Journal of Vocational in Aquaculture
Available online at https://journal.ipb.ac.id/index.php
ISSN 3109-7731
JAVA
Research Article
OPEN ACCES

Application of Cat's Whiskers (*Orthosiphon aristatus*) Leaf Extract in Tambaqui (*Colossoma macropomum*) Infected with *Aeromonas hydrophila*

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Received: 23 Apr 2025
Revised: 9 Mei 2025
Accepted: 20 Jun 2025

https://doi.org/10.29244/java.v2i1.63842

Abstract

Treatment using environmentally friendly natural materials, such as *Orthosiphon aristatus* (cat's whiskers) leaf extract, can inhibit the growth of *Aeromonas hydrophila* in Tambaqui juvenile (*Colossoma macropomum*). This study aimed to determine the effect of *Orthosiphon aristatus* leaf extract on Tambaqui juvenile infected with *Aeromonas hydrophila* and to identify the optimal concentration based on blood profile responses during treatment. The research was conducted from April to May 2024 at the Fish Hatchery and Breeding Laboratory, Faculty of Marine and Fisheries, Universitas Syiah Kuala. A Completely Randomized Design (CRD) was used, consisting of five treatments and four replications. The extract concentrations were as follows: Treatment A (control), B (2 ppt), C (3 ppt), D (4 ppt), and E (5 ppt), administered via immersion. The clinical symptoms observed after treatment indicated that the extract had a positive effect on the fish: they regained active swimming behavior, feeding response improved, red patches faded, mucus secretion decreased, and scales returned to normal. Abdominal swelling subsided, and eye conditions normalized. ANOVA results showed a significant difference in survival rate ($P<0.05$), with the highest survival observed in Treatment D (4 ppt), at $86.75 \pm 5.31\%$. In conclusion, *Orthosiphon aristatus* leaf extract at a concentration of 4 ppt provided the best outcomes in terms of clinical symptoms, survival rate, leukocyte and erythrocyte counts, and hemoglobin levels, indicating its potential as an antibacterial agent for Tambaqui juvenile.

Keywords: *Aeromonas hydrophila*, blood profile, *Orthosiphon aristatus*, tambaqui.

I. INTRODUCTION

Freshwater tambaqui (*C. macropomum*) is a high-value species that is widely consumed and cultivated due to its significant economic importance. This species is favored by aquaculture practitioners for several reasons, including its relatively fast growth rate (Fatchurochman *et al.,* 2017). However, the development of aquaculture often faces challenges such as disease outbreaks, particularly at the seed (juvenile) stage, which is highly susceptible to infections. Fish diseases are generally caused by infectious pathogens, including parasites, fungi, bacteria, and viruses (Arisa *et al.,* 2021; Putra *et al.,* 2021; Putra *et al.,* 2021a, 2021b, 2021c; Salsabilla *et al.,* 2021; Suratno & Putra, 2022).

One most common disease affecting tambaqui is red sore disease, caused by *A. hydrophila* , also known as Motile Aeromonas Septicemia (MAS)

(Pratama *et al.,* 2017). The uncontrolled and prolonged use of synthetic drugs at inappropriate dosages can lead to negative consequences, such as environmental pollution, the accumulation of harmful residues in fish tissues, and potential health risks to consumers (Lukistyowati & Syawal, 2013).

In response to the challenges faced by fish farmers, it is essential to identify alternative materials for disease control that are both safe and environmentally friendly, particularly those derived from medicinal plants or beneficial bacteria (Abbas *et al.,* 2023; Dachi *et al.,* 2019; Muhammadar *et al.,* 2018). The use of herbal plants is recommended as a potential alternative for combating bacterial infections through the application of natural antibiotic compounds. These natural antibiotics offer several advantages, including environmental friendliness and biodegradability. Additionally, herbal ingredients have been reported to influence blood profiles, improve egg hatchability, and enhance fish growth performance (Afriani *et al.,* 2023; Evendi *et al.,* 2017; Gunawan *et al.,* 2024; Liu *et al.,* 2020, 2019; Putra *et al.,* 2019). One plant with potential as a natural antibiotic is cat's whiskers (*O. aristatus*), known for their antimicrobial and anti-inflammatory properties.

