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Short Communication

Effects of the Combination of Honey and *Euphorbia hirta* on Controlling *Aeromonas hydrophila* in Catfish (*Clarias gariepinus*)

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Abstract

Honey and *Euphorbia hirta* are natural ingredients that may serve as alternative treatments for *Clarias gariepinus* catfish infected with *Aeromonas hydrophila* bacteria. Both honey and *E. hirta* contain bioactive compounds with known antibacterial properties. This study aims to investigate the effects of combining honey and *E. hirta* on various hematological parameters, including erythrocytes, leukocytes, MCH, MCV, as well as histopathological and morphological changes in *C. gariepinus* catfish infected with *A. hydrophila*. This study used an experimental design involving three treatment combinations of honey and E. hirta, which were A (2:1), B (1:1), C (1:2) and a control with three replications. The treatments were administered to the fish through immersion. Honey was diluted at a 50% concentration in distilled water. Meanwhile, *E. hirta* was prepared as a coarse powder and boiled at a 3% concentration. The results showed that the combination of honey and *E. hirta* affected the hematological, histopathological, and morphological parameters of *C. gariepinus* catfish within the normal ranges. The most effective treatment was found to be treatment C (1:2). Therefore, the combination of honey and *E. hirta* is potential as a therapeutic option for bacterial infections in fish.

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1. Introduction

The use of natural ingredients for treating fish diseases is widely recognized as a viable alternative among farmers (Pandey *et al.*, 2012; Caruso *et al.*, 2017; Liao *et al.*, 2022). This is attributed to the lack of negative impacts associated with natural treatments compared to synthetic antibiotics, as well as the high concentration of bioactive compounds contained in natural ingredients (Su *et al.*, 2020; Rahimi *et al.*, 2022). In addition, natural ingredients have demonstrated efficacy in treating fish diseases, especially bacterial infections (Hammed *et al.*, 2015; Aisiah *et al.*, 2020; Rosidah *et al.*, 2021).

Clarias gariepinus, commonly known as catfish, is frequently cultivated in Indonesia, including East Nusa Tenggara. This is because catfish are easy to cultivate, grow rapidly, and easily adapt to various aquatic environments (Andriyono *et al.*, 2022; Andriani *et al.*, 2023; Liufeto *et al.*, 2023). However, bacterial infections pose a significant challenge in aquaculture, often resulting in substantial losses for farmers (Enyidi and Maduakor, 2017; Afolabi *et al.*, 2020; Mufidah *et al.*, 2022). A previous study reported a total loss of 4.0 million kg of regional catfish due to diseases, with 1.0 million kg caused by *Aeromonas hydrophila* infections (Peterman and Posadas, 2019).

Aeromonas hydrophila is a prevalent pathogen affecting *C. gariepinus*, causing symptoms such as wounds and damage to the fins and skin (Kusdarwati *et al.*, 2017; Zubaidah *et al.*, 2019). This bacterial infection can cause changes in blood cell counts as the hemolysin toxin produced by *A. hydrophila* can damage red blood cells, resulting in lysis and a subsequent decrease in their numbers. Therefore, the blood profile of fish serves as an indicator of their health (El-Salam *et al.*, 2018; Abd-Allah *et al.*, 2019; Esmaeili, 2021; Seibel *et al.*, 2021; Yanuhar *et al.*, 2021). Moreover, the health status of fish can be assess through the examination of vital organs such as the liver, kidneys, and gills. The extent of damage present in these tissues can provide valuable insights into the overall health and well-being of the fish (Rašković *et al.*, 2013; Avrilia *et al.*, 2022). Although morphological observations are a straightforward method to determine fish health, they should be complemented by blood and tissue analyses (Strzyzewska *et al.*, 2016). Therefore, observations on hematology, histopathology, and morphology are essential indicators for determining fish health (Hamouda *et al.*, 2019; Velichkova *et al.*, 2019; Rosidah *et al.*, 2020).

Honey is a natural ingredient that is being developed as an antibacterial ingredient for treating fish diseases (Andleeb *et al.*, 2014; Nolan *et al.*, 2019; Almasaudi, 2021; Balázs *et al.*, 2023; Zakaria *et al.*, 2023). Research has shown that honey can control bacterial infections on fish, due to the presence of bioactive compounds such as alkaloids, saponins, and terpenoids (Salosso, 2019a, 2019b; Da Cunha *et al.*, 2020). Honey disrupts bacterial protein synthesis by causing protein leakage into the bacterial suspension, thereby compromising the permeability of the bacterial cytoplasmic membrane (Al-Sayaghi *et al.*, 2022; Erwan *et al.*, 2022).

