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Review Article

Effect of Curry Leaf (*Murraya koenigii*) as Edwardsiellosis Treatment on Gourami Fish (*Osphronemus goramy*)

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ABSTRACT

The primary objective of this study was to explore the potential of *Murraya koenigii* as a natural alternative to synthetic antimicrobial agents for the treatment of edwardsiellosis in gourami fish (*Osphronemus goramy*). This research employed a combination of both experimental and descriptive methods. The research followed a two-stage approach. In the first stage, antibacterial compounds were extracted and characterized from *M. koenigii* leaves, followed by an assessment of their antibacterial activity against *Edwardsiella tarda*. The second stage involved applying the crude extract of *M. koenigii*, known for its antibacterial properties, to gourami fish infected with *E. tarda*. The findings revealed that *M. koenigii* predominantly contains alkaloids, terpenoids, saponins, flavonoids, and tannins, all of which exhibit antibacterial activity. The application of the crude *M. koenigii* extract had a substantial impact on the hematological profile of the infected gourami fish. The most effective dose in treating Edwardsiellosis in gourami fish was found to be 600 mg/L, as it significantly improved various hematological parameters, including hematocrit, hemoglobin, erythrocyte count, leukocyte count, lymphocytes, monocytes, and neutrophils. Additionally, histopathological analysis showed improvements in tissue conditions, with lower scores for degeneration, congestion, and necrosis. Furthermore, the survival rate of gourami fish reached its highest at 83.33% when treated with the 600 mg/L dose of *M. koenigii* extract. These results suggest the potential of *M. koenigii* as an effective alternative for the treatment of Edwardsiellosis in gourami fish. However, further research is needed to assess the practical application of *M. koenigii* extract in gourami fish culture.

Keywords: Gouramy Fish, Bacterial Fish Diseases, Edwardsiellosis, Fish Diseases Treatment, *Murayya koenigii*

INTRODUCTION

Gourami fish (*Osphronemus goramy*) is a freshwater fish species that has been cultured in the Southeast Asian region; its maintenance cost is relatively low, and it has adaptability with low dissolved oxygen content. Gourami fish also has a relatively stable high selling price (2.28 USD/kg) (Al-Baiquni, 2019). However, an intensive maintenance strategy to pursue production targets evokes damage to the immune system and susceptibility to diseases which evokes high economic losses if not resolved (Abdel-Latif et al., 2020).

Edwardsiella tarda is a species of the Gram-negative bacteria, which poses a significant challenge to the aquaculture industry. It is responsible for causing edwardsiellosis, a disease that has been identified as a major threat to various economically valuable fish species globally (Li et al., 2019).

This pathogen has led to substantial losses in the aquaculture sector, with significant impacts observed in countries such as the United States of America, Japan, and various European and Asian countries. In scenarios where fish are raised in ponds with limited water circulation, losses due to *E. tarda* infections can be particularly severe, sometimes reaching as high as 50% (Kole et al., 2017).

The effective treatment of edwardsiellosis, caused by *E. tarda* infection, has been achieved through the use of antibiotics such as tetracycline and kanamycin. However, the widespread and inappropriate use of antibiotics has led to significant problems. These challenges encompass the development of antibiotic resistance in *E. tarda* and the potential presence of antibiotic residues in human populations (Xu et al., 2019). As highlighted by Harikrishnan et al. (2020), the use of antibiotics and chemical agents in aquaculture can also pose risks, including concerns related to water toxicity, public health, and environmental damage.

One potential solution to address the aforementioned challenges involves the utilization of a natural bioactive crude extract derived from curry leaves, *Murraya koenigii*, which contains antibacterial compounds. *M. koenigii* is a plant belonging to the Rutaceae family and is commonly found in tropical and subtropical regions. Extensive phytochemical analyses of *M. koenigii* have revealed a wide range of natural compounds, including alkaloids, sesquiterpenes, essential oils, and alkenes (Patil et al., 2024). Different parts of the *M. koenigii* plant exhibit various biological activities, including anti-inflammatory, antioxidative, nephroprotective, hepatoprotective, anti-listerial, and antibacterial properties (Ma et al., 2019). To gain a deeper understanding of the bioactive components of *M. koenigii*, researchers conducted GC-MS (Gas Chromatography-Mass Spectrometry) analysis. They investigated the chemical composition of the ethanol extract of *M. koenigii* using GC-MS and compared the mass spectra of the identified compounds in the extract to the National Institute of Standards and Technology (NIST) library (Azhagu et al., 2021). The analysis of the ethanol extract of *M. koenigii* revealed the presence of two primary compounds: 1-methyl-pyrrolidine-2-carboxylic acid, which constituted 69.00% of the extract, and ethyl α-glucopyranoside, making up 13.36% of the extract (Salikutty et al., 2012).