This plant is widely found in Java and has long been used in traditional medicine. According to Rukmana and Mulyowati (2015), *O. aristatus* leaves contain phenolic compounds capable of inhibiting the growth of *Salmonella typhi*. Humani *et al.,* (2013) reported that *O. aristatus* leaf extract naturally contains various bioactive compounds, including flavonoids, polyphenols, active proteins, glycosides, essential oils, potassium, and terpenoids. Similarly, Wulandari (2011) identified saponins, flavonoids, and polyphenols among its chemical constituents. Suteja *et al.,* (2016) stated that flavonoids are active chemical compounds in plants that function as antibacterial agents by disrupting the cytoplasmic membrane. Therefore, *O. aristatus* may serve as an effective therapeutic and preventive agent against *A. hydrophila* infections in fish.

II. MATERIALS AND METHODS

2.1 Research Procedure and Experimental Design

This study was conducted in May 2024 at the Fish Hatchery and Breeding Laboratory, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh. The experimental design employed was a Completely Randomized Design (CRD), consisting of five treatments with four replications each. The dosage for each treatment was determined based on the study by Abidin *et al.,* (2020). The treatments administered to Tambaqui juvenile (*C. macropomum*) were as follows:

A: Control (no extract immersion)
B: *O. aristatus* leaf extract at 2 ppt
C: *O. aristatus* leaf extract at 3 ppt
D: *O. aristatus* leaf extract at 4 ppt
E: *O. aristatus* leaf extract at 5 ppt

Before preparing the experimental containers, all equipment was thoroughly cleaned, rinsed, and dried. The containers used were 25-liter plastic jars, totaling 20 units. Each container was labeled according to the treatment group and equipped with an aeration system to ensure adequate oxygen supply. Each was filled with 15 liters of water and arranged randomly.

The test fish used in this study were Tambaqui juvenile (*C. macropomum*), obtained from Ulee Lheue, Banda Aceh, totaling 300 individuals with an average length of 8–10 cm. The stocking density was set at 1 fish per liter, or 15 fish per container. The fish were acclimated for 14 days prior to treatment. During the initial 3 days of acclimation, they were fed commercial pellets ad libitum three times daily—morning, noon, and evening.

The preparation of *O. aristatus* leaf extract was conducted at the Chemistry Laboratory, Faculty of Teacher Training and Education (FKIP), Universitas Syiah Kuala. Dried *O. aristatus* leaves were ground into powder using a blender and then sieved. The powdered leaves were macerated in 96% ethanol for three days. The resulting mixture was filtered to obtain the filtrate, which was subsequently concentrated using a rotary evaporator until the solvent had completely evaporated. The thick extract obtained was weighed and then diluted to prepare a series of concentrations according to the experimental requirements (Abidin, 2020).

2.2 Bacterial Preparation and Infection Procedure

Bacterial infection was carried out after the fish had been acclimatized for three days. The infection was induced using *A. hydrophila* bacteria obtained from the Faculty of Agriculture. The bacterial challenge was performed using the immersion method for 24 hours at a bacterial density of 10^{-8} CFU/mL (Sari *et*

al., 2022). Following the immersion period, the fish were transferred into containers filled with freshwater. During the infection process, clinical symptoms were observed. The clinical signs of *A. hydrophila* infection included surface lesions in the form of red patches, darkened body coloration, abdominal swelling, damaged or protruding eyes, and sluggish movement (Rochani *et al.,* 2021). After 24 hours of exposure to the bacteria, the fish were treated by immersion in various concentrations of *O. aristatus* leaf extract (Indriani *et al.,* 2014).

