

Histological changes in the gills and liver of a Nile tilapia (*Oreochromis niloticus*) exposed to Cat Whisker leaf extract (*Orthosiphon aristatus*)

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Abstract

Bacterial infectious diseases are a serious obstacle in the cultivation of tilapia (*Oreochromis niloticus*). The use of herbal ingredients such as cat whisker leaves (*Orthosiphon aristatus*) is an alternative to environmentally friendly treatment because of its antibacterial compound content. This study aims to analyze the antibacterial activity of cat whisker leaf extract against *Pseudomonas aeruginosa* bacteria and its effect on the histopathology of liver organs and gills of tilapia. This study used an experimental method with 4 extract dose treatments (50 ppm, 75 ppm, 100 ppm, and 125 ppm) and negative controls, each with 3 replicates. The first stage included the extraction of cat whisker leaves with ethanol solvent, determination of minimum inhibition concentrations through Optical Density (OD) observation using UV/Vis spectrophotometry, and LC-50 24-hour toxicity test. The second stage involves bacterial infection in fish, followed by soaking in the extract for 24 hours and maintenance for 7 days. The parameters observed included histopathology of the liver and gills and survival rate (SR). The results showed that cat whisker leaf extract was able to inhibit the growth of *Pseudomonas aeruginosa*, which can be seen from the difference in the degree of histological damage to the liver and gills between treatments. The highest survival of tilapia at the end of the maintenance period was found at a dose of 100 ppm with a value of 90% which is the optimal dose to improve the survival of infected fish.

Keywords: Histological changes, Nile tilapia, *Orthosiphon aristatus*, *Pseudomonas aeruginosa*

1. Introduction

Tilapia (*Oreochromis niloticus*) is a leading commodity in Indonesia with the potential to support national food security (Tran et al., 2017). Diseases are the most common obstacle faced by tilapia farmers, resulting in the death of the cultivated fish. Fish disease is a physical, morphological, and functional condition resulting from changes in normal conditions caused by both internal and external factors (Azhar et al., 2022). According to Reverter et al. (2020), the emergence of diseases in fish is the result of weak host factors (fish), presence of pathogenic microorganisms, and poor environmental quality.

Pathogens, such as viruses, bacteria, fungi, protozoa, and parasites are a group of infectious diseases that often occur in fish (Bricknell, 2017). One infectious disease that often attacks tilapia is bacterial disease (Latif, 2020). *Pseudomonas aeruginosa* is the causative agent in tilapia. It is an anaerobic gram-negative bacterium in the form of a pink rod, with polar flagellation and unipolar motility (Mohammed et al., 2023). Aziz and Abdullah (2021) stated that the symptoms shown by fish affected by diseases due to bacterial infections are usually in the form of loss of appetite, injuries to the body surface, bleeding in the gills, an enlarged abdomen filled with fluid, loose scales, and tail fins. If a histological test is carried out, swelling and damage to the liver, kidneys, and spleen of the fish will be observed. According to Des Roza (2018), bacterial infections can be prevented by sterilizing water and equipment that will be used, maintaining good water quality, avoiding stress caused by high stocking density and poor handling, and providing good quality feed.

Efforts to control diseases in fish have been made using chemicals, such as antibiotics. The continuous use of synthetic antibiotics can have a negative impact on the aquatic environment, pathogen resistance, and antibiotic residues, which affect consumer health,

making their use ineffective (Pham et al., 2020). Bacterial diseases in fish can be treated using natural ingredients from plants that have anti stress and immunostimulant properties and contain antibacterial compounds that can be used as medicines for fish affected by diseases (Reverter et al., 2020). One plant that can be used to overcome bacterial diseases in farmed fish is the whisker leaf of cats (*Orthosiphon aristatus*).

According to Faramayuda and Mariani, (2021), cat whisker leaf extracts contain many natural chemical compounds including flavonoids, polyphenols, active proteins, glycosides, essential oils, potassium, terpenoids, and alkaloids. Flavonoids are chemically active compounds in plants that function as antibacterial compounds and can inhibit the function of the bacterial cytoplasmic membrane in bacteria (Górniak et al., 2019). Alkaloid compounds can act as antibacterial agents by interfering with the constituent components of peptidoglycan in bacterial cells, causing the cell wall layer to remain intact and causing cell death in bacteria (Othman et al., 2019).

2. Materials and Methods

2.1. Time and Place

This study was conducted from January to May 2024 in the Parasitic and Fish Disease Laboratory, Productivity and Water Quality Laboratory, and Fish Hatchery Technology and Management Laboratory. Faculty of Marine Sciences and Fisheries, Hasanuddin University.