Similarly, *Euphorbia hirta* is a natural ingredient that has antibacterial properties (Tran *et al.*, 2020; Issa *et al.*, 2021). *E. hirta* is known to contain antibacterial compounds including flavonoids, alkaloids, tannins, saponins, and terpenoids (Ahmad *et al.*, 2017; Jakhar and Dahiya, 2017; Oseni *et al.*, 2019; Silalahi, 2021; Puspitasari *et al.*, 2022). Its antibacterial activity has been demonstrated through inhibition zones against *Escherchia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Shanmugam *et al.*, 2017). The minimum inhibitory concentration (MIC) test of *E. hirta* against *A. hydrophila* showed moderate antibacterial activity compared to *Zingiber officinale*, *Annona reticulata*, and *Perilla fruescens* (Dao *et al.*, 2020).

Both honey and *E. hirta* have shown potential in controlling *A. hydrophila* infections in fish, aiding their defense against bacterial and other microbial infections (Salosso *et al.*, 2020; Dawan *et al.*, 2021; Kari *et al.*, 2022; Semwal *et al.*, 2023). Previous in vitro studies have confirmed the antibacterial activity of *E. hirta* against *A. hydrophila* through an inhibition zone (Salosso and Jasmanindar, 2014). However, honey must be diluted to be used effectively due to the aqueous environment of fish. Therefore, it is beneficial to combine honey with other ingredients, such as *E. hirta*, which contains a higher concentration of active compounds. Several studies have explored the effectiveness of the combination of honey and *E. hirta* in preventing (Jumina *et al.*, 2024) and treating (Salosso *et al.*, 2023) *A. hydrophila* infection in *C. gariepinus* catfish. Nevertheless, these studies primarily focused on hematological parameters, such as erythrocyte count, leukocyte count, and hemoglobin level, as well as fish survival rate. There has been limited research on the changes in histopathological, morphological, and hematological parameters, such as mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). MCV and MCH measurements are crucial for evaluate the ratio of hematocrit and hemoglobin in erythrocytes. Therefore, this study aims to investigate the effects of combining honey and *E. hirta* in the form of liquid, on the hematology, histopathology and morphology of *C. gariepinus* catfish infected with *A. hydrophila*.

2. Materials and Methods

2.1 Materials

2.1.1 The equipments

The equipments used in this study were a blender, Whatman filter paper number 42, aquarium size 60 cm x 35 cm x 35 cm, thermometer, pH meter, One Med disposable syringe size 1 cc, eppendorf tube, small bottle, rotation microtome, and Olympus microscope.

2.1.2 The materials

The materials used in this study were C. gariepinus catfish from the Dry Laboratory of the Faculty of Animal Husbandry Marine and Fisheries, *A. hydrophila* from The Fish Quarantine Center, Forest honey from North Central Timor (Kefa) Regency, *E. hirta* plants from Liliba Village Kupang City, 3.8% Na-citrate, distilled water, H-pro pellet feed, formalin, alcohol, xylene, paraffin wax, hematoxylin and eosin (HE).

2.1.3 Ethical approval

This experiment was performed on the basis of approval by the laboratory animals use the research ethics committee of faculty of veterinary medicine [060/KEH/SK/XI/2021], Universitas Nusa Cendana, Indonesia.

2.2 Methods

2.2.1 Honey and E. hirta preparation

Forest honey samples were obtained from North Central Timor (Kefa) Regency, East Nusa Tenggara Province. Meanwhile, *E. hirta* plants were obtained from Liliba Village, Kupang City. This wild plant grows in the tropics. These two samples were collected during the dry season. The preparation of honey and *E. hirta* followed established methods (Salosso *et al*., 2023). Pure Kefa forest honey was diluted with distilled water at a 50% concentration. This dilution was achieved by mixing 500 ml of honey with 500 ml of distilled water, resulting in 1,000 ml of a 50% honey solution, which was then prepared for blending with *E. hirta* according to the specified treatment ratios. On the other hand, the *E. hirta* plants were washed and dried before being cut into two to three cm pieces and blended into a coarse powder. This powder was boiled in distilled water at a 3% concentration (3 grams of powder per 100 ml of distilled water) and left for six hours. According to Salosso and Jasmanindar (2014), aqueous extracts of fresh leaves exhibit antibacterial activity. As a result, using boiled water extracts facilitates the application to fish. After boiling, the extract was left for six hours to maximize the dissolution of active compounds. The resulting extract was then filtered using Whatman filter paper number 42.