2.3 Treatment of Infected Fish

The treatment was conducted using the immersion method, in which fish were immersed in water containing *O. aristatus* leaf extract at the designated concentrations for each treatment group, for a duration of 20 minutes. Following the immersion process, the treatment water was replaced with standard maintenance water. The fish were then reared for 14 days, during which clinical symptoms were monitored, including swimming activity, feeding response, and external morphological conditions.

2.4 Observed Research Parameters

2.4.1 Clinical Symptoms

The observed clinical symptoms included both physical damage and behavioral changes in the fish, particularly their feeding response. The clinical signs associated with A. hydrophila infection included darkened body coloration, rough skin with hemorrhages that could progress into ulcers, reduced swimming ability, frequent gasping at the surface due to gill damage and respiratory distress, a slightly swollen abdomen, frayed and whitish fins, and damaged or protruding eyes. Behavioral and physical changes were monitored daily throughout the treatment period (Cahyono, 2011).

2.4.2 Survival Rate

The survival rate of Colossoma macropomum was monitored by recording the number of surviving fish each day throughout the treatment period. The survival rate was calculated using the following formula, as described by Effendie (2013):

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

Where :

SR = Survival rate of the fish (%)

Nt = Number of fish alive at the end of the experiment (individuals)

No = Number of fish at the beginning of the experiment (individuals)

2.4.3 Hematological Profile

Blood sampling of the test fish was conducted after infection with *A. hydrophila* and following treatment on days 1, 7, and 14 (H1, H7, H14). The effects of *O. aristatus* leaf extract on the hematological profile were assessed based on red blood cell (erythrocyte) count, white blood cell (leukocyte) count, and hemoglobin concentration. Blood was drawn from the caudal artery using a 1 mL syringe pre-treated with EDTA to prevent coagulation. Each blood sample was then transferred into a microtube for subsequent hematological analysis (Dianti *et al.,* 2013).

2.4.4 White Blood Cell (Leukocyte) Count

The leukocyte count was conducted using the method described by Blaxhall and Daisley (1973). First, a blood sample was drawn into a pipette containing a white mixing bead up to the 0.5 mark. Turk's solution was then added to the 11.0 mark. The pipette was shaken in a figure-eight motion for 3–5 minutes to ensure thorough mixing. The first two drops of the diluted sample were discarded, and the subsequent drop was placed onto a hemocytometer and covered with a cover slip. The diluted blood entered the counting chamber by capillary action. The total number of white blood cells (leukocytes) was counted under a microscope at 100× magnification. Leukocytes were counted within four small squares of the hemocytometer grid, and the total count was calculated using the following formula (Hartika *et al.,* 2014):

$$\textit{Leukocyte count}\ (cells/mm^3) = \frac{N\ x\ D\ X\ 10}{4}$$

Where:

N = Number of leukocytes counted

D = Dilution factor (typically 20)

10 = Conversion factor for volume (mm^3)

4 = Number of large squares used (on the Neubauer counting chamber)

2.4.5 Red Blood Cell (Erythrocyte) Count

The procedure for erythrocyte counting was conducted following the method described by Blaxhall and Daisley (1973). Blood samples were collected using a red-tipped erythrocyte pipette containing a mixing bead, with blood drawn up to the 1.0 mark. Hayem's solution was then added until the 10.0 mark.

The mixture was gently shaken in a figure-eight motion for 3–5 minutes to ensure complete homogenization.

After mixing, the first two drops of the diluted blood were discarded. A single drop of the thoroughly mixed solution was then placed onto a Neubauer hemocytometer and covered with a cover slip. The fluid entered the counting chamber through capillary action. Erythrocytes were observed under a microscope at 100× magnification. The erythrocyte count was performed by counting the cells within five small central squares of the Neubauer grid. The total number of erythrocytes was calculated using the following formula:

$$Erythrocyte\ count\ (cells/mm^3) = \frac{N\ x\ D\ X\ 10^4}{L}$$

Where:

N = Number of erythrocytes counted
D = Dilution factor (typically 200)
10^4 = Volume conversion factor (mm^3)
L = Volume of the counting chamber (typically 0.02 mm^3 when counting 5 small squares)