2.2. Tools and Materials

The tools used in this study were basins, hoses, aeration, thermometers, pH meters, micropipettes, laminar airflow, hot plate, incubator shakers, ovens, vortices, analytical scales, test tubes, Petri dishes, Erlenmeyer flasks, measuring cups, ose needles, microscopes, Bunsen, and Eppendorf tubes.

The materials used in this study were tilapia (*Oreochromis niloticus*) obtained from the Gowa Makassar Freshwater Fish Seed Center; *Pseudomonas aeruginosa* bacteria obtained from the Banten Serang Fish Health and Environment Testing Center; cat whisker leaves obtained from Anggareja Enrekang District, Ethanol, chloramphenicol, filter paper, 70% alcohol, aquades, plasti wrap, aluminum foil, Tryptic Soy Agar (TSA), Tryptic Soy Broth (TSB), formalin 10%, hayem solution, turk solution, and pellets.

2.3. Research Design

This study used an experimental method based on a Completely Randomized Design (CRD). The experiment involved five different treatments, each replicated three times, resulting in a total of 15 randomly arranged experimental units. The treatments consisted of a control negative of fish infected with *Pseudomonas aeruginosa* bacteria without the administration of cat's whiskers leaf extract and four test groups with different extract concentrations. These treatment groups included Treatment A at 50 ppm, Treatment B at 75 ppm, Treatment C at 100 ppm, and Treatment D at the highest concentration of 125 ppm.

2.4. Extract Production

The extraction process using the kinetic maceration method has been previously described (Zainuddin et al., 2019; 2020). Extraction was performed for 3 × 24 h at room temperature. The extraction process was carried out with 96% ethanol solvent. A total of 500 g of simplicia was soaked with 1,500 ml of solvent (1:3) in an Erlenmeyer flask, extracted using a shaker incubator with medium rotation, and then filtered using Whatman no. 1 filter paper to separate the filtrate and residue. The filtrate was then concentrated separately using a rotary evaporator at 90 rpm under vacuum to allow the solvent to evaporate perfectly. The extract was then placed in a clean sterile vial bottle.

2.5. Optical Density (OD) Observation using UV/Vis Spectrophotometer

Optical Density (OD) observation using UV/Vis spectrophotometry was carried out to determine the minimum concentration of cat whisker leaf extract (*Orthosiphon aristatus*) required to inhibit the growth of *Pseudomonas aeruginosa* bacteria after 24 h of incubation period (Ashraf et al., 2018). An inhibition test was performed by preparing a test tube filled with 4.5 ml of sterile TSB medium (Anarkhis et al., 2022).

2.6. Toxicity Test LC50-24 Hours

The LC50 dose was determined using the extract dose based on the results of the minimum inhibition test, which was 50, 100, 150, 200, and 250 ppm. Fifty tilapia were divided into five containers containing 10 liters of water with different concentrations of the extract and observed for 24 h. The fish were kept in containers in the form of basins, with a water volume of 10 L. The number of test animals used was 10 heads/containers with a size between 10-12 cm with a density of 1 head/liter. Tilapia were acclimatized to a new location. The acclimatization process was carried out for three days, during which commercial feed was provided while still considering water quality. Feeding was carried out 2 times a day in the morning at 08.00 and in the afternoon at 16.00.

2.7. Infection of *Pseudomonas aeruginosa* Bacteria in Tilapia (*Oreochromis niloticus*)

Infection of *Pseudomonas aeruginosa* bacteria was carried out using a method described by Budiando et al. (2022) by soaking tilapia for 15 min in 10 liters of water that had been mixed with a bacterial suspension at a concentration of 107 CFU/ml. Fish were observed to behave in the form of fish movement. After the fish were indicated to be infected with *Pseudomonas aeruginosa*, they were transferred to a basin containing a solution of cat whisker leaf extract at different concentrations and soaked for 24 h.

2.8. Soaking Extract *Orthosiphon aristatus*

Coarse extracts of cat whisker leaves (*Orthosiphon aristatus*) were added to the maintenance medium to prepare coarse extracts of cat whisker leaves (*Orthosiphon aristatus*), and calculations were performed based on the volume of the maintenance medium and the dose of the extract used. The extract was weighed, placed in a maintenance container, and then immersed for 24 h. The fish were then transferred to a tank filled with fresh water (0 ppt) for 7 days of storage.

2.9. Histological Gills and Liver *Oreochromis niloticus*

Tilapia that had been infected with *Pseudomonas aeruginosa* and soaked in cat whisker leaf extract (*Orthosiphon aristatus*) at different doses for 24 h were collected from the liver and gills, which were histologically tested to observe changes in the structure of the liver and gills after seven days of maintenance.