2.2.2 Preparation of C. gariepinus catfish

C. gariepinus catfish were sourced from the Dry Laboratory of the Faculty of Animal Husbandry, Marine and Fisheries, Universitas Nusa Cendana (Kupang, East Nusa Tenggara). The fish, averaging 11 ± 0.98 cm in length, were housed with six fish per rearing tank with a volume of 30 L of water. The fish were acclimated for seven days with continuous aeration. The water quality was maintained at a temperature of 28-31ºC and pH of 8-8.2, in accordance with the Indonesian National Standard (SNI 01-6484.3-2000). During the acclimatization process, the fish were fed pelleted feed twice a day in the morning and evening.

2.2.3 Infection of C. gariepinus with A. hydrophila

C. gariepinus catfish were fasted the day before the treatment. They were then infected with *A. hydrophila* at a density of 10^6 CFU mL^{-1} through injection at the base of the tail, administering 0.1 ml per fish. Within six to 12 hours, all fish showed symptoms of infection with different characteristics including body discoloration, wounds around the injection spot, and abnormal swimming movements. The fish were subsequently transferred to treatment aquariums.

2.2.4 Catfish C. gariepinus treatment

C. gariepinus catfish that showed symptoms of infection were transferred to aquariums containing a combination of honey and *E. hirta* for treatment through immersion. Each aquarium was filled with 10 L of water. This study used a completely randomized design. The treatment ratios of honey and *E. hirta* used were 2:1 (treatment A), 1:1 (treatment B), and 1:2 (treatment C). A control group (infected fish without honey and *E. hirta* treatment) was also included, with each treatment and control having three replicates. Immersion was carried out for 1.5-2 minutes and repeated for 10 days (Salosso *et al*., 2023). The duration is considered safe for the fish as they did not show symptoms of stress. The 10-day repetition was based on the observed recovery symptoms of the fish. Subsequently, the fish were transferred to rearing aquariums. On the 12th day, observations of hematological, histopathological and morphological parameters were carried out.

2.2.5 Blood sampling and hematological observation of C. gariepinus catfish

Blood samples of *C. gariepinus* catfish were colelcted from the area in front of the caudal fin using a 1 ml syringe previously rinsed with 3.8% Na-citrate. The collected blood samples were placed into an Eppendorf tube containing 10% anticoagulant EDTA (ethylene diamine tetra acetic acid) (Kefas *et al*., 2015). The hematological observations of *C. gariepinus* catfish included erythrocytes (red blood cells), leukocytes (white blood cells), mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV). The methods for observing erythrocytes and leukocytes followed the procedure outlined by (Sheikh *et al*., 2022). Meanwhile, MCH and MCV measurements were conducted according to the method by (Al-Zahaby *et al*., 2017). These observations were performed before infection, after infection, and after treatment of the *C. gariepinus* catfish.

2.2.6 Organ sampling and histopathological observation of C. gariepinus catfish

The organs of the *C. gariepinus* catfish were collected, including the liver, kidneys and gills, using surgical instruments. The collected organ samples were placed in small bottles containing 10% formalin for 24 hours. Subsequently, the tissues were dehydrated with alcohol and cleared in xylene and embedded in paraffin wax. The tissues were immersed in molten paraffin at a temperature of 56-60ºC for two hours, followed by sectioning into 4-5µm thick slices using a rotation microtome. Thereafter, the sections were stained with hematoxylin and eosin (HE) (Maftuch *et al*., 2018). The preparations were examined under a microscope at 400x magnification to analyze the tissue damage descriptively.

2.2.7 Morphological observation of C. gariepinus catfish

Morphological observations of the *C. gariepinus* catfish were carried out by looking at changes in the body of the fish from the onset of infection until the symptoms of recovery appeared. The parameters observed were body color, fin condition, scale condition, eye and body shape, as well as the presence of scars, especially at the injection spot (Chen *et al*., 2019).