2.4.6 Hemoglobin Concentration Measurement

The procedure for measuring hemoglobin concentration was conducted using the Sahli method. First, a blood sample was drawn using a Sahli pipette up to the 20 mm^3 mark (equivalent to 0.2 mL). The tip of the pipette was then carefully wiped with tissue paper. The blood was transferred into an Hb-meter tube containing 0.1 N hydrochloric acid (HCl) up to the red mark at scale 10. The mixture was stirred using a glass rod for 3–5 minutes to ensure complete mixing. Distilled water (aqua dest) was then added dropwise into the tube until the color of the solution matched the standard comparator in the Hb-meter. Hemoglobin concentration was recorded in grams per deciliter (g/dL).

III. RESULTS

3.1 Clinical Symptoms

Clinical symptoms in pomfret (*C. macropomum*) were visually assessed, with a focus on morphological changes such as surface lesions (reddish patches), scale loss, alterations in skin pigmentation, damaged or protruding eyes, abdominal swelling, excessive mucus secretion, and lethargic movement. Variations in clinical symptoms among the different treatment groups are presented in Table 1.

3.2 Hematological Test

The results of leukocyte, erythrocyte, and hemoglobin measurements throughout the study are presented in Table 2. Blood cell parameters were used as indicators to assess the severity of infection in juvenile tambaqui (*C. macropomum*) infected with *A. hydrophila* . By day 14, the most favorable outcomes were recorded in Treatment D (4 ppt dosage), where the leukocyte count reached 160,700 cells/mm^3, the erythrocyte count was 175,700 cells/mm^3, and hemoglobin levels also showed a marked improvement (Table 2).

3.3 Survival Rate

Based on the results of the Analysis of Variance (ANOVA), the administration of *O. aristatus* leaf extract to juvenile tambaqui (*C. macropomum*) infected with *A. hydrophila* had a significant effect ($P < 0.05$) on survival rate. The highest survival was recorded in Treatment D, reaching 87%. Detailed data on survival rates over the 14-day post-infection period are presented in Table 3.

3.4 Water Quality

Water quality parameters, including pH, temperature, and dissolved oxygen (DO), were measured throughout the duration of the experiment. The results of these measurements are presented in Table 4.

IV. DISCUSION

Observations of healthy (control) fish showed normal morphological features, characterized by bright body coloration, stable swimming patterns, and an active feeding response. In contrast, visual examination of clinical symptoms in Tambaqui juvenile (*C. macropomum*) infected with *A. hydrophila* revealed notable morphological alterations on the body surface. The observed symptoms included scale loss, pale or darkened skin, excessive mucus secretion, reddish patches, abdominal swelling, sluggish movement, and decreased appetite. These findings are consistent with the report by Rochani *et al.,* (2014), who stated that fish infected with *A. hydrophila* exhibit clinical signs such as skin darkening, hemorrhagic lesions that may progress to ulcers, dropsy (abdominal distension), damaged or protruding eyes, frayed fins, and lethargy.

This is further supported by Haryani *et al.,* (2012), who reported that fish infected with *A. hydrophila*