2.10. Survival Rate

The survival rate (SR) is the percentage of fish survival compared to the number of stocks, and is expressed as a percentage. The survival rate was calculated using the following formula (Cheikyula and Ojekwu, 2003).

2.11. Data Analysis

The analysis was performed by examining histopathological changes in the gills and liver of tilapia that had been infected with *Pseudomonas aeruginosa* and soaked in cat whisker leaf extract at different doses.

3. Results and Discussion

3.1. Optical Density (OD) Observation using UV/Vis Spectrophotometer

The results of the absorbance value Optical density measured with a spectrophotometer showed the ability of cat whisker leaf extract (*Orthosiphon aristatus*) to inhibit the growth of *Pseudomonas aeruginosa*. Absorbance measurements are presented in **Table 1**.

Table 1. Optical density results of cat whiskers leaf extract at different doses measured from spectrophotometer absorbance values to inhibit the growth of *Pseudomonas aeruginosa*

Dose Treatment (ppm)	Absorbance
50	0.387
100	0.394
150	0.398
200	0.565
250	0.587
Control(+)	0.047
Control(-)	0.748

Note: Positive control (K+) using chloramphenicol 5 ppm chloramphenicol and negative control (K-) without extract.

These results showed that the lowest absorbance value of several concentrations of cat whisker leaf extract was obtained at 50 ppm, with a value of 0.387 ppm. The 50 ppm dose was close to the positive control value of 0.047. The absorbance value for each concentration showed that there was a change in the absorbance value, which was not significantly different with increasing extract concentration. Thus, there are antibacterial compounds in the whisker leaf extract (*Orthosiphon aristatus*) that can inhibit *Pseudomonas aeruginosa* growth.

Cat whisker leaf extract (*Orthosiphon aristatus*) at 50 ppm was the lowest dose that inhibited *Pseudomonas aeruginosa* growth. Turbidity was measured using a spectrophotometer to estimate the concentration of the bacterial cells in the liquid culture. The higher the turbidity, the more bacterial cells present and the greater the bacterial growth (Salihoglu et al., 2019). Cat whisker leaf extract, which contains antibacterial compounds, can affect the turbidity of the liquid culture solution of bacteria by inhibiting the growth or killing bacteria, thereby reducing the number of bacterial cells in the liquid culture and will reduce turbidity. This explains how changes in turbidity can be used to assess the effectiveness of tested antibacterial compounds (Lim et al., 2021).

3.2. Toxicity Test LC50-24 Hours

The results of the LC50 toxicity test using the 24-hour immersion method on several concentrations of cat whisker leaf extract in 10 tilapia (*Oreochromis niloticus*) at different concentrations are presented in **Table 2**.

Table 2. Results of the Toxicity Test LC50-24 Hours of Cat Whisker Leaf Extract (*Orthosiphon aristatus*) in tilapia (*Oreochromis niloticus*) at different concentrations

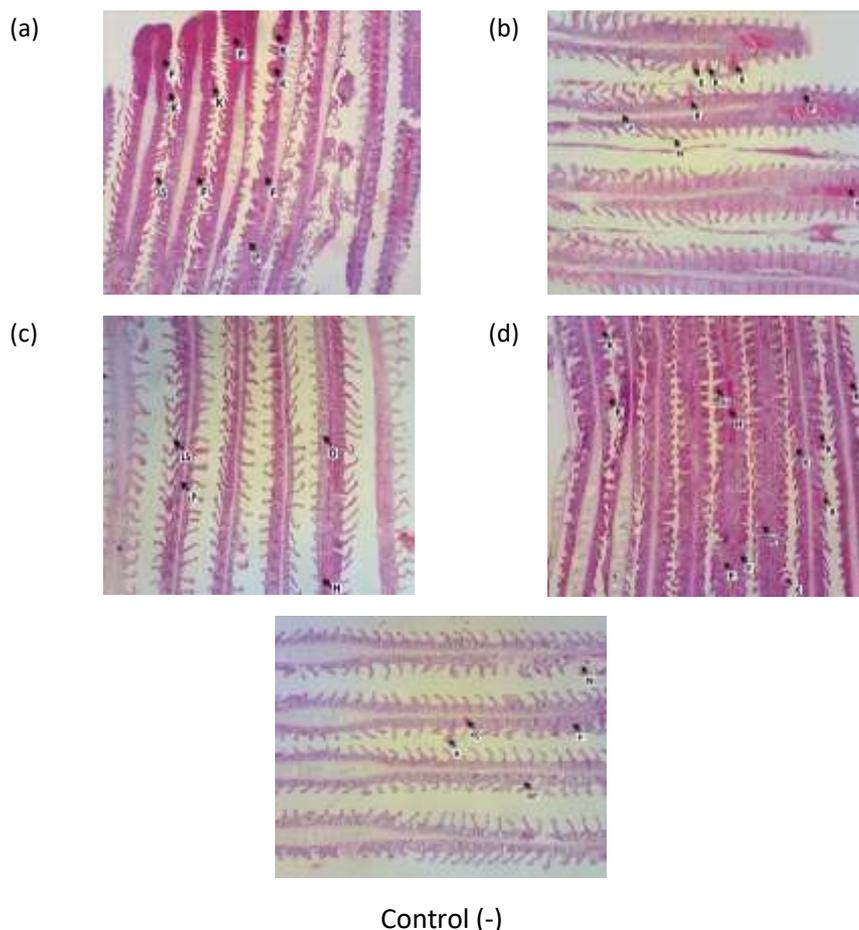
Extract Concentration (ppm)	Total Test Fish	Total Dead Fish
50	10	0
100	10	0
150	10	7
200	10	9
250	10	10

