**Journal of Fish Health**
Volume 5, Issue 4, November 2025
https://doi.org/10.29303/jfh.v5i4.8609

# Application of Black Turmeric Rhizome (*Curcuma aeruginosa* Roxb) Ethanol Extract to Treat *Aeromonas hydrophila* Infection in Catfish (*Clarias gariepinus*)

Riri Mulfiani, Indriyani Nur, La Ode Baytul Abidin*

Aquaculture Department, Fisheries and Marine Science Faculty, Halu Oleo University
HEA Mokodompit Street, Anduonohu, Kendari City, Indonesia

**Correspondence**:
baytul.abidin@uho.ac.id

**Received:**
November 3rd, 2025
**Accepted:**
November 8th, 2025
**Published:**
November 27th, 2025

**Keywords**:
Catfish, *Curcuma aeruginosa*, Ethanol Extract, *Aeromonas hydrophila*

*Aeromonas hydrophila* is a pathogenic bacterium that frequently attacks, causes death, and harms catfish farmers. The continuous use of antibiotics for disease control has been shown to have negative impacts on pathogenic bacterial resistance, antibiotic residues in fish, aquatic environmental pollution, food safety, and causes allergies in human consumers. The use of phytopharmaceuticals is known to be more environmentally friendly and sustainable because they contain active compounds that are more easily degraded, thus leaving no synthetic antibiotic residues in fish tissues and water. This research aimed to determine the effect of black turmeric (*Curcuma aeruginosa* Roxb) rhizome ethanol extract on *treating A. hydrophila* infection in catfish. Fish were raised for 14 days. For the first seven days, they were kept under normal conditions. On day 7, the fish were infected with *A. hydrophila*. On day 8, the diseased fish were immersed in black turmeric rhizome ethanol extract. The experiment used group randomized design with treatment of control or without black turmeric extract (BT0), 35 mg/L, 40 mg/L and 45 mg/L black turmeric extract (BT35, BT40, and BT45). The result showed that treatments were not significantly different (P>0,05) on recovery rate, survival rate, and WBC, but significantly different in hematocrit percentage (P<0,05). The highest of fish recovery rate and survival rate (100%) were obtained in BT40. This research concluded that immersion dosage of 40 mg/L black turmeric rhizome ethanol extract could treating catfish infected with *A. hydrophila*.

## INTRODUCTION

Diseases caused by bacterial infections are a major problem in fish farming. *Aeromonas hydrophila* is a pathogen that frequently causes infectious diseases in freshwater fish, including catfish (*Clarias gariepinus*). The disease caused by this bacterium is known as Motile Aeromonas Septicemia (MAS). Infected catfish exhibit clinical symptoms such as skin inflammation, skin ulcers that penetrate the muscle, fin damage, and hemorrhage (Abidin *et al*., 2022; Armin *et al*., 2023). This disease has been reported to cause mass mortality in farmed fish (Zhang *et al*., 2016).

Control measures for infectious diseases in fish generally still use antibiotics. However, the continuous use of antibiotics has been shown to have negative impacts on pathogenic bacteria, the treated fish, the environment, and even consumers. The use of antibiotics to control infectious diseases has been reported to cause bacterial resistance to commonly used antibiotics (Suyamud *et al*., 2024), the emergence of antibiotic residues in fish, which impact food safety and cause allergies in humans as consumers (Rico & Van den Brink, 2014; Liu *et al*., 2017). Furthermore, antibiotics that are not fully decomposed in aquatic systems can cause aquatic environmental pollution, disrupt the balance of aquatic ecosystems, alter the composition of natural microbiota in the water, and harm non-target organisms such as plankton (Cherian *et al*., 2023).