https://doi.org/10.29244/java.v2i1.63842

Table 1. Clinical Symptoms After Bacterial Infection

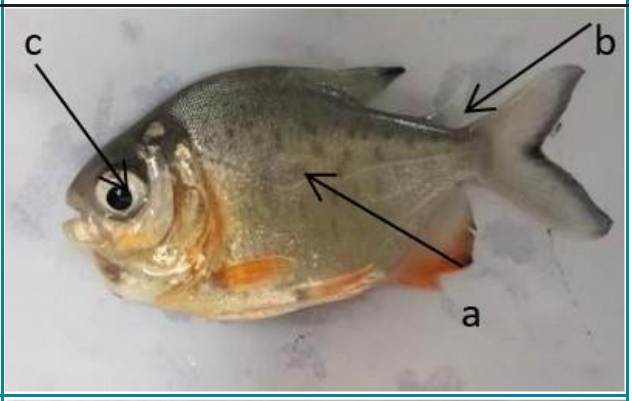
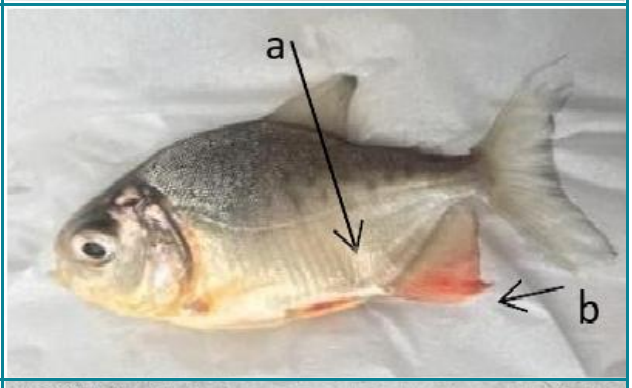
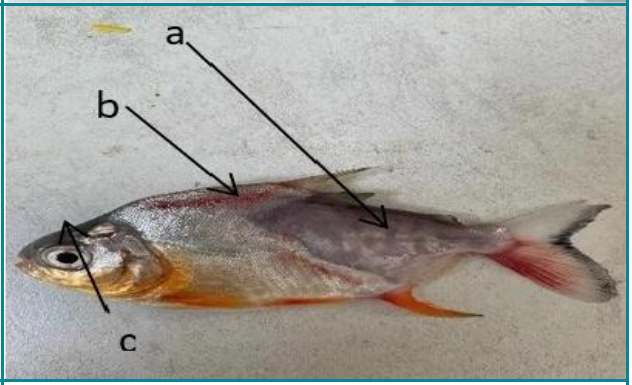
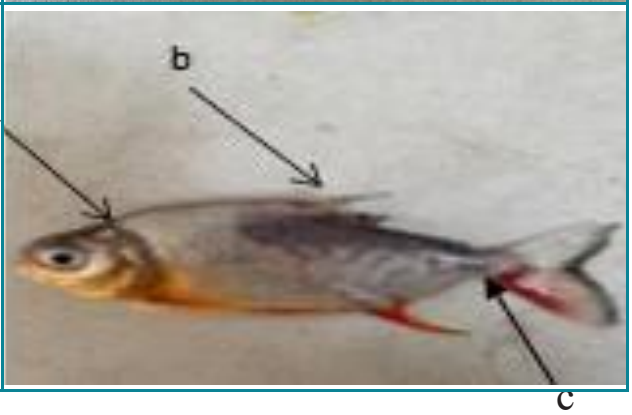
No	Treatment	Symtoms	Figure
1	A (Control)	Normal	
2	B (2 ppt)	a. Color change to pale, b. Excessive mucus secretion, c. Protruding eyes.	
3	C (3 ppt)	a. Pale coloration b. Excessive mucus secretion.	
4	D (4 ppt)	a. Scale detachment, b. Red patches c. Swollen eyes	
5	E (5 ppt)	a. Scale detachment, b. Red patches, dan c. Excessive mucus secretion	

Table 2. Clinical symptoms after bacterial infection

Treatment	Leukocyte Count (10^3cells/mm^3)			Erythrocyte (10^6cells/mm^3)			Hemoglobin (g/dL)		
	H1	H7	H14	H1	H7	H14	H1	H7	H14
A (Control)	83.700	85.500	83.050	82.400	90.900	84.120	6	5	7
B (2 ppt)	256.300	223.300	180.900	232.700	199.600	185.900	4	5,6	5
C (3 ppt)	220.600	200.500	190.400	246.400	220.050	192.960	3	5	6
D (4 ppt)	255.500	198.700	160.700	265.200	180.900	175.700	4	6	7
E (5 ppt)	235.200	210.600	200.800	256.400	219.700	195.400	2	6	6,2

exhibit behavioral changes such as lethargic movement and reduced appetite, attributed to metabolic disruptions and post-infection stress. Additional behavioral manifestations in infected fish include anorexia and isolation from the group. *A. hydrophila* is the causative agent of motile *A. septicemia,* commonly known as red sore disease (Dar *et al.,* 2022). This condition is characterized by the appearance of reddish, ulcerative lesions on the surface of the fish's body.