2.3 Analysis Data

Hematological data were analyzed using Microsoft Excel and ANOVA. If significant effects were identified, subsequent analysis was performed using the Duncan's test. Meanwhile, histopathological and morphological data were analyzed descriptively.

3. Results and Discussion

3.1 Results

3.1.1 Hematological parameters of C. gariepinus

The hematological analysis of *C. gariepinus* catfish infected with *A. hydrophila* and treated with a combination of honey and *E. hirta* revealed different results in terms of the mean counts of erythrocytes, leukocytes, MCH, and MCV (Table 1). Among the treatments, treatment A, with a ratio of honey to *E. hirta* of 2:1, showed a mean erythrocyte count of 2.37 ± 0.06 $\times 10^6$ cells/mm^3, which was closest to the level observed in healthy fish. This value was similar to those found in treatments B and C. For leukocyte, treatment C yielded a mean count of 29,733 ± 1,096.97 cells/mm^3, which was closest to that of healthy fish. In terms of MCH and MCV, treatment B yielded values closest to the healthy range, with MCH at 47.89 ± 1.16 pg and MCV at 101.48 ± 8.88 fL.

3.1.2 Histopathological changes in liver, kidney, and gills

The histopathological analysis of the liver, kidney and gills of *C. gariepinus* catfish infected with *A. hydrophila* and treated with a combination of honey and *E. hirta* revealed different results. For the liver tissue (Figure 1), treatment C showed features closest to those of normal tissue. In contrast, treatment A showed noticeable vacuole degeneration and congestion, while treatment B showed noticeable vacuole degeneration. The liver tissue of the Control group showed severe necrosis. For the kidney tissue (Figure 2), treatment C also showed features closest to those of normal tissue. In contrast, treatment A showed congestion and necrosis, while treatment B showed necrosis. The kidney tissue of the Control group showed severe necrosis. For the gill tissue (Figure 3), treatment C also showed features closest to those of normal tissue, although secondary lamellae adhesion was observed. Treatment A showed secondary lamella adhesion, hyperplasia of hyaline cartilage, and necrosis, while treatment B showed secondary lamella adhesion, inflammation, and hyperplasia of hyaline cartilage. In contrast, the gill tissue of the Control group showed severe necrosis, inflammation, secondary lamella adhesion, and hyperplasia of hyaline cartilage.

3.1.3 Morphological observation and recovery signs

The morphological analysis of *C. gariepinus* catfish infected with *A. hydrophila* and treated with a combination of honey and *E. hirta* revealed similar results (Figure 4). The morphology of the fish was comparable to that of healthy fish across all treatments, although minor scars were present. In contrast, the morphology of the control group showed visible wounds on the body, red spots on the scales, and protruding eyes.

close to the normal range in *C. gariepinus* catfish infected with *A. hydrophila*.

Erythrocytes are crucial for the transportation of oxygen and carbon dioxide in fish (Solomon *et al*., 2015; Gunanti *et al*., 2019). Infected fish, particularly

Table 1. Results of hematological analysis of *C. gariepinus*

Parameters	Healthy fish	Unhealthy fish	12 days after immersion			
			Control	A	B	C
Erythrocyte ($\times 10^6$ cell/mm^3)	2.83 ± 0.31	1.8 ± 0.26	1.73 ± 0.15^a	2.37 ± 0.06^b	2.23 ± 0.06^b	2.27 ± 0.15^b
Leukocyte (cell/mm^3)	28,467 ± 2119.75	34,870 ± 3315.12	35,333 ± 2550.16^c	32,200 ± 1558.85^b	29,867 ± 1001.67^a	29,733 ± 1096.97^a
MCH (pg)	46.00 ± 3.46	52.24 ± 2.19	50.80 ± 3.78^a	49.37 ± 3.97^a	47.89 ± 1.16^a	50.38 ± 1.84^a
MCV (fL)	100.07 ± 2.71	107.29 ± 4.70	107.29 ± 4.70^a	107.15 ± 2.05^a	101.48 ± 8.88^a	101.60 ± 2.00^a

Description: Data are expressed as mean ± SD. Control (infected fish without treatment), A (more honey with a ratio of 2:1), B (balanced between honey and *E. hirta* with a ratio of 1:1), C (more *E. hirta* with a ratio of 1:2). Values in the same row with different superscripts showed significant differences ($p < 0.05$).