Toxicity tests with soaking of the whisker leaf extract at concentrations of 50 ppm and 100 ppm showed that no fish died, which means that at that dose, the cat whisker leaf extract was not toxic and did not cause death in tilapia. Immersion of the extract at a concentration of 150–250 ppm resulted in the death of tilapia in the range of 7-10 fish,

which means that immersion at this concentration is toxic to tilapia, causing up to 100% death. Toxicity tests using the extract immersion method for fish survival are important to assess the toxicity potential of natural material extracts or chemical compounds against aquatic organisms. Fish were immersed in extract solutions of different concentrations to determine the toxic effects that might arise and their impact on fish survival of the fish (Tanna et al., 2020).

3.3. Histological Gills

Histopathological changes in tilapia gills (*Oreochromis niloticus*) infected with *Pseudomonas aeruginosa* and treated with different doses of whisker leaf extract (*Othosiphon asristatus*) are shown in **Figure 1**.



Note : Symbol (LP) primary lamella, (LS) secondary lamella, (K) parasite cysts, (F) lamella fusion, (H) hemorrhage, (D) Dilatation, (DLP) primary lamella dilation, and (N) necrosis.

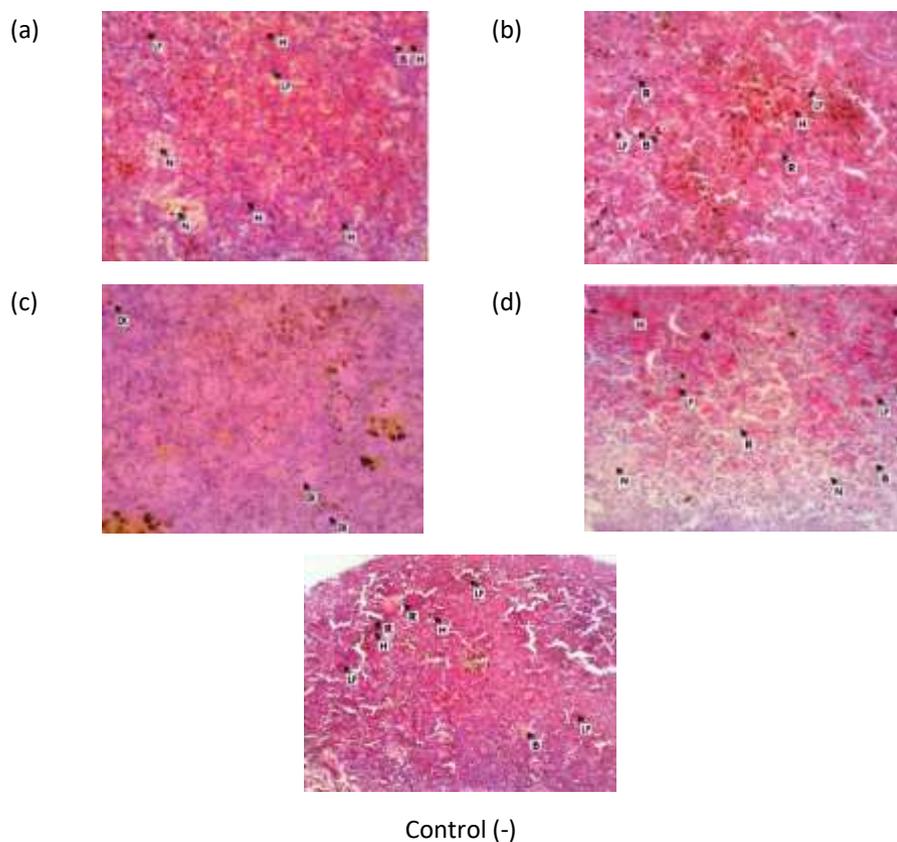
Figure 1. Histology results of tilapia gills infected with *Pseudomonas aeruginosa* bacteria and soaked in cat whisker leaf extract with different doses. Treatment at (a) 50 ppm; b) 75 ppm; c) 100 ppm; d) 125 ppm