Numerous investigations have explored the potential antibacterial properties of *M. koenigii*, but its specific use as an antibacterial agent against *in vitro* antibacterial activity was investigated by Syaifurrisal et al. (2021) and its application in the treatment of fish has never been explored. It is imperative to initiate research and studies focused on the identification of active compounds within *M. koenigii* with antibacterial properties. Furthermore, these studies should investigate the feasibility of employing these compounds as potential drug candidates for the treatment of *E. tarda* infections in gourami fish. This research should encompass comprehensive hematological and histopathological examinations to determine the potential therapeutic effects.

MATERIAL AND METHODS

Experimental fish and bacteria

Experimental gourami fish samples (7-10 cm) were provided from fish farms located in Tulungagung, East Java, Indonesia. To conduct antibacterial activity testing, a pure culture of *E. tarda* was acquired from BUSKIPM Jakarta. The culture and revitalization of *E. tarda* bacteria were facilitated using TSB (Tryptic Soy Broth) as the growth medium. The LD_{50} (Lethal Dosage 50) test was carried out to determine the density and time required for bacteria to kill 50% of the experimental fish (Rattanachaikunsopon & Phumkhachorn, 2010). The LD_{50} test uses a pure culture of *E. tarda* bacteria with a density 10^9 cells/ml, 10^8 cells/ml, 10^7 cells/ml, 10^6 cells/ml, and 10^5 cells/ml. The *E. tarda* used in the study had a density of 2.53×10^7 cells/mL, as determined by the LD_{50} results. In this test, fish were put into treatment containers with a stocking density of 10 fish/container.

Antibacterial activity testing was carried out using a disc test to examine the inhibitory power of the crude extract of *M. koenigii* leaves on *E. tarda* bacteria. According to Ulmursida et al., (2017) in testing how big the impact of giving the extract is and if the diffusion method shows positive results, the clear zone area results will be obtained by reducing the diameter of the clear zone by the diameter of the paper disc (6 mm). *E. tarda* cell damage was analyzed by comparing SEM photos (normal conditions and after the bacteria were treated with crude extract of *M. koenigii* leaves so that a picture of bacterial cell wall damage was visible. According to Nursidika et al., (2014), determining the antimicrobial mechanism can be done using the SEM method. The working principle of SEM is the creation of images based on the detection of new (secondary) or reflected electrons that appear or emerge from the sample surface when the sample surface is scanned using an electron beam.

Experimental design

To infect experimental fish with *E. tarda* bacteria, the experimental fish were soaked in a pure culture of *E. tarda*. Soaking was carried out in an aquarium measuring 30 x 30 x 30 cm with a density of 10 fish in 20 liters of water at a temperature of 27-30°C. Observations of fish death were then carried out for 96 hours, according to the statement of Wulandari et al. (2014), since this was the time commonly used in short-scale bioassays. This research employed a combination of both experimental and descriptive methods. The experimental approach was employed to obtain quantitative data for this study, while the descriptive method was utilized to comprehensively depict the natural bioactive components found in *M. koenigii* and their effects on the hematological and histopathological aspects of gourami fish. In this study, various treatments were administered, including negative control (K-) infected fish that were not treated, positive control (K+) normal fish that were not infected, as well as doses of 500 mg/L, 600 mg/L, 650 mg/L, and 700 mg/L (three repetitions, 10 fish each). The selection of these dosages was based on prior *in vitro* research conducted by Syaifurrisal et al. (2021).

Extraction of *M. koenigii*

The *M. koenigii* leaves utilized in this study were collected from the Ampel Religious Tourism area of Surabaya, Indonesia, and extracted with ethanol as the maceration solvent. To ascertain the presence of active compounds in the extract, several tests were conducted by the methodology outlined by Dewi et al. (2019). These tests encompassed phytochemical screening, UV-Vis (Ultraviolet-Visible) analysis, and FTIR (Fourier-transform in-

frared) spectroscopy. The primary objective of phytochemical screening was to detect the presence of active compounds in the plant extract such as saponins, tannins, flavonoids, alkaloids, and terpenoids, within the crude extract of *M. koenigii* leaves.

The extract was then also tested for LC_{50} (Lethal Concentration) to determine the concentration of the extract that could cause 50%, to determine a dose that was safe and did not cause death in experimental fish (Suciati et al., 2012). In this test, fish were put into treatment containers with a stocking density of 10 fish/container. Then the aquarium is filled with extract according to the treatment. The behavior, number of fish that died, and the time were recorded. The test was repeated if fish in the control treatment experienced mortality above 10% (Taufik & Setiadi, 2012).