To mitigate the negative impacts of antibiotic use in aquaculture, the use of medicinal plants, also known as phytopharmaceuticals, can be an alternative solution for fish treatment. The use of phytopharmaceuticals is known to be more environmentally friendly and sustainable because they contain active compounds that are more easily degraded, thus leaving no synthetic antibiotic residues in fish tissues and water (Awad & Awaad, 2017). Furthermore, phytopharmaceuticals also have immunostimulant effects (Harikrishnan *et al*., 2011) and a complex mode of action, enabling them to simultaneously possess antibacterial, antiparasitic, antifungal, and antiviral properties (Yilmaz & Tan, 2022).

One of phytopharmaceutical whose efficacy in treating fish diseases can be tested is black turmeric. Phytochemical tests of black turmeric rhizome extract revealed that it contains active compounds such as alkaloids, flavonoids, terpenoids, and saponins (Amaliah, 2019; Marliani *et al*., 2021). Alkaloids are known to work by inhibiting bacterial metabolism, inhibiting nucleic acid and protein synthesis, inhibiting cell wall synthesis, and disrupting cell membrane permeability (Yan *et al*., 2021). In controlling bacterial infections, flavonoids play a role in inhibiting the gyrase enzyme, thus preventing the continuation of bacterial DNA replication (Yan *et al*., 2024). Flavonoids are also useful as anti-inflammatories, thus suppressing the impact of lesions that occur during infection (Choy *et al*., 2019). Terpenoids have antitumor, anti-inflammatory, antibacterial, antiviral, antioxidant, anti-aging, and neuroprotective effects (Yang *et al*., 2020). Black turmeric also contains curcuminoids, which have therapeutic, anti-inflammatory, antioxidant, and anticancer effects (Ravindran *et al*., 2013). Black turmeric is also known to contain essential oils with antibiotic properties (Kamazeri *et al*., 2012). Based on the description above, this research aimed to determine the effect of different concentrations of black turmeric extract on treating diseases caused by *A. hydrophila* infection in catfish.

One of phytopharmaceutical worthy of testing for its efficacy in treating fish diseases is black turmeric. The results of phytochemical tests on black turmeric rhizome extract revealed that it contains active compounds such as alkaloids, flavonoids, terpenoids, and saponins (Amaliah, 2019; Marliani *et al*., 2021). Alkaloids are known to work by inhibiting bacterial metabolism, inhibiting nucleic acid and protein synthesis, inhibiting cell wall synthesis, and disrupting cell membrane permeability (Yan *et al*., 2021). In controlling bacterial infections, flavonoids play a role in inhibiting the gyrase enzyme, thus preventing the continuation of bacterial DNA replication (Yan *et al*., 2024). Flavonoids are also useful as anti-inflammatories, thus suppressing the impact of lesions that occur during infection (Choy *et al*., 2019). Terpenoids have antitumor, anti-inflammatory, antibacterial, antiviral, antioxidant, anti-aging, and neuroprotective effects (Yang *et al*., 2020). Black turmeric also contains curcuminoids, which have therapeutic, anti-inflammatory, antioxidant, and anticancer effects (Ravindran *et al*., 2013). Black turmeric is also known to contain essential oils with antibiotic properties

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## METHODS

### Materials

The test animals used were catfish fingerlings weighing between 16 to 30 grams, totaling 60 individuals, obtained from a Community Hatchery Unit in Cialam Jaya Village, South Konawe Regency. The *Curcuma aeruginosa* (black turmeric) rhizomes used were sourced from a traditional market in Kendari City. During the rearing period, the fish were fed with commercial pellet feed containing approximately 35% protein.

### Research Design

This research employed a Group Randomized Design consisting of four treatments and three groups, detailed as follows:

- BT0 : Control group (no *C. aeruginosa* extract)
- BT35 : Treatment with 35 mg/L *C. aeruginosa* extract
- BT40 : Treatment with 40 mg/L *C. aeruginosa* extract
- BT45 : Treatment with 45 mg/L *C. aeruginosa* extract

Note: Dosage determination is based on the results of the Brine Shrimp Lethality Test (BSLT) of *C. aeruginosa* extract at a concentration of 37.73 mg/L (Zulfiah *et al*., 2020). Grouping based on fish weight:

- Group 1 : Fish weighing 16–20 g
- Group 2 : Fish weighing 21–25 g
- Group 3 : Fish weighing 26–30 g

### Preparation of Rearing Containers

The containers used were aquariums measuring 30 cm × 30 cm × 30 cm, each equipped with an aeration system (12 units total). Every aquarium was thoroughly cleaned using a brush and detergent, then rinsed with clean water. Afterward, each aquarium was filled with water up to a height of 20 cm. The test fish were introduced into the tanks 24 hours later, with five fish placed in each container.