3.2 Discussion

3.2.1 Therapeutic Efficacy of Honey and Euphorbia hirta

Honey is known to contain bioactive compounds such as flavonoids, alkaloids, and saponins (Nwankwo *et al*., 2014). In contrast, *E. hirta* is predominantly composed of bioactive compounds such as phenols, flavonoids, tannins, and triterpenoids (Salosso and Jasmanindar, 2014). These compounds are recognized for their antibacterial properties against *A. hydrophila* (Sopiah *et al*., 2018; Bariyyah *et al*., 2019). Pure honey is difficult to apply directly to fish due to its need for dilution, which reduces its antibacterial effectiveness. Therefore, *E. hirta* was added to enhance the antibacterial activity of honey (Salosso *et al*., 2023).

The analysis of fish blood aims to assess their health status (Fazio, 2019; Witeska *et al*., 2022). Blood serves as a defense system in fish, playing a key role in combating bacterial infections (Dangeubun and Metungun, 2017; Radityo *et al*., 2022). In this study, the combination of honey and *E. hirta* was effective in achieving hematological parameter values, such as erythrocytes, leukocytes, MCH and MCV, that were those suffering from bacterial infections, often exhibit a significant decrease in erythrocyte counts (Aly *et al*., 2020). In this study, the combination of honey and *E. hirta* was found to increase the erythrocyte count to the level comparable to that of healthy fish. This increase suggested that the combination of honey and *E. hirta* inhibited bacterial growth, due to the bioactive compounds present in these substances. These compounds have antibacterial properties that can eliminate bacteria, thereby inhibiting the inflammatory process (Stan *et al*., 2021).

3.2.2 Hematological responses and antibacterial action

Leukocytes play an important role in the defense system of fish (Salkova *et al*., 2022). Bacterial infections increase leukocytes counts in fish as a defensive response to the pathogens (Fransira *et al*., 2020). In this study, the combination of honey and *E. hirta* significantly reduced leukocyte counts to near-normal levels compared to the Control group. An increase in leukocytes is a common response to infection, reflecting the reaction to the presence of pathogens in the body. However, leukocyte production typically subsides once the bacterial load decreases.

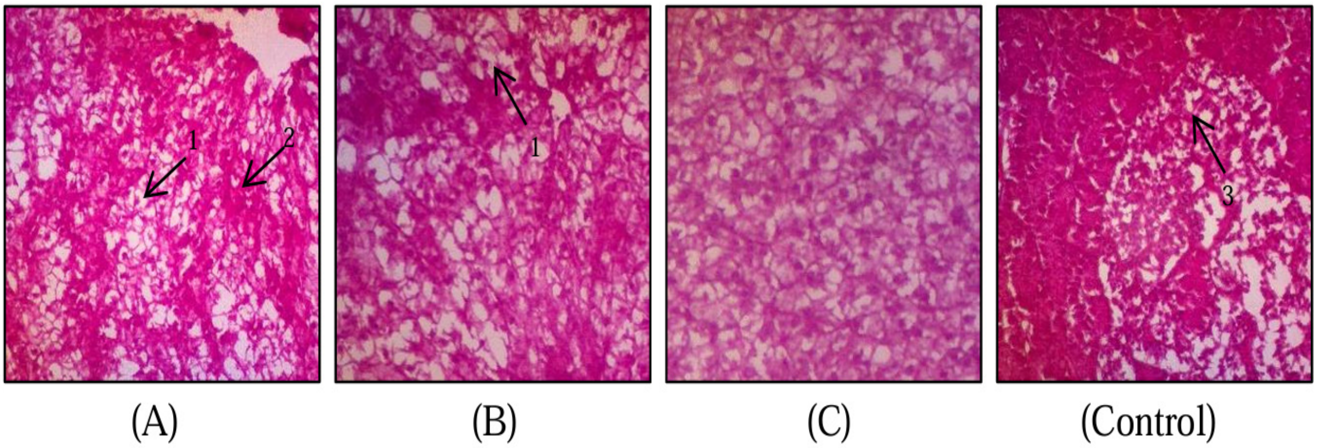

Figure 1. Histopathology of the liver of *C. gariepinus*; (A) treatment A; (B) treatment B; (C) treatment C; (Control) infected fish without treatment (H & E, 100X). (1) vacuole degeneration, characterized by swelling of the cells; (2) congestion, characterized by blood accumulation in the circulating vein; (3) necrosis, characterized by an overall reduction in the size of the nucleus.