Histopathology analysis

Edwardsiellosis can be observed with symptoms such as lesions in muscle tissue, skin, and internal organs, such as kidney tissue (Harikrishnan et al., 2020). Kidney tissue can be an important organ in the study of pathogenic mechanisms and immune responses in fish (Rauta et al., 2012; Bailey et al., 2020). The relationship between the severity of histological changes due to bacterial infection and the immune response can be elucidated by the study of these organs. Some studies report kidney tissue is used as histopathology analysis for edwardsiellosis in fish (Cheng et al., 2020; Kalindamar et al., 2020). To determine the characteristics of kidney tissue, histopathological observations of gourami fish were carried out. The staining method used Haematoxylin Eosin previously fixed with 10% formalin and calculations were used to assess the impact of *M. koenigii* extract on edwardsiellosis, as described by Maftuch et al. (2015). Also, the normal and pathological semi-quantitative approach was used to compare normal tissue samples with tissue that had been affected by the pathogen. This involved determining the extent of staining in stained areas and manually calculating the percentage of affected tissue, following the method outlined by Maftuch et al. (2015). This histopathological analysis was performed with three replications, and for each treatment, five distinct views were examined to ensure a comprehensive assessment.

Blood analysis

Hematological observations encompassed the assessment of several parameters, including hematocrit value, hemoglobin levels, erythrocyte count, leukocyte count, monocyte count, lymphocyte count, and neutrophil count, following the methodology outlined by Maftuch (2018). These observations were conducted by extracting blood from the lateral line of the gourami fish using a 1 ml syringe, which had been preloaded with 0.5 µL of EDTA as an anticoagulant. The blood cell count was then determined. This test aimed to evaluate the condition of infected gourami fish after the administration of *M. koenigii* extract. The blood analysis was performed with three repetitions and monitored before infection, after infection, and after treatment.

Survival rate

To assess the effectiveness of *M. koenigii* in treating Edwardsiellosis, the survival rate was calculated with the formula given by Kasnir et al. (2023). The research spanned 7 days for survival rate assessment, involving three replications for each treatment group and a sample of 10 fish in each replication.

$$SR = \frac{Nt}{No} x\ 100\%$$

Notes:

SR: Survival Rate (%)

Nt: The quantity of fish at a specific time point t (individual)

No: The initial count of fish at the start of the experiment (individual)

Data analysis

In this study, data analysis was carried out in a completely randomized design (CRD) using the Statistical Package for the Social Sciences (SPSS) 25.0 for Windows software application.

RESULTS AND DISCUSSION

Active compound present in *M. koenigii*

The phytochemical tests conducted on the crude extract of *M. koenigii* leaves are summarized in Table 1. The results of the phytochemical screening indicated that the crude extract derived from *M. koenigii* leaves contained various active compounds, including flavonoids, alkaloids, saponins, terpenoids, and tannins. These outcomes are consistent with the findings reported by Fauziah et al. (2014), who also identified alkaloids, steroids, flavonoids, tannins, and phenolic compounds as the natural bioactive constituents present in the crude extract of *M. Koenigii*.

Table 1. Phytochemical Test Results.

Active Compound	Result	Description
Flavonoid	+	Formed pink color
Saponin	+	Formed non-permanent foam
Alkaloid		
- Dragendroff	+	An orange precipitate formed
- Meyer	+	A white precipitate formed
- Buchardat	+	A brown precipitate formed
Tannin	+	A blackish brown color is formed
Terpenoid		
- Steroid	-	Formed a bluish green color
- Triterpenoid	+	A brownish-orange color is formed

To validate the presence of the active constituents identified in the phytochemical test results, further analysis of the crude extract from *M. koenigii* was conducted using both a UV-Vis spectrophotometer (Table 2) and an FTIR (Fourier-transform infrared) spectrophotometer (Table 3). The UV-Vis observations indicated that the dominant compounds in the extract were derived from the alkaloid group (with a peak at 3.893), saponin group (peaking at 3.824), and terpenoid group (peaking at 4.016). These groups exhibited notably high absorbance values. Conversely, flavonoids (with a peak at 0.022) and tannins (peaking at 1.590) displayed comparatively lower absorbance values. According to Hammado and Illing (2013), absorbance values can provide in-

sights into the concentration of bioactive compounds within the extract. A higher absorbance value suggests that the absorption band contains specific compounds with a stronger absorption affinity. Furthermore, the FTIR observations unequivocally confirmed the presence of compounds such as flavonoids, alkaloids, saponins, terpenoids, and tannins.

Table 2. UV-Vis peak point data from *M. koenigii* extract.