### *Curcuma aeruginosa* Rhizome Extraction

The rhizomes of black turmeric were first cleaned, then thinly sliced and oven-dried at approximately 50°C for four days. Once dried, the rhizomes were ground into a fine powder. The extraction process used the maceration method, where 500 grams of rhizome powder were soaked in 1000 ml of ethanol for 24 hours, repeated three times. The resulting solution was filtered using filter paper. The filtrate was then concentrated using a rotary evaporator at ±50°C and further dried in an oven at the same temperature to obtain a paste-form extract.

### Fish Rearing and Bacterial Challenge Test

The test fish were reared for 14 days. During the first 7 days, the fish were reared under normal conditions. On the seventh day, the fish were infected with *A. hydrophila* at a concentration of $10^7$ CFU/mL, administered via intramuscular injection. After the injection, the fish were monitored daily for the next 7 days. Throughout the maintenance period, fish were fed at 5% of their biomass, with feeding done twice a day.

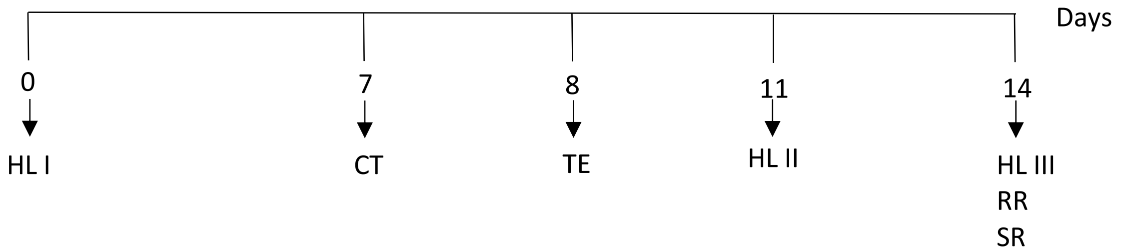


Figure 1. Treatment Time and Research Parameters Observation

Where:
CT : challenge test with *A. hydrophila*
TE : treatment with black turmeric rhizome extract
OB I : first observation of hematocrit and leukocyte
OB II : second observation of hematocrit and leukocyte
OB III : third observation of hematocrit and leukocyte
RR : recovery rate
SR : survival rate

**Treatment Procedure**

Treatment was carried out by immersion. The catfish that had been injected with *A. hydrophila* for 24 hours were immersed in black turmeric ethanol extract at concentrations of 0 mg/L, 35 mg/L, 40 mg/L, and 45 mg/L for 30 minutes. After immersion, the fish were returned to their respective rearing containers.

**Observation**

**Recovery Rate (RR)**

RR (%) = (Number of recovered fish / Number of sick fish) × 100%

Note: Recovered fish are those that show visible signs of improvement and no longer exhibit clinical symptoms (Hasmin *et al*., 2025).

**Survival Rate (SR)**

$$\mathrm{SR}\ (\%) = \frac{\mathrm{Nt}}{\mathrm{N0}} \times 100\%$$

Where:
Nt = Number of fish alive at the end of the rearing period
N0 = Number of fish at the beginning of the rearing period (Hasmin *et al*., 2025)