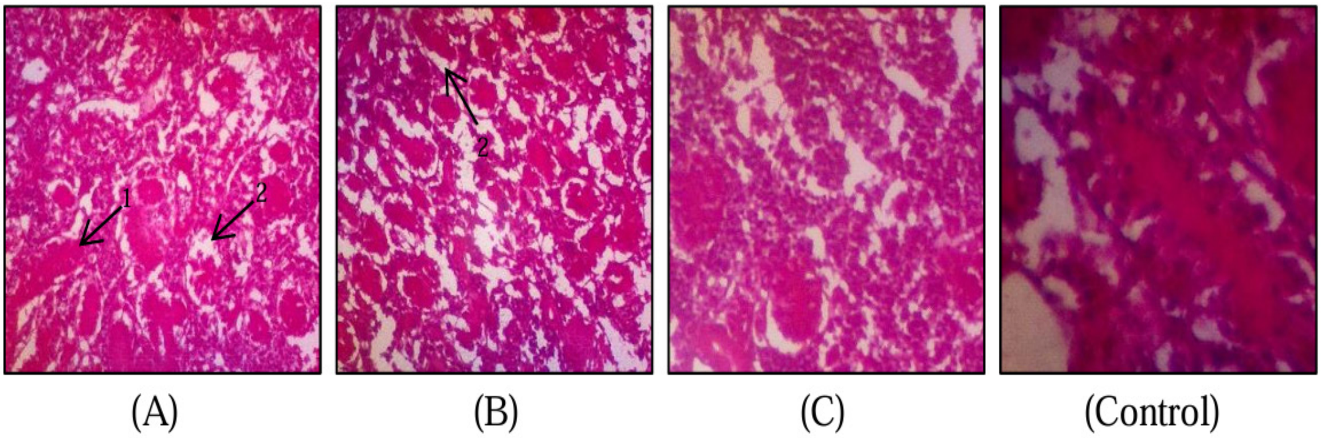

Figure 2. Histopathology of the kidneys of *C. gariepinus*; (A) treatment A; (B) treatment B; (C) treatment C; (Control) infected fish without treatment (H & E, 400X). (1) congestion, characterized by blood accumulation; (2) necrosis, characterized by cell fading.

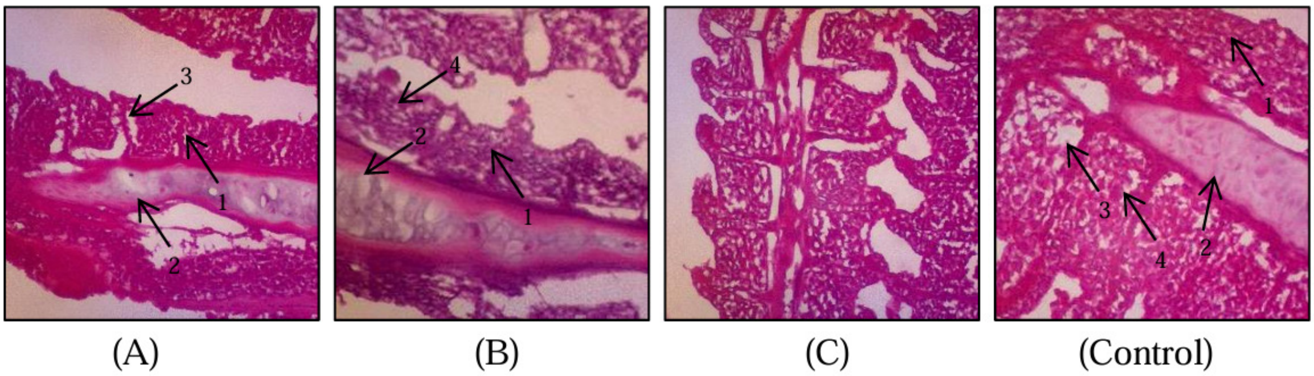

Figure 3. Histopathology of the gill of *C. gariepinus*; (A) treatment A; (B) treatment B; (C) treatment C; (Control) infected fish without treatment (H & E, 100X). (1) secondary lamella adhesion, characterized by the fusion of secondary lamellae; (2) hyperplasia of hyaline cartilage, characterized by tissue thickening; (3) necrosis, characterized by unclear cell membrane; (4) inflammation, characterized by blood clots.