Long Waveform (nm)	Absorbance	Compound	Literature
662.0	0.022	Flavonoid	(Obaseki et al., 2017)
294.9	1.590	Tanin	(Lestari & Sidik, 2013)
220.0	3.480	Alkaloid	(Hammado & Illing, 2013)
217.0	3.577	Alkaloid	(Hammado & Illing, 2013)
213.0	3.893	Alkaloid	(Hammado & Illing, 2013)
211.0	3.824	Saponin	(Amanati et al., 2017)
205.1	4.016	Terpenoid	(Agustini et al, 2019)
202.1	3.879	Terpenoid	(Agustini et al., 2019)

Table 3. FTIR spectrophotometer wavelength absorption data from *M. koenigii* extract.

FTIR wavelength (cm^{-1})	Functional groups	Group
1453.868	N-H	Alkaloid (Hammado & Illing, 2013)
1627.321	C=O	Alkaloid (Aksara et al, 2013)
1453.868	C-H alifatik	Alkaloid (Hammado & Illing, 2013)
1163.814	N-H bending	Alkaloid (Aksara et al, 2013)
1069.868	N-H bending	Alkaloid (Aksara et al, 2013)
928.175	Tekuk C- H	Alkaloid (Santi, 2010)
2924.574	-OH	Terpenoid (Agustini et al., 2019)
2855.169	C-H alifatik	Terpenoid (Agustini et al., 2019)
1713.556	C=O	Terpenoid (Agustini et al., 2019)
1314.393	C-O-H	Tanin (Sari et al., 2015)
679.341	C-H *bending*	Tanin (Sari et al., 2015)
583.419	C-H *bending*	Tanin (Sari et al., 2015)
1209.989	C-O	Saponin (Bintoro et al, 2017)
835.125	C-H	Flavonoid (Ningrum et al, 2017)

Histopathology analysis

The histopathological images presented in Figure 1 offer valuable insights. The kidney tissue of healthy fish appears normal, showing no signs of cellular damage. The cross-section of the network, including the glomerulus and tubular tissue exhibits a healthy state. The distal tubule tissue and hematopoietic tissue also display normal conditions, devoid of any damage. This contrasts with infected fish, where congestion is apparent. The reddish color observed in kidney cells is indicative of congestion resulting from an increased presence of blood clots within the blood vessels, as explained by Parameswari et al. (2013). In severe cases, congestion can lead to ruptured blood vessels, ultimately causing cell death or necrosis, characterized by changes in cell nuclei, where the nuclei become denser and darker (a condition known as pyknosis) (Abdelfadeel et al., 2023). Coagulative necrosis, described by Adinata et al. (2012), involves densely packed and fixed protoplasmic cells that can still be observed under a microscope.

Takashima and Hibiya (1995) define necrosis as a state of reduced tissue activity marked by the progressive loss of cell components, accompanying cell degeneration in organisms. In histopathological terms, the use of *M. koenigii* is shown to inhibit *E. tarda* infection in gourami fish. Many prior studies have explored the application of *M. koenigii* in treating pathogenic bacteria such as *Escherichia coli, Aeromonas hydrophila, Citrobacter freundii, Vibrio parahaemolyticus, Vibrio vulnificus, Staphylococcus aureus, Streptococcus agalactiae*, and *Streptococcus anginosus* in fish *in vitro* (Heny et al., 2011; Najiah et al., 2011). However, this research is the first to demonstrate the effectiveness of *M. koenigii* in treating *E. tarda* infection in gourami fish. Notably, kidney necrosis was observed to increase in average scores at the 650 mg/L and 700 mg/L treatments, indicating that the extract reached its optimal efficacy for treatment following *E. tarda* infection. Doses exceeding this optimal point appeared to result in more severe kidney tissue damage.

According to Kumar et al. (2012), proximal tubular epithelial cells are highly susceptible to the effects of toxic substances. This vulnerability arises because toxic substances tend to accumulate at higher levels in the proximal tubules due to active processes of absorption and secretion in this region. Additionally, the proximal tubules have elevated levels of cytochrome P450 enzymes, which play a role in either detoxifying or activating toxic substances (Ge et al., 2022). Consequently, these tubules are often the primary targets of the adverse effects caused by toxic substances. Continuous exposure of the proximal convoluted tubule to toxic substances can result in cellular injury, ultimately leading to cell death, known as necrosis (Moore et al., 2021).

In the overall evaluation of the comparison to the positive control group, the treatment with *M. koenigii* extract appeared to have a positive impact on mitigating the extent of kidney necrosis (Figure 2). This positive effect can be attributed to the bioactive compounds present in *M. koenigii*'s crude extract, such as alkaloids and flavonoids, which can stimulate the fish's immune defense system. Previous studies have indicated that alkaloids and flavonoids possess antibacterial properties (Donadio et al., 2021; Yan et al., 2021). Alkaloids can disrupt bacterial cell membranes, inhibiting bacterial growth (Jubair et al., 2021), while flavonoids can cause physical membrane disruption and act as inhibitors of ATP synthase (Donadio et al., 2021).