**Hematocrit**

Hematocrit (He) is obtained using the method of Anderson & Siwicki (1995). A blood sample is drawn into a microhematocrit capillary tube, filling three-quarters of its length. Crystoceal seals one end of the tube to a depth of approximately 1 mm, and the tube is then centrifuged at 5,000 rpm for 5 minutes. A ruler is used to measure the hematocrit level in the tube. The formula used to calculate the hematocrit percentage is as follows:

$$\mathrm{He}(\%) = \frac{\mathrm{a}}{\mathrm{b}} \times 100\%$$

**Leukocyte Count (WBC)**

The leukocyte count/white blood cells (WBC) is determined using the method of Blaxhall & Daisley (1973). A white bead helps draw blood into the pipette when it reaches the 0.5 mark. Then, Turk's solution is added until the mixture reaches the 11 mark. The pipette is shaken in a figure-eight motion for about 3-5 minutes to mix the solution thoroughly. After the first drop of blood is discarded, the next drop is placed on a hemocytometer equipped with a cover glass. A microscope is used to count leukocytes. Counting takes place inside four large 1 mm × 1 mm squares. The formula for calculating the number of leukocytes is as follows:

$$WBC = n \times 50 \text{ cells/mm}^3$$

Where:
WBC : Number of leukocytes in 1 µL (cells/mm$^3$)
n : Number of leukocytes in 4 counting chambers
V : Volume of 4 counting chambers (0.4 mm$^3$)
P : Dilution factor (20×)

**Data Analysis**

Data on blood parameters (hematocrit and WBC), recovery rate, and survival rate were analyzed using Analysis of Variance (ANOVA). If significant differences were found, further analysis was conducted using the Least Significant Difference (LSD) test.

## RESULTS

In general, catfish infected with *A. hydrophila* and then treated with black turmeric rhizome ethanol extract showed better development compared to the control. Although statistical analysis showed no significant differences between treatments in either parameter (P>0.05), BT40 was proven to increase the recovery and survival rates of the test fish to 100% (Figures 2 and 3).

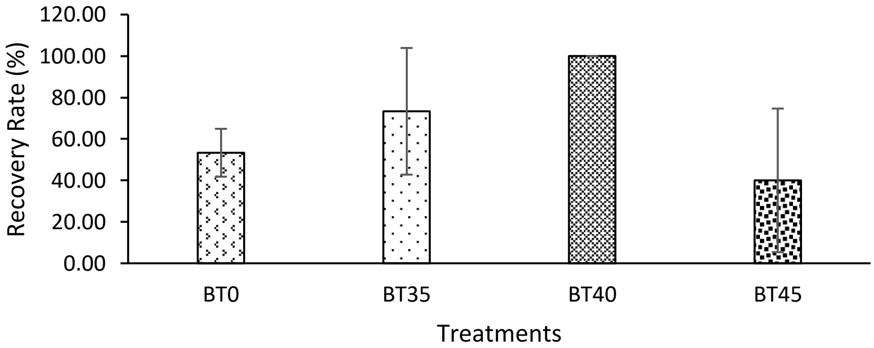


Figure 2. Recovery Rate of Catfish After Being Infected by *A. hydrophila* and then Treated with *C. aeruginosa* Ethanol Extract

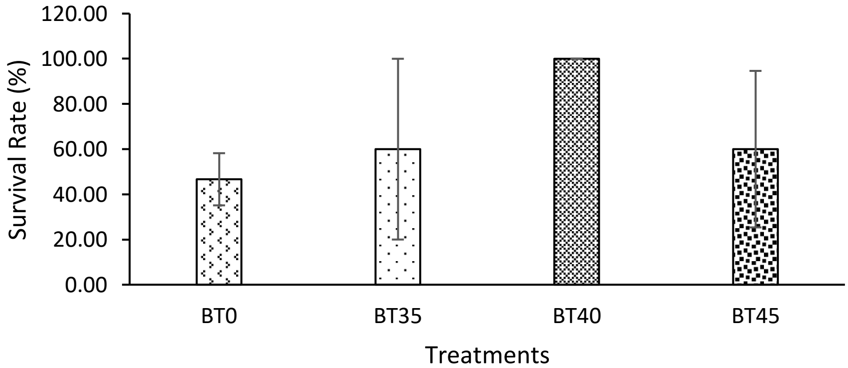


Figure 3. Survival Rate of Catfish at the End of Rearing

Observations on day 11 showed a decrease in the hematocrit in catfish in all treatments. However, the hematocrit in fish treated with black turmeric rhizome ethanol extract was higher and significantly different from the control (P<0.05). The same pattern was again observed on day 14 (Figure 4).

