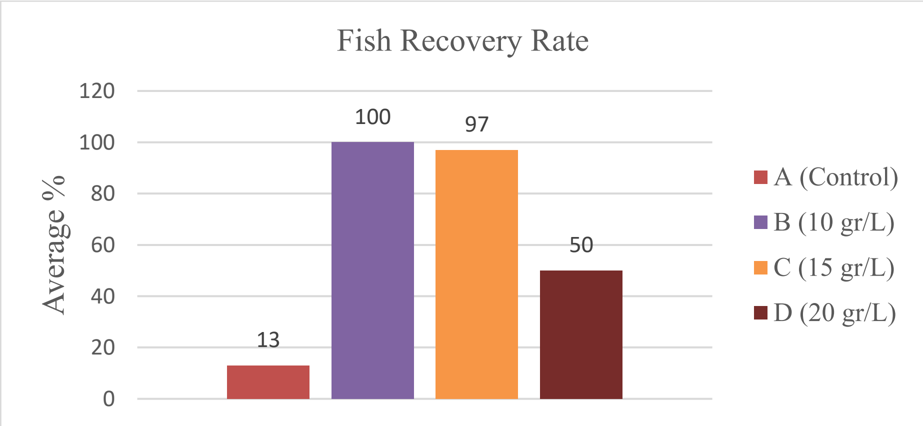

Figure 2. Graph of Fish Recovery Rates

The health status of fish can be determined from clinical symptoms, which is the easiest and most efficient method. According to Pratama *et al* (2017) Clinical symptoms caused by *A. hydrophila* bacteria include peeling skin, red spots all over the body, dull or bluish gills, exophthalmos (protruding eyeballs), bleeding in the dorsal, pectoral, ventral, and caudal fins, bleeding from the anus, and loss of appetite.

Survival Rate

Survival rate is the percentage comparison of the number of organisms alive at the beginning of maintenance and at the end of maintenance (Rihi, 2019). Observation of fish survival in this study was conducted daily, starting from the first day the fish were infected with *A. hydrophila* bacteria until treatment and the last day of the study.

Based on the results of research on the effectiveness of soaking young teak leaves (*Tectona grandis*) in solution on catfish (*Clarias* sp.) infected with *A. hydrophila* bacteria that had been kept for 19 days, the following graph shows in the table 5 below:

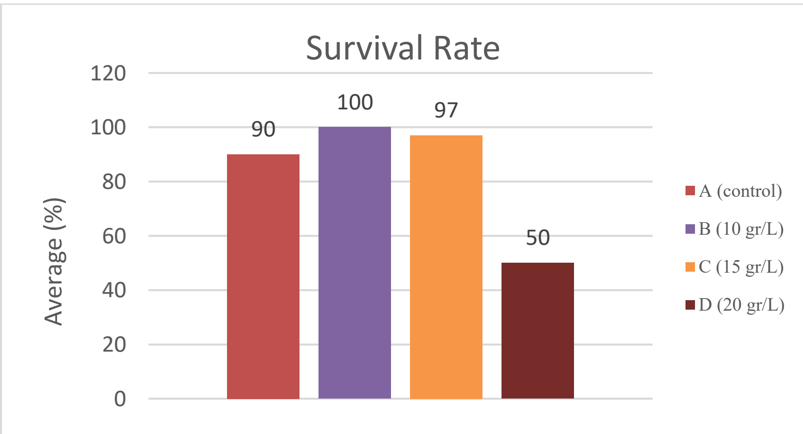

Figure 3. Survival rate graph for Catfish (*Clarias* sp.)

Based on graph 5 above, it shows that the survival rate of catfish in treatment A (control) injected with *A. hydrophila* and not treated with young teak leaves had a survival rate of 90%, where treatment A (Control) had the second lowest survival rate after treatment D (20 g/L), which had a survival rate of 50%. The highest survival rate was found in treatment B (10 g/L) with a survival rate of 100%, and treatment C (15 g/L) had a survival rate of 97%.