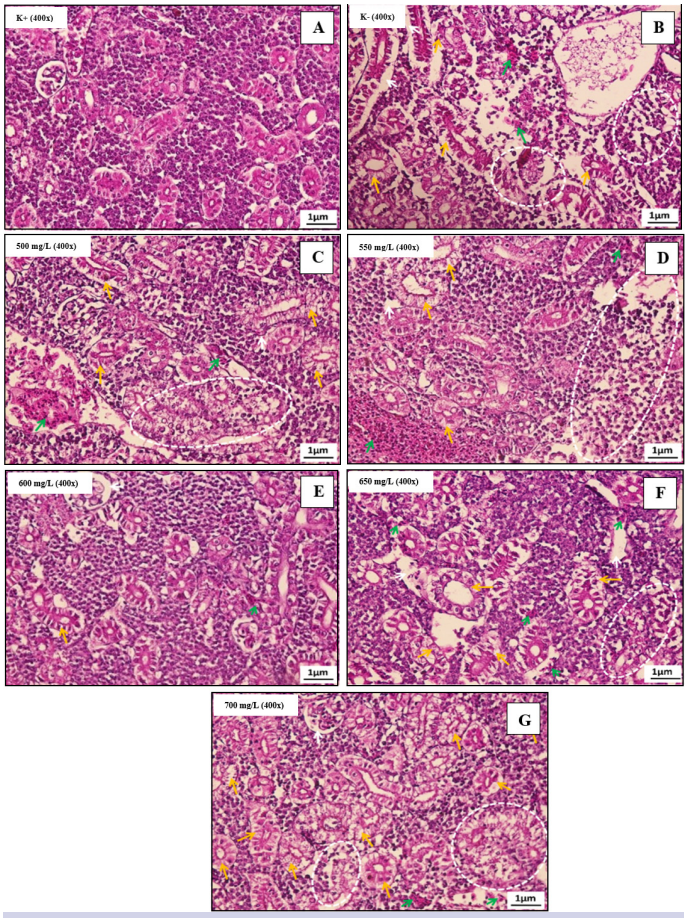

Figure 1. Kidney Histopathological Overview of Gourami Fish / 400x. (A) Normal (non-infected) /K+, (B) Infected fish (not-treated) / K-, (C) treated with 500 mg/L. (D) treated with 550 mg/L. (E) treated with 600 mg/L. (F) treated with 650 mg/L. (G) treated with 700 mg/L, (Yellow Arrow: Degeneration; Green Arrow: Congestion; White Circle: Necrotic Area)

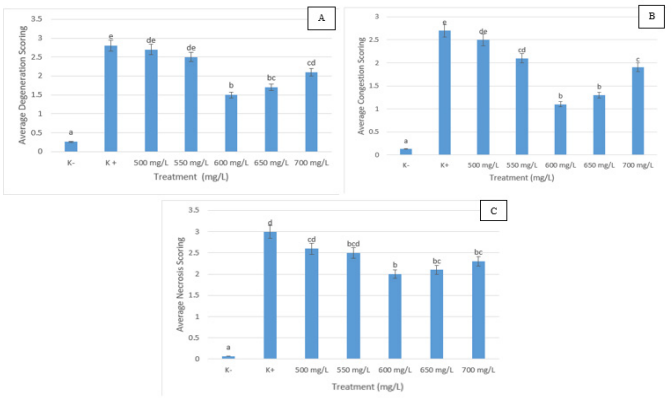

Figure 2. Histopathological Scoring Test Results (A) Degeneration, (B) Congestion, and (C) Kidney Necrosis of Gourami Fish.

The treatment with the lowest average score for abnormal histopathological findings was observed at a dose of 600 mg/L, which resulted in 1.5 for degeneration, 1.1 for congestion, and 2 for necrosis. This indicates that 600 mg/L was the most effective dosage of *M. koenigii* for treating edwardsiellosis in gourami fish. According to Kim & Yang (2018), employing natural active ingredients at the appropriate dosage can stimulate the immune system and enhance protection against infections. Interestingly, after the decrease in damage scores observed in the extract concentrations ranging from 500 mg/L to 600 mg/L, there was a noticeable increase in congestion at the 650 mg/L and 700 mg/L treatment levels. This suggests that the extract's effectiveness had reached its peak at the optimal dosage, and doses higher than this optimum could become toxic, causing more severe kidney tissue damage. At high doses, the secondary metabolites present in the crude extract of *M. koenigii* leaves can be toxic to gourami fish. According to Rand et al. (2015), as the dose of the extract administered increases, the damage to tissue cells also intensifies. This study also showed that no test animals succumbed during the treatment process because the density of bacteria and the concentration of the extract dose used had been through acute LD_{50} and LC_{50} toxicity tests to assess the acute safety of a drug or substance to be used.