Based on the histological observations of tilapia gill organs infected with bacteria and soaking of cat whisker leaves at different concentrations, the administration of 50 ppm resulted in the fusion of secondary lamella (F) and parasite cysts (K). At 75 ppm, parasitic cysts are observed in the secondary lamella (LS), lamella fusion (F), and hemorrhage (H). At a concentration of 100 ppm, dilatation was observed in the primary lamella (D) and inflammation occurred in some gills in the primary lamella (LP). At a concentration of 125 ppm, dilatation of the primary lamella (DLP) and secondary lamella (DLS) was obtained, the majority of which were filled with inflammatory cells, secondary lamella fusion (F), and the presence of parasitic cysts (K). In the negative control group, cysts were observed in the secondary lamellae (K), necrosis (N), and primary lamellar dilatation (DLP).

Fish gills play important roles in osmoregulation and detoxification. The quality of maintenance water containing bacteria can affect the health of gills and, ultimately, the overall health of fish (Moll et al., 2020). Damage to the gills due to bacterial infection has been suspected (Liu et al., 2020). This has a serious impact on the health of fish because disruption of the gills can affect the efficiency of Respiration and balance of gases in the blood of fish. In addition, pathogenic bacteria can directly attack the gill tissue when the maintenance water contains bacteria that cause gill damage, such as inflammation, cell damage, and necrosis (Van loo et al., 2020). Furthermore, Van loo et al. (2020) explained that structural damage to the gill organs can also occur because bacteria can produce toxins or enzymes that damage the physical structure of the gills, such as lamellae and filaments, thereby interfering with respiration and excretion.

3.4. Histological Livers

The results of histopathological observations of tilapia livers (*Oreochromis niloticus*) infected with *Pseudomonas aeruginosa* treated with different doses of whisker leaf extract (*Orthosiphon aristatus*) are shown in **Figure 2**.



Note : Symbol (H) Hemorrhage, (N) Necrosis, (LP) Hepatic congestion, (Dk) Turbid degeneration, (R) inflammatory cells and (B) bile stasis.

Figure 2. Histology results of tilapia liver infected with *Pseudomonas aeruginosa* bacteria and soaked in cat whisker leaf extract with different doses. Treatment a (50 ppm); b (75 ppm); c (100 ppm); d (125 ppm)

Based on histopathological observations of tilapia livers infected with *Pseudomonas aeruginosa*, changes were observed after treatment with different doses of whisker leaf extract. Treatment with the 50-ppm extract revealed several areas that experienced hemorrhage and necrosis, as well as hepatic congestion with bile secretion (B). At 75 ppm, there were inflammatory cells (R), hemorrhage (H), necrosis (N), hepatic congestion (LP), and only one sign of bile secretion (B). At a concentration of 100 ppm, the parenchyma was turbid Degeneration (DK). At a concentration of 125 ppm, hemorrhage (H), accumulation of inflammation (R), several hepatocyte cells, and secreted bile (B). In the control treatment group (-), inflammatory cells (R) and hemorrhage yeast were observed

in the dorsal area of the tissue (H). There were several hepatocyte dams with one sign indicating the presence of bile stasis.

After the administration of cat whisker extract at a concentration of 50 ppm, there were many areas that experienced hemorrhage damage and necrosis. Some bacteria produce exotoxins that can damage the endothelial cells in the blood vessels of the liver (Kumar and Singh, 2022). Severe hemorrhage damage to the liver can interfere with normal liver functions, such as metabolism and detoxification, resulting in a decline in the overall health of tilapia (Fahmi et al., 2019).

Toxins produced by bacteria can also interfere with cell function and cause liver cell death, leading to necrosis (Valerio et al., 2010) The treatment of giving cat whisker leaf extract at concentrations of 75 ppm and 125 ppm has a lot of hepatic congestion and few signs that bile is secreted. Hepatocyte-secreted bile stasis plays an important role in the digestive system, which is crucial for tilapia health, including liver function (Wolf and Wolfe, 2005).