This decrease suggests that the bioactive compounds in honey and *E. hirta* effectively combat pathogenic bacteria (Salosso *et al*., 2020). Specifically, flavonoids present in these substances can inhibit nucleic acid synthesis and alter the permeability of the bacterial membrane, thereby reducing pathogenicity (Xie *et al*., 2014).

MCH and MCV are critical components of the erythrocyte index used to detect anemia in fish (Javed *et al*., 2016; Oparaku *et al*., 2024). This study observed a decrease in MCH and MCV 12 days after immersion post-infection. This erythrocyte index can maintain erythrocyte balance, which plays a role in protecting fish physiology against anemia (Das *et al*., 2021). The secondary metabolites present in the combination of honey and *E. hirta* contribute to bacterial inhibition, thereby reducing the incidence of infection. According to Fransira *et al.* (2019), phenolic compounds possess antibacterial properties, including inducing cytoplasmic leakage in bacteria.

2015; Kaur *et al*., 2018). In this study, the liver tissue of the Control group appeared disorganized with notable damage, including vacuole degeneration and necrosis. Vacuole degeneration, characterized by enlarged liver cells, is a temporary condition that can return to normal (Fahmi *et al*., 2019). Meanwhile, necrosis is characterized by cell death due to acute damage and caused by blood clots (Tresnati and Djawad, 2012). Conversely, the liver tissue treated with a combination of honey and *E. hirta* showed minor damage, such as vacuole degeneration and congestion. Congestion refers to the accumulation of blood in the tissues (Dane and Şişman, 2017). These differences suggested that the combination of honey and *E. hirta* reduced liver damage in *C. gariepinus* catfish. This is due to the antibacterial properties of the compounds in these natural ingredients, such as tannins. Tannins precipitate proteins and damage bacterial cell walls, causing leakage of intracellular materials and impaired metabolic processes, including inhibition of specific enzyme activities and biosynthesis due to damage of

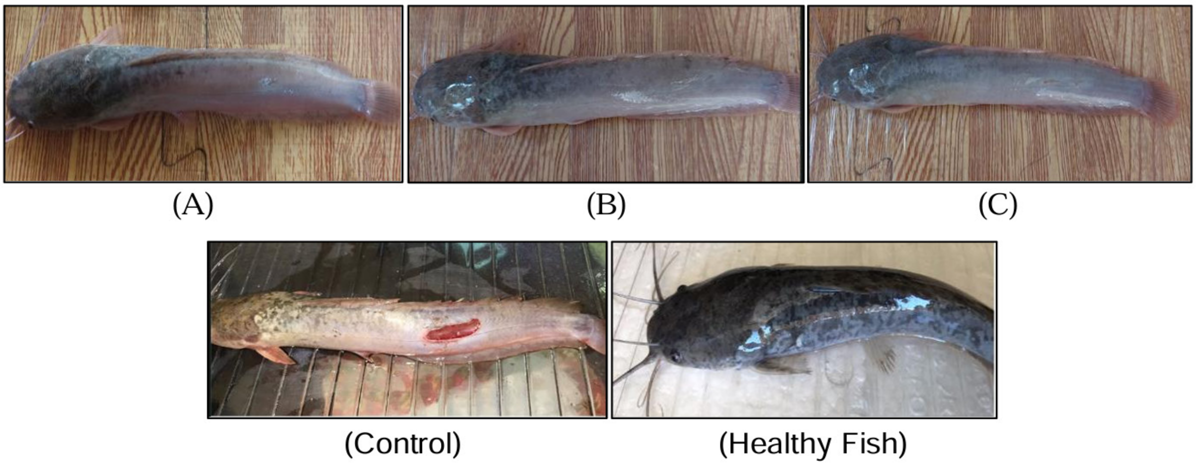

Figure 4. Morphology of catfish *C. gariepinus* post-treatment; (A) treatment A; (B) treatment B; (C) treatment C; (Control) infected fish without treatment; (Healthy Fish).