SEM test (*Scanning Electron Microscopy*)

The SEM results offer insights into the effects of the treatment on the structural changes in bacterial cells (Figure 3). Figure 3A shows the morphology of *E. tarda* bacteria with no apparent damage to their cell walls, while Figure 3B presents morphological images of *E. tarda* bacteria that have experienced lysis due to instability in their cell walls. For bacterial fish pathogens, after entering the host body, the first step for inducing an infection is the attachment, colonization, and biofilm formation of the microorganism on the host cells and tissues which is generally linked to their cell wall proteins. According to Hussain et al (1997), accumulative growth on polymer surfaces resulting in biofilm formation; it was contributing to pathogenicity by reducing the efficacy of host defenses and antimicrobial killing. This instability disrupts bacterial metabolism, depletes ATP reserves, and decreases cell acidity. Several studies have elucidated this phenomenon, which occurs when antibacterial compounds induce changes or lysis in the protein structure, cell wall stability, and bacterial plasma membrane. This is a result of antibacterial compounds (bacteriostatic effect) binding to proteins through hydrogen bonds (Zhou et al., 2022). Alkaloid compounds, known for their antibacterial properties, can interfere with the formation of cross-bridges within the peptidoglycan constituent components of bacterial cells. This interference prevents the complete formation of the cell wall layer, ultimately leading to the removal of the bacterial cells from the host's cell surface as explained by Ernawati and Sari (2015). Antimicrobials are bacteriostatic if they only inhibit bacterial growth as compound application continues. However, if it is stopped or finished, bacterial growth will increase again (Soelama et al., 2015).

Hematology

The collected data included measurements of hematocrit, hemoglobin, and erythrocyte levels, as depicted in Figure 4. The

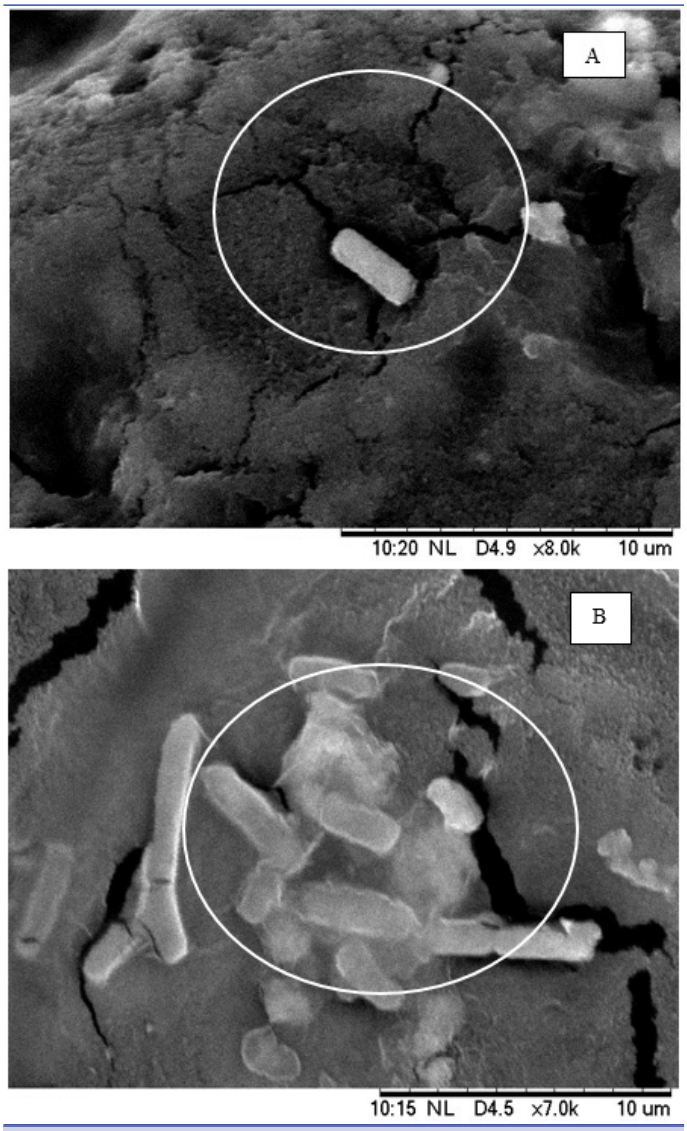

Figure 3. Morphology of *E. tarda* bacteria after SEM test (Lysis) (A) Without treatment, (B) With treatment of *M. koenigii*.