Based on the clinical symptoms observed in juvenile tambaqui (*C. macropomum*) following infection with *A. hydrophila*, all treatment groups exhibited excessive mucus secretion on the body surface. This response is believed to be a physiological mechanism, in which the fish activate their immune defenses to combat the bacterial invasion, particularly as part of their nonspecific immune response. This finding is consistent with Rochani *et al.,* (2021), who reported that excessive mucus production is a clinical sign observed in common carp infected with *A. hydrophila*, serving as a physiological reaction to the presence of pathogens. Given the emergence of clinical symptoms, treatment was administered through immersion in *O. aristatus* (cat's whiskers) leaf extract for 20 minutes. Post-treatment observations revealed noticeable improvements in the condition of the fish. Both morphological and behavioral indicators showed gradual recovery following the immersion. Behavioral improvements were marked by a progressive increase in feeding activity and a return to normal swimming behavior over time.

Observational data collected over the 14-day post-immersion showed significant improvement in fish condition. The immersion method, as noted by Haryani *et al.,* (2012), is an effective treatment approach, particularly for large-scale juvenile populations. This was supported by observations on day 7 post-immersion, where fish in Treatment D (4 ppt) had regained normal body coloration and demonstrated an increased appetite. The most rapid recovery was recorded in Treatment D (4 ppt), with full recovery observed by day 8 post-immersion. In contrast, a high mortality rate occurred

Table 3. Survival rate data of tambaqui (*Colossoma macropomum*) juveniles over a 14-day period.

Treatment	Survival Rate (%)
A (Control)	$80{,}00 \pm 5{,}71^{c}$
B(2 ppt)	$56{,}50 \pm 4{,}04^{a}$
C(3 ppt)	$61{,}75 \pm 6{,}70^{ab}$
D(4 ppt)	$86{,}75 \pm 5{,}31^{c}$
E (5 ppt)	$68{,}25 \pm 6{,}18^{b}$

Table 4. Water quality parameters of tambaqui (*Colossoma macropomum*) juveniles over a 14-day period

Treatment	pH	DO (mg/L)	Temperature (˚C)
A (Control)	7,0-8,0	5 - 6,11	29-30
B (2 ppt)	7,0-8,0	5 - 6,11	29-30
C (3 ppt)	7,0-8,0	5 - 6,11	29-30
D (4 ppt)	7,0-8,0	5 - 6,11	29-30
E (5 ppt)	7,0-8,0	5 - 6,11	29-30

in Treatment B (2 ppt), likely due to the extract dosage being insufficient to effectively combat the infection.

Based on the clinical symptom observations over a 14-day period, it can be concluded that cat's whiskers (*O. aristatus*) leaf extract at the concentrations used in Treatments B (2 ppt), C (3 ppt), and E (5 ppt) was ineffective in treating *A. hydrophila* infection. This ineffectiveness may be attributed to the fact that higher concentrations of certain active compounds can exert toxic effects on the test fish, while the 2 ppt concentration was insufficient to treat *A. hydrophila* -infected juvenile tambaqui. As a result, the recovery of clinical symptoms in fish under these treatments was slower compared to Treatment D (4 ppt), which demonstrated the most effective outcome.