3.5. Survival Rate (SR)

The results of the annova test showed that administration of cat whisker leaf extract at different doses to tilapia infected with *Pseudomonas aeruginosa* as shown in **Figure 3**.

The results showed that the administration of cat whisker leaf extract (*Orthosiphon aristatus*) to tilapia (*Oreochromis niloticus*) infected with *Pseudomonas aeruginosa* had different effects on the survival rate (SR) depending on the dose administered. A dose of 100 ppm showed the highest SR of 90%, indicating the maximum effectiveness of cat whisker leaf extract at that dose in fighting bacterial infections and maintaining fish survival. Cat whisker leaf extract contains various bioactive compounds such as flavonoids, tannins, and saponins, which have antibacterial activity (Chung et al., 2019). These compounds can inhibit the growth of, or kill, pathogenic bacteria. By reducing bacterial infections in tilapia, cat whisker leaf extract increases the resistance of fish to disease and stress. This will increase tilapia survival of tilapia (Sumarmin, 2021).

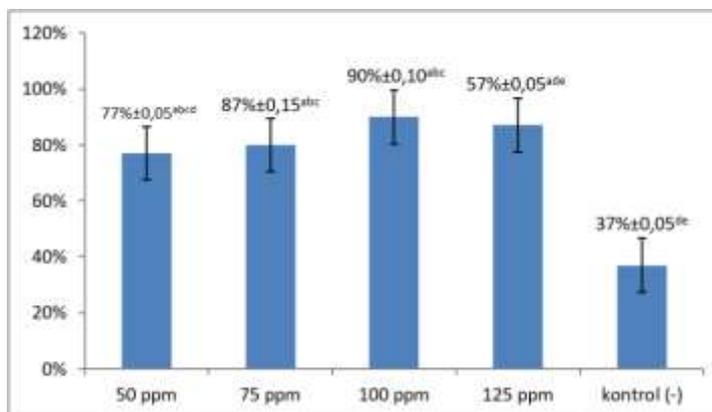


Figure 3. Survival Rate of Tilapia (*Oreochromis niloticus*) after 7 days of maintenance with different doses of extract *Orthosiphon aristatus*. Different letters have a real difference ($P < 0.05$)

The low survival rate in negative controls may be caused by several factors, such as the absence of antibacterial agents or immunostimulants that can help fish fight infection with *Pseudomonas aeruginosa*. Bacterial infections can increase stress in fish both physically and physiologically. Stress can be caused by infections, inflammation, or metabolic changes. Prolonged stress can lower fish endurance and exacerbate the impact of infection, leading to increased mortality (Austin and Zhang, 2020). Infection with *Pseudomonas aeruginosa* can weaken the immune system of fish, making them more susceptible to secondary infections. Ability to fight pathogens. If antibacterial agents are not used to reduce the bacterial load, the immune system cannot function properly and worsen fish health of fish (Sekkin and Kum, 2011)..

4. Conclusions

Based on the results of the study, the administration of cat whisker leaf extract (*Orthosiphon aristatus*) has been proven to be effective as an alternative treatment for tilapia infected with *Pseudomonas aeruginosa* bacteria. A dose of 100 ppm is an optimal concentration that is able to minimize histopathological damage to the liver and gills, and produces the highest survival rate of 90%. The success of this treatment is supported by the content of active compounds in cat whisker leaf extract which has antibacterial, antistress, and immunostimulant properties, so that it is able to increase the response of the fish's body to bacterial infections.

Conflicts of Interest

There are no conflicts to declare.

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AI Writing Statement

The authors did not use any artificial intelligence-assisted technologies in the writing process.