optimal hematocrit value was achieved in the treatment with a dose of 600 mg/L, reaching 32.67%. Regression analysis revealed that the crude extract of *M. koenigii* had a 73% positive effect on improving hematocrit values, which were notably lower after infection, at 19.50%. Similarly, the hemoglobin level showed a significant increase in the 600 mg/L treatment, reaching 4.504 g%. *M. koenigii* demonstrated a 78% effect in improving hemoglobin values, which had declined to 2.652 g% after infection. The highest increase in erythrocyte values was also observed in the treatment with an extract concentration of 600 mg/L, reaching 3.27 x 10^6 cells/mm^3. The crude extract of *M. koenigii* exhibited a remarkable 78% effect in enhancing erythrocyte values, which had dropped to 1.73 x 10^6 cells/mm^3 after infection. These findings indicate that the extract concentration of 600 mg/L was the most effective in increasing blood parameters after *E. tarda* infection in gourami fish. The increase in hematocrit, hemoglobin, and erythrocyte levels in the blood of gourami fish can be attributed to the bioactive properties of the extract, such as terpenoids. According to Sundaryono (2011), terpenoids can stimulate erythropoiesis, the process of erythrocyte formation in the bone marrow. The results of UV-Vis observations in this study showed that the dominant compounds in the extract came from the alkaloid group (with a peak of 3.893), the saponin group (peak of 3.824), and the terpenoid group (peak of 4.016). These groups showed very high absorption values.

According to Anderson and Siwick (1993), when fish are infected, their hematocrit levels tend to decrease. A hematocrit value below 20% indicates erythrocyte deficiency. Zorriezahra et al. (2010) stated that a low erythrocyte count and hematocrit level are indicative of anemia in fish. Hardi et al. (2011) also explained that an increase in total erythrocytes in fish blood indicates a homeostatic response in the fish's body. This response aims to produce more blood cells to replace erythrocytes that may have undergone lysis due to infection. Conversely, the decline in hematocrit, hemoglobin, and erythrocyte values at doses of 650 mg/L and 700 mg/L was attributed to excessive dosages causing stress in the fish. This stress was evident from the lower survival rates of the test fish at these two higher doses compared to the 600 mg dose. According to Awad and Awaad (2017), the more extracts used will not necessarily help treatment efforts due to bacterial infections. The active components contained in an extract are toxic to test animals if the concentration is too high. Using natural ingredients at the right dosage can stimulate the immune system and increase protection against infection.

Treatment with various doses of *M. koenigii* also caused different effects on the leukocyte, lymphocyte, monocyte, and neutrophil values of the fish samples (Figure 5). Following treatment with *M. koenigii*, there was a notable decrease in leukocyte values. The most effective dose observed during the study was 600 mg/L, which reduced leukocyte count from 15,7x10^4 cells/mm^3 to 9.6 x 10^4 cells/mm^3. Regression analysis revealed that the 600 mg/L dose of *M. koenigii* had a 61.59% positive effect on improving leukocyte values. This reduction in leukocyte count indicated that the *M. koenigii* extract played a role in the process of treating gourami fish after they were infected with *E. tarda* bacteria. According to Rieger & Barreda (2011), an increase in the number of leukocytes in the blood is an indication of leukocytes mounting an immune response to bacteria, while a decrease in leukocyte count signifies healing and the cessation of the inflammatory process.

Lymphocyte values showed a significant decrease to 48% after the fish were infected with bacteria. However, after treatment, lymphocyte values began to increase, with the most substantial increase occurring at an extract concentration of 600 mg/L, reaching a 68.88% increase. This rise in lymphocyte count following treatment indicated that the compounds present in the crude extract of *M. koenigii* could enhance the gourami fish's immune system in combating *E. tarda* bacterial infection. As stated by Kim & Yang (2018), natural ingredients, when used at the right dosage, can stimulate the immune system and enhance protection against infections.