Histopathological analysis aims to determine the structural changes that occur in tissues organs due to infectious diseases (A'Yunin *et al*., 2020; Zhong *et al*., 2022), which is crucial for understanding the function of the organs. The liver functions as a detoxifier (Hastuti *et al*., 2019), the kidneys regulates body fluid concentration (Outtandy *et al*., 2019), and the gills serve as respiratory organs (Foyle *et al*., 2020). In this study, the combination of honey and *E. hirta* yielded results that were comparable to normal tissues in *C. gariepinus* catfish infected with *A. hydrophila*.

3.2.3 Histopathological and morphological recovery mechanisms

In healthy fish, the liver shows intact tissue with neatly arranged hepatocyte cells (Feist *et al*., the cell membrane (Huang *et al*., 2018; Sartika *et al*., 2021).

In healthy fish, the distal tubules and glomeruli of the kidneys are in good condition (Shahid *et al*., 2021). In this study, the kidney tissue of the Control group showed severe necrosis, which is associated with severe bacterial infections. Conversely, the combination of honey and *E. hirta* resulted in kidney tissue with reduced congestion and necrosis. This finding is consistent with other studies indicating that natural treatments could restore the kidney tissue to near-normal conditions in fish infected with bacteria (El-Salam *et al*., 2018; Aisiah *et al*., 2020). This is attributed to the alkaloid compounds which induce bacterial cell death and help restore kidneys function.

Moreover, alkaloids are known to inhibit bacterial cell wall synthesis (Cushnie *et al*., 2014).

In healthy fish, the gills show regular, undamaged filaments and lamellae (Mustafa *et al*., 2017). In this study, the gill tissue of the Control group showed severe damage, including necrosis, secondary lamella adhesion, inflammation, and hyperplasia of hyaline cartilage. Secondary lamella adhesion, characterized by the fusion of secondary lamellae, causes an obstruction in oxygen uptake (Aliza *et al*., 2021). Hyperplasia is characterized by tissue thickening in response to infection (Rosidah *et al*., 2020). Inflammation of the gill tissue is also known to result from bacterial infections (A'Yunin *et al*., 2020), causing swelling and tissue damage. In contrast, the gill tissue of *C. gariepinus* catfish treated with a combination of honey and *E. hirta* showed less severe damage. This finding is consistent with other studies showing that antibacterial natural ingredients can reduce gill tissue damage in fish (Maftuch *et al*., 2018). The phenolic compounds are known as an antibacterial by causing bacterial cell leakage (Aldulaimi, 2017).

Healthy *C. gariepinus* catfish showed normal morphology with no red spots, wounds, or discoloration. Converselt, *C. gariepinus* catfish in the control group showed morphological changes. For pathogenic bacteria, these symptoms are typically observed between six to 12 hours after infection with *A. hydrophila* bacteria (Kusdarwati *et al*., 2017; Adah *et al*., 2021). These morphological changes are caused by a toxin produced by *A. hydrophila*, namely hemolysin. Hemolysin is known to be able to break down red blood cells, thereby damaging the skin of the fish (Syahidah, 2021). In contrast, the body color of *C. gariepinus* catfish treated with a combination of honey and *E. hirta* returned to normal and the injuries were absent after 10 days of treatment. This is attributed to the bioactive compounds, such as saponins, which possess anti-inflammatory properties (Zhong *et al*., 2022).

4. Conclusion

This study showed that the administration of a combination of honey and *E. hirta* affected the hematological, histopathological, and morphological parameters of *C. gariepinus* catfish infected with *A. hydrophila* bacteria, as evidenced by the erythrocyte and leukocyte counts as well as MCH and MCV levels within the normal ranges. In addition, the histopathological and morphological examinations revealed significant improvements of the liver, kidneys, and gills. The most effective treatment was found to be treatment C, which *E hirta* is more than honey (1:2). Therefore, it can be used as a potential therapeutic option for treating bacterial infection in fish.

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Authors' Contributions

YS devised the main conceptual ideas, collected data, and designed the study. ATR reviewed and monitored the study. AD collected the data and provided critical revisions to the manuscript. IF analyzed data and drafted the manuscript. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

Declaration of Artificial Intelligence (AI)

The authors affirm that no artificial intelligence (AI) tools, services, or technologies were employed in the creation, editing, or refinement of this manuscript. All content presented is the result of the independent intellectual efforts of the authors, ensuring originality and integrity.

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