References

- Abdel-Latif, H. M., Dawood, M. A., Menanteau-Ledouble, S., & El-Matbouli, M. (2020). The nature and consequences of co-infections in tilapia: A review. *Journal of Fish Diseases*, 43(6), 651–664. <https://doi.org/10.1111/jfd.13164>
- Anarkhis, N., Prajitno, A., & Maftuch, M. (2022). Effect of Crude Aloe Vera Extract on the Histopathology of Gurame Fish (*Osphronemus Gouramy*) Infected with the Bacteria *Edwardsiella tarda*. *Technium*, 4(10), 207-216. <https://doi.org/10.47577/technium.v4i10.8112>
- Ashraf, K., Sultan, S., & Adam, A. (2018). *Orthosiphon stamineus* Benth. is an outstanding food medicine: Review of phytochemical and pharmacological activities. *Journal of Pharmacy & Bioallied Sciences*, 10(3), 109-118. https://doi.org/10.4103/jpbs.JPBS_253_17
- Austin, B., & Zhang, X. H. (2020). *Vibrio* and *Pseudomonas* infections in aquaculture. *Aquaculture*, 528, 735533. <https://doi.org/10.1016/j.aquaculture.2020.735533>
- Azhar, F., Scabra, A. R., & Lestari, D. P. (2022). Penanggulangan Penyakit Bakterial Pada Ikan Nila Menggunakan Ekstrak Daun Sirih (*Piper Betle L.*) Di Desa Gontoran Lombok Barat. *Jurnal Pepadu*, 3(2), 287-291. <https://doi.org/10.29303/pepadu.v3i2.2483>
- Aziz, S., & Abdullah, S. (2021). Common bacterial diseases of fish: prevention and control strategies. *Veterinary Pathobiology & Public Health*, 30, 352-364. <https://doi.org/10.47278/book.vpph/2021.030>
- Bricknell, I. (2017). Types of pathogens in fish, waterborne diseases. In *Fish diseases*. Academic Press 3(2), 53-80 <https://doi.org/10.1016/B978-0-12-804564-0.00003-X>
- Budianto, B., Faadhilah, I. F., Ekawati, A., Anggaita, S., A'yunin, Q., & Andayani, S. (2022). Effectiveness of *Bacillus subtilis* on survival rate of Nile tilapia (*Oreochromis niloticus*) infected with *Pseudomonas fluorescens*. *Jurnal Sumberdaya Akuatik Indopasifik*, 6(2), 81–88. <https://doi.org/10.46252/jsai-fpik.unipa.2022.Vol.6.No.2.215>
- Cheikyula, J. O., & P.C. Ojekwu. (2003). Growth responses and survival of the gold fish *Carassius auratus* (Cyprinidae) fry reared on *Moina* (Cladocera) and *Cylops* (Copepoda). *Journal of Aquatic Sciences*. 18(1), 43-46. <https://doi.org/10.4314/jas.v18i1.19941>
- Chung, W. Y., Md Kamal, N. H., Tye, G. J., Lim, S. K., Khoo, B. Y., Chung, L. Y., & Goh, K. L.