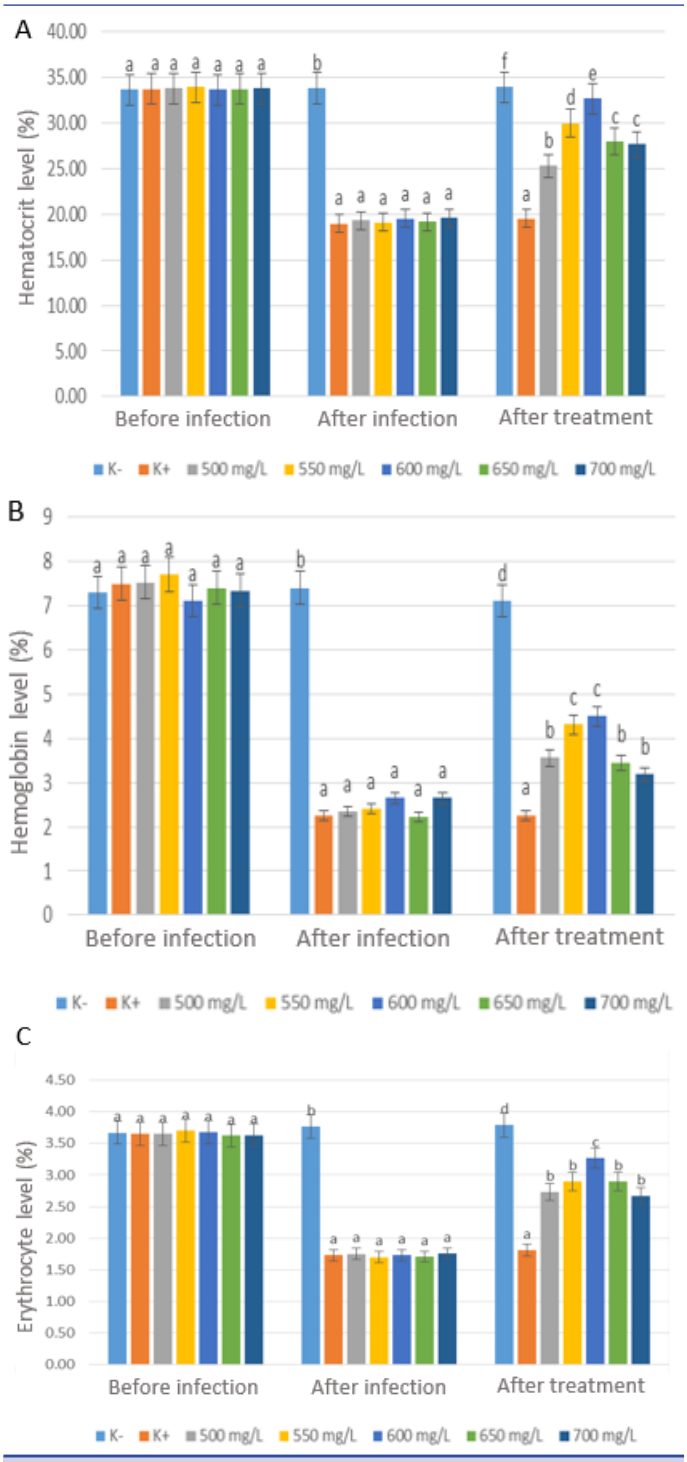

Figure 4. he value of hematocrit (A), hemoglobin (B), and erythrocytes (C) from gourami fish in this study. (K+) Normal (non-infected), (K-) Infected fish (not-treated).

Monocyte and neutrophil values showed a decrease after treatment with *M. koenigii* extract. The best reduction in monocyte values occurred at a concentration of 600 mg/L from 15% to 11.3%. Meanwhile, the highest neutrophil reduction activity occurred at a treatment concentration of 600 mg/L from 17% to 12.6%. The crude extract of *M. koenigii* leaves exhibited the capability to inhibit the growth of *E. tarda* bacteria, leading to a decline in the number of monocytes. This is consistent with Fujaya (2004), suggesting that monocytes leave the bloodstream and migrate to infected areas, where they phagocytize bacteria due to their superior phagocytic capabilities compared to neutrophils. Monocytes serve as macrophages, engaging in the immune system's function of engulfing and destroying pathogenic cells, microorganisms, and foreign substances.

At doses of 650 mg/L and 700 mg/L, the improvement in leukocyte, lymphocyte, monocyte, and neutrophil values was not optimal. This aligns with previous research findings, such as those by Awad & Awaad (2017), which suggest that increasing extract doses may not necessarily enhance treatment efficacy against bacterial infections. Active ingredients within an extract can inhibit pathogen replication and stimulate the innate immune system's defense mechanisms. According to Venkatalakshmi et al. (2016), active compounds like flavonoids can stimulate the release of adrenocorticotropic hormone (ACTH), which, in turn, triggers the adrenal glands to produce cortisol. This cortisol acts as an immunosuppressant. Batool et al. (2020) stated that curry leaf extract exhibited effective immunomodulation in experimental animals through antioxidant and immunosuppressant mechanisms, which are crucial in medicine for reducing an excessive immune response that can occur during infection.

Survival Rate

After the administration of *M. koenigii* extracts, the survival rate values were monitored in the experimental groups, and different values were achieved (Figure 6). The highest mean survival rate, at 83.33%, was observed in the 600 mg/L treatment group. This survival rate value closely approached that of the negative control group (not treated), which recorded a survival rate of 93.33%. According to Mulia (2012), providing a booster like an herbal extract can trigger an increase in antibody production because the test fish already possess immune memory, allowing the booster to generate a more robust immune response.

Conversely, the lowest mean survival rate was observed in the positive control treatment group (received infectious agent only and not treated), which recorded a survival rate of 23.33%. The decline in gourami fish survival rates in the 650 mg/L and 700 mg/L treatment groups indicated that the dosage administered had reached its maximum point. Setyani et al. (2018) explained that high mortality occurs when there is a substantial amount of organ damage resulting from bacterial infection