The results of the survival rate observation of Tambaqui juvenile (*C. macropomum*) over a 14-day rearing period indicated that the administration of cat's whiskers (*O. aristatus*) leaf extract had a significant impact on fish survival. The highest survival rate was recorded in Treatment D, reaching 87%. This improvement is attributed to the antibacterial properties of the active compounds present in the extract, which effectively inhibited the growth of *A. hydrophila*, thereby reducing the severity of infection and facilitating the gradual recovery of the fish. The effectiveness of cat's whiskers leaf extract is presumed to result from the presence of bioactive compounds such as flavonoids, tannins, saponins, and other active constituents. This assumption is supported by Yulianti *et al*., (2015), who reported that cat's whiskers leaves contain antibacterial agents. These antibacterial compounds specifically target bacterial pathogens, with flavonoids in particular known to disrupt bacterial cell wall function, ultimately leading to cell lysis (Sari *et al.,* 2022). In contrast, the lowest survival rate, at 57%, was observed in Treatment B. This may be attributed to the low concentration of the extract administered, which was likely insufficient to inhibit the growth of *A. hydrophila* or to support the recovery of infected fish. Furthermore, improved feed efficiency has been linked to enhanced growth performance and increased immune response (Yu *et al.,* 2023). This finding is supported by Sari *et al.,* (2024) and Zulfahmi *et al.,* (2023), who demonstrated that the administration of fermented *Gliricidia sepium* leaf flour and turmeric extract can improve growth, egg hatchability, and larval survival rates in pomfret. Environmental factors, such as light intensity, also play a role in influencing fish survival rates. Fluctuations in light intensity can affect water quality, whereas optimal lighting conditions can help degrade pollutants and improve overall water parameters. Proper lighting has been shown to improve survival rates, growth performance, aquaculture productivity, and disease resistance (Qu *et al.,* 2022).

The leukocyte count of juvenile tambaqui (*C. macropomum*) on day 1 (H1) in Treatment A (normal/ control) was recorded at 83,700 cells/mm³. As stated by Yuliana *et al.,* (2021), the normal leukocyte range for pomfret is between 75.56×10^3 and 86.8×10^3 cells/ mm³. Following infection with *A. hydrophila* on H1, the highest leukocyte count was observed in Treatment D, reaching 255,500 cells/mm³. This significant increase is attributed to the entry of *A. hydrophila* into the fish's body, which stimulated the immune system to activate leukocyte production as part of its defensive response (Prabakaran *et al.,* 2006). By day 7 (H7), leukocyte levels began to gradually decline after immersion in cat's whiskers (*O. aristatus*) leaf extract. A significant reduction in leukocyte count was observed by day 14 (H14). Leukocytes are components of the nonspecific immune system that aid in the elimination of pathogens through phagocytosis (Ipa *et al.,* 2019). In fish, leukocytes play a vital role in immune responses to parasitic infections, inflammation, and physiological stress. Healthy fish generally show lower leukocyte counts (Daneshvar *et al.,* 2012). Leukocyte levels in fish are influenced by various factors, including species, age, physical activity, stress, and reproductive status (Yanto *et al.,* 2015). In this study, leukocyte counts increased in all treatment groups except for the control (Treatment A), indicating an immune response triggered by infection. When pathogens invade the host, leukocyte production increases at the site of infection as part of the body's defense mechanism (Ipa *et al.*, 2019).

Erythrocyte observations during the maintenance period of juvenile tambaqui (*C. macropomum*) showed that on day 1 (H1), the normal erythrocyte count in Treatment A (control) was 82,400 cells/mm³. On the same day, post-infection with *A. hydrophila*, the highest erythrocyte count was recorded in Treatment D, reaching 265,200 cells/mm³. However, by day 7, after immersion in cat's whiskers (*O. aristatus*) leaf extract, a significant decrease in erythrocyte count was observed in Treatment D, dropping to approximately

180,900 cells/mm³. This reduction is likely associated with the optimal dosage administered in this treatment. Normal erythrocyte counts can vary among fish species and are influenced by factors such as sex, age, environmental conditions, and nutritional status (Yanto *et al.,* 2015). Erythrocyte levels play an important role in determining an organism's capacity to adapt and survive in low-oxygen environments (Kartashev *et al.*, 2023). Erythrocyte count is also regarded as a key indicator of fish health. Low erythrocyte levels may suggest anemia, while elevated counts can indicate physiological stress (Aziz, 2019). The normal erythrocyte range in fish is approximately 1.91–2.83 × 10^6 cells/mm³ (Thrall *et al.,* 2022). *A. hydrophila* is known to exert a cytolytic effect on erythrocytes, leading to hemolysis (Ahmed *et al.,* 2018). Hemolysins produced by *A.hydrophila* cause the lysis of red blood cells, resulting in hemorrhaging on the skin of infected fish. The lysis of erythrocytes releases various nutrients and ions essential to the fish (Azizah *et al.,* 2023).