- (2019). *Orthosiphon stamineus* Benth. leaf extract inhibits cell proliferation, angiogenesis and metastasis in colorectal cancer. *Nutrients*, 11(8), 1879. <https://doi.org/10.3390/nu11081879>
- Fahmi, U., Andriani, I., Salmah, S., Hatta, T. H., Omar, S. B. A., & Sari, D. K. (2019). Histopathology of liver and intestine of pangkalan bare fish (*Oryzias matanensis*) Polluted by nickel and iron in Lake Matano, South Sulawesi. *IOP Conference Series: Earth and Environmental Science*, 370(1), 1-8. IOP Publishing. <https://doi.org/10.1088/1755-1315/370/1/012078>
- Faramayuda, F., & Mariani, T. S. (2021). Chemical Compound Identification of Two Varieties Cat of Whiskers (*Orthosiphon aristatus* Blume Miq) from In Vitro Culture. *Sarhad Journal of Agriculture*, 37(4), 2-10. <https://doi.org/10.17582/journal.sja/2021/37.4.1355.1363>
- Górniak, I., Bartoszewski, R., & Króliczewski, J. (2019). Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochemistry Reviews*, 18(1), 241- 272. <https://doi.org/10.1007/s11101-018-9591-z>
- Kumar, V., & Sharma, R. (2021). Impact of bacterial infections on fish health and aquaculture productivity. *Journal of Fish Diseases*, 44(6), 855-869. <https://doi.org/10.1111/jfd.13321>
- Lim, J. W., Kim, N. Y., Seo, J. S., Jung, S. H., & Kang, S. Y. (2021). Antibacterial compounds against fish pathogenic bacteria from a combined extract of *Angelica gigas* and *Artemisia iwayomogi* and their quantitative analyses. *Fisheries and Aquatic Sciences*, 24(10), 319-329. <https://doi.org/10.47853/FAS.2021.e31>
- Liu, Y., Wang, Z., Zhang, T., & Wang, Y. (2020). Gill health in aquaculture: Effects of bacterial infections and environmental stressors. *Aquaculture*, 518, 734739. <https://doi.org/10.1016/j.aquaculture.2019.734834>
- Mohammed, R. M., Muhammed Ameen, S., & Abdullah, S. M. A. (2023). Record of aerobic bacterial species from the cyprinid fish *cyprinus carpio* from fish farms in sulaimani province, kurdistan region, iraq. *Applied Ecology & Environmental Research*, 21(6), 1-18. https://doi.org/10.15666/aeer/2106_56075624
- Moll, S., Gudim, M., Anwar, M., & Perera, C. (2020). Impact of bacterial water contamination on gill pathology and fish health in aquaculture. *Aquaculture*, 518, 734773.
- Othman, L., Sleiman, A., & Abdel-Massih, R. M. (2019). Antimicrobial activity of polyphenols and alkaloids in middle eastern plants. *Frontiers in Microbiology*, 10(911), 1-28. <https://doi.org/10.3389/fmicb.2019.00911>
- Pham, D. K., Chu, J., Do, N. T., Brose, F., Degand, G., Delahaut, P., & Scippo, M. L. (2015). Monitoring antibiotic use and residue in freshwater aquaculture for domestic use in Vietnam. *Ecohealth*, 17(1), 11-20. <https://doi.org/10.1007/s10393-014-1006-z>
- Reverter, M., Sarter, S., Caruso, D., Avarre, J. C., Combe, M., Pepey, E., & Gozlan, R. E. (2020). Aquaculture at the crossroads of global warming and antimicrobial resistance. *Nature communications*, 11(1), 1-8. <https://doi.org/10.1038/s41467-020-15735-6>
- Roza, D. (2018). Diagnosis dan pengendalian penyakit infeksius pada induk kuda laut, *Hippocampus* kuda di hatchery. *Jurnal Ilmu dan Teknologi Kelautan Tropis*, 10(2), 353–364. <https://doi.org/10.29244/jitkt.v10i2.20173>
- Salihoglu, N. K., Karaer, G., & Tasdemir, Y. (2019). Determination of bacterial growth with spectrophotometer and colony counting methods. *Pamukkale University Journal of Engineering Sciences*, 25(9), 1062-1066. <https://doi.org/10.5505/pajes.2019.93195>
- Sekkin, S., & Kum, C. (2011). Antibacterial drugs in fish farms: application and its effects. In F. Arl (Ed.), *Recent Advances in Fish Farms*. InTech. <https://doi.org/10.5772/26919>
- Sumarmin, R. (2021). The Effect of Cat Whisker Leaf Extract (*Orthosiphon aristatus*) on Estrus Cycle Recovery of Mice (*Mus musculus* L.). *Jurnal Serambi Biologi*, 6(2), 15-19.
- Tanna, K., Bhavsar, D. D., & Mankad, D. A. (2020). Aquatic toxicity impacts on behaviour and survival of fresh water fish: A review. *International Journal of Fisheries and Aquatic Studies*, 8(3), 235-243.
- Tran, N., Rodriguez, U. P., Chan, C. Y., Phillips, M. J., Mohan, C. V., Henriksson, P. J., & Hall, S. (2017). Indonesian aquaculture futures: An analysis of fish supply and demand in

- Indonesia to 2030 and role of aquaculture using the Asia Fish model. *Marine Policy*, 79, 25-32. <https://doi.org/10.1016/j.marpol.2017.02.002>
- Valerio, E., Chaves, S., & Tenreiro, R. (2010). Diversity and impact of prokaryotic toxins on aquatic environments: A review. *Toxins*, 2(10), 2359-2410. <https://doi.org/10.3390/toxins2102359>
- Van Loo, J., De Boeck, G., De Schryver, P., & Willems, A. (2020). Effects of bacterial exposure on gill health and function in aquaculture fish. *Aquaculture*, 518, 734788. <https://doi.org/10.1016/j.aquaculture.2019.734788>
- Wolf, J. C., & Wolfe, M. J. (2005). A brief overview of nonneoplastic hepatic toxicity in fish. *Toxicologic pathology*, 33(1), 75-85. <https://doi.org/10.1080/01926230590890187>
- Zainuddin EN., H.Anshary., H. Huyyirnah., R Hiola dan D.V. Baxa. 2019. Antibacterial Activity Of *Caulerpa racemosa* against Pathogenic Bacteria Promoting ice-ice Disease in The Red Alga *Gracilaria verrucosa*. *Journal of Applied Phycology*, 31, 3201–3212. <https://doi.org/10.1007/s10811-019-01805-w>
- Zainuddin, E. N., Tassakka, A. C. M., Manggau, M., & Syamsuddin, R. 2020. Preliminary study of cultivated algae from South Sulawesi as antibacterial agent against fish pathogenic bacteria. In *IOP Conference Series: Earth and Environmental Science*. 564(1): 1-10. <https://doi.org/10.1088/1755-1315/564/1/012060>