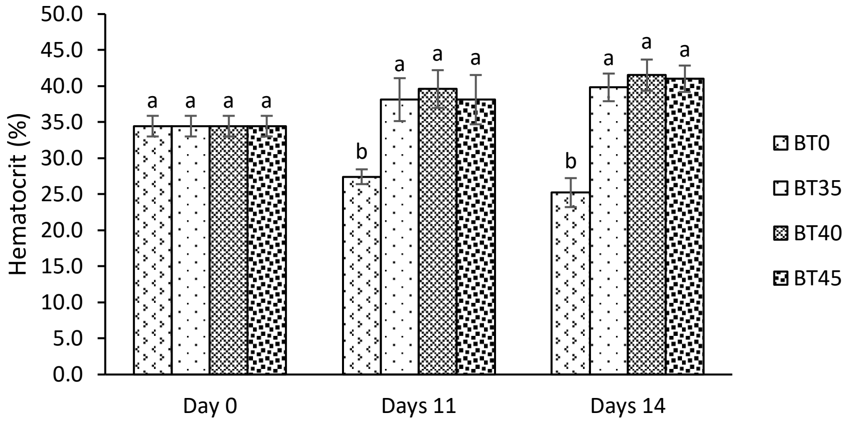


Figure 4. Hematocrit of Catfish Before and After Infection by *A. hydrophila*

The WBC of catfish in this observation showed a decrease in the number observed on both days 11 and 14. On day 14, actually larger WBC were obtained in the three treatments given black turmeric rhizome ethanol extract, but the results of the analysis showed that there was no significant difference between the treatments (P>0.05).