On day 14, the hemoglobin (Hb) levels in Tambaqui juvenile (*C. macropomum*) across treatments were recorded as follows: Treatment A (5.5 g/dL), Treatment B (5.4 g/dL), Treatment C (6.0 g/dL), and Treatment D (6.2 g/dL). These values fall within the normal hemoglobin range for fish, which is approximately 6–9 g/dL (Salasia *et al.,* 2001). This is consistent with the findings of Mali *et al.,* (2023), who reported that increased hemoglobin levels can be influenced by the presence of flavonoid and tannin compounds, which act as antioxidants capable of protecting hemoglobin from oxidative damage, thereby helping to maintain higher Hb levels. Hemoglobin (Hb) is the primary component of erythrocytes (red blood cells) (Kartashev *et al.,* 2023). It plays a crucial role in transporting oxygen to body tissues (Puteri *et al.,* 2016), and also contributes to nutrient transport into cells and the removal of metabolic waste products (Ipa *et al.,* 2019). In the present study, the lowest hemoglobin level was observed in Treatment E on day 1 (H1), which may have resulted from the pathogenic activity of *A. hydrophila* . This bacterium produces various extracellular enzymes and toxins, including aerolysin, lipase, chitinase, amylase, gelatinase, hemolysin, and enterotoxins (Murwani *et al.,* 2017). These extracellular compounds are known to induce hemolysis—the destruction of red blood cells (Keman, 2018).

The recorded water quality parameters throughout the experiment were as follows: temperature ranged from 29–30 °C, dissolved oxygen (DO) from 5.0–6.11 mg/L, and pH from 7.0–8.0. These values fall within the favorable range for the survival of juvenile tambaqui (*C. macropomum*), where the optimal temperature is 28–32 °C, DO levels range between 5.0–7.0 mg/L, and the ideal pH lies between 7.5–8.5 (Effendie, 2013). Water quality was assessed using several principal indicators, including DO, pH, temperature, nitrogenous compounds, and electrical conductivity (EC). Among these, DO is one most critical indicator of aquatic quality, as sufficient levels are necessary to support the passive diffusion of oxygen into the bloodstream of fish. When DO concentrations drop below the physiological needs of the fish, energy metabolism becomes inefficient, resulting in stunted growth, poor feed utilization, and diminished swimming capability. pH is another critical parameter, as a decline in pH is often linked to nitrification activity. Extremely low pH levels can lead to the accumulation of ammonia at concentrations toxic to fish. Temperature, a fundamental physical attribute of water, is closely tied to DO levels. It influences photosynthetic processes and the breakdown of organic material, both of which impact oxygen demand and the ionization of ammonia (Deswati *et al.,* 2021). Increasing water temperature may lead to a reduction in DO levels (Lentera, 2002). Therefore, water quality is a crucial factor in ensuring fish health and survival. Therefore, ongoing monitoring of these parameters is crucial to maintain a stable and suitable environment for rearing juvenile tambaqui.

V. CONCLUSION

The findings of this study indicate that the application of cat's whiskers leaf extract (*O. aristatus*) on juvenile tambaqui (*C.macropomum)* infected with *A. hydrophila* had a significant effect on survival rate, with the highest survival observed in Treatment D (4 ppt), reaching 86.75 ± 5.31%. Additionally, Treatment D resulted in the greatest improvements in hematological parameters, including erythrocyte, leukocyte, and hemoglobin levels. These results suggest that *O. aristatus* extract has strong potential as an effective antibacterial agent for disease management in juvenile tambaqui.

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