The variance analysis data shows that the immersion of young teak leaf solution has no significant effect on the survival rate of catfish (Fcount > Ftable α = 0.05). The results of the variance analysis of catfish survival rates can be seen in Table 5 below:

Table 6. Analysis of the variance in the survival rate of catfish (*Clarias* sp.)

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
SR	Between Groups	4825.000	3	1608.333	2.881	.103
	Within Groups	4466.667	8	558.333		
	Total	9291.667	11			

Based on Table 6, the results of the ANOVA analysis on the survival of catfish (Clarias sp.) show no significant effect (Sig > 0.05) on the survival of catfish (Clarias sp.). Therefore, the decision rejects H1 and accepts H0, meaning that the treatment of immersion using a solution of young teak leaves does not affect the survival of catfish (Clarias sp.). The results of the study show that the highest survival rate was in treatment B (10 g/liter) with a value of 100.

Excessive doses of young teak leaf solution can also be toxic, which is in line with the statement by Widyaningsih *et al* (2022) that excessive concentrations can be toxic to fish. This is because tannins, which are antibacterial, can become toxic at high concentrations because tannins can reduce iron compounds (Fe), where Fe can cause kidney damage, and tannins can bind to proteins and other minerals, preventing the body from utilizing them.

In a study conducted by Lili *et al* (2018) using cherry leaf extract to treat fish infected with Aeromonas hydrophila bacteria, it was found that excessive doses can be toxic to fish and even cause death. This is in line with the results of the study, which found that the highest doses, D (80 ppm) and E (100 ppm), resulted in the lowest survival rates.

Water Quality

In aquaculture, water quality plays an important role because the entire life cycle of the cultivated biota takes place in water. In addition to being clear and free of pollution, water used for aquaculture must also take into account the physical, biological, and chemical properties of the water. Water quality greatly affects the survival rate, growth, development, reproduction, and health of fish (Koniyo, 2020), The water quality parameters observed were pH, temperature, and DO. Water quality measurements were taken twice during the study, at the beginning and at the end of the study.

Table 7. Water quality measurement results during the study

Parameters	Treatment			
	A	B	C	D
	(Control)	(10 gr / L)	(15 gr / L)	(20 gr / L)
Temperature (^{0}C)	31.6	32.6	32.1	31.9
pH	7.8	7.8	8.0	7.7
DO (mg/L)	5.9	5.8	5.1	6.9

This is consistent with the results of the study, where the survival rate in treatments C and D was much lower due to mortality caused by toxicity at high doses. Higher doses can be toxic to fish, which is in line with the statement. Widyaningsih *et al* (2022) That too high a concentration can be toxic to fish. This is because tannins, which are antibacterial, can become toxic at high concentrations because they can reduce iron (Fe) compounds, which can cause kidney damage. Tannins can also bind with proteins and minerals, preventing the body from utilizing them. And where the water quality during the herbal therapy test was in the high range, it triggered stress in the fish and made them weak and eventually died. (Rochani *et al*., 2021).

CONCLUSION

Based on the research conducted, it was found that herbal therapy using young teak leaf solution was effective in treating catfish infected with A. hydrophila bacteria with an optimal dose in treatment B (10 g /L), with a decrease in the number of bacteria by 0.06 x 10^7 in the kidneys and 0.02 x 10^7 in the liver, with a survival rate and cure rate of 100%. This result is influenced by the concentration in each treatment, the highest concentration has the lowest survival rate and the lowest concentration has the highest survival rate, this is because bioactive compounds always become toxic when given at high or excessive doses such as in treatment D (20 gr/L) namely stress or shock in fish where the body that was initially infected with bacteria meets a high dose of treatment that triggers stress in fish and high doses can make fish gasp due to tannins that accumulate in the gills, causing hypoxia or lack of oxygen and eventually death. The results of this study are expected to be easy to apply and useful for farmers.

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