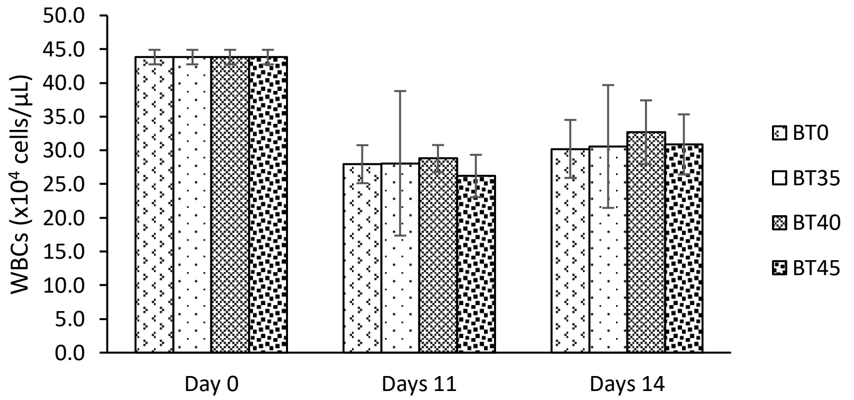


Figure 5. The WBC of Catfish Before and After Infection by *A. hydrophila*

## DISCUSSION

Observations of the recovery rate in this study were conducted based on visual diagnosis by observing changes in the clinical symptoms of the test fish, where fish that began to recover experienced active movement again, a return to normal appetite, hemorrhagic and ulcer wounds in the test fish had healed (Abidin *et al*., 2022; Armin *et al*., 2023). The BT40 treatment resulted in the highest fish recovery rate, reaching 100% after immersion in black turmeric rhizome extract. This value obtained was better than the BT35 and BT45 treatments, which were 73.3 and 40.0, respectively. This indicates that the BT40 treatment had a more optimal impact on the recovery of the test fish from MAS disease. The active compounds contained in the ethanol extract of black turmeric are thought to have eliminated pathogenic bacteria. According to Amaliah (2019) and Marliani *et al*. (2021), black turmeric contains active compounds such as alkaloids, flavonoids, terpenoids, and saponins. Xie *et al*. (2015) explained that the direct antibacterial activity of flavonoids is primarily associated with the mechanisms of inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, and inhibition of bacterial energy metabolism. Thus, treatment with ethanol extract of black turmeric rhizome has successfully increased the recovery of catfish infected with *A. hydrophila*. The BT45 treatment resulted in a lower recovery rate than the BT0 (control) treatment, suggesting that the dose had reached an overdose level for the test animals. However, excessive application of medicinal plant extracts affects tissue damage and physiological activity of the fish (George *et al*., 2023). In this study, the recovery rate appeared to significantly contribute to the survival rate of the fish. The BT40 treatment, which resulted in a 100% recovery rate, also resulted in a 100% survival rate of the test fish.

The hematocrit level of catfish before being challenged with pathogenic bacteria was 34.5%. On days 4 and 7 post-challenge, the hematocrit level of BT0 fish decreased compared to before the challenge. Meanwhile, in the BT35, BT40, and BT45 treatments, the hematocrit level increased. Hemorrhage and ulcers due to *A. hydrophila* infection are suspected to be responsible for the decreased hematocrit level (Abidin *et al*., 2002). In addition, low hematocrit levels may also occur because A. hydrophila secretes hemolysin, which causes hemolysis of erythrocytes (Kusdarwati *et al*., 2021; Xiong *et al*., 2021). Lower hematocrit levels can indicate an unhealthy condition of the fish. Treatment with black turmeric rhizome extract on catfish infected with *A. hydrophila* bacteria has contributed to maintaining hematocrit levels. The content of active compounds such as flavonoids, terpenoids, and curcuminoids with anti-inflammatory properties (Choy *et al*., 2019; Yang *et al*., 2020; Ravindran *et al*., 2013) is thought to have prevented inflammation in erythrocyte cells so that hematocrit levels are maintained in better conditions compared to the control (BT0). In addition, active compounds with antimicrobial properties such as alkaloids, flavonoids, and terpenoids (Shamsudin *et al*., 2022; Das & Ruhal, 2025) are thought to play a role in eliminating pathogenic cells, thereby preventing the impact of *A. hydrophila* attacks on erythrocytes.

The WBC are cells that play a role in the immune system. Bacterial infections in fish can cause an inflammatory response characterized by changes in leukocyte activity and profile (Titus *et al*., 2024). On the fourth day after pathogen infection, WBC decreased in all treatments. This pattern aligns with the research findings of Azlan *et al*. (2021) that showed a decrease in WBC after infection. Bacterial infections in fish trigger mass migration of WBC from hematopoietic organs to inflamed tissues (Titus *et al*., 2024). The decrease in WBC is likely related to their function in localizing and eliminating pathogens through phagocytosis (Soliman *et al*., 2021). On the 7th day after experimental pathogen infection, WBC increased in all treatments, including the control (BT0). Fish given black ginger rhizome extract had higher WBC counts than those in the control. The contribution of black ginger rhizome extract to pathogen elimination is thought to have helped reduce the physiological stress on fish during pathogen elimination, thus allowing hematopoietic organs to produce higher WBC counts compared to the BT0.

## CONCLUSION

The conclusion of this research is that the ethanol extract of black turmeric rhizome (*C. aeruginosa* Roxb) can be used to treat *A. hydrophila* infections in African catfish. A dose of 40 mg/L of ethanol extract of black turmeric rhizome applied through the immersion method was the best dose in this research for treating catfish infected with *A. hydrophila*.

## ACKNOWLEDGEMENT

This research was conducted independently and funded by the research team, without financial support from any party. This published article is part of Riri Mulfiani's undergraduate thesis at the Aquaculture Department. We would like to thank the laboratory staff of the Faculty of Fisheries and Marine Sciences University of Halu Oleo for their assistance in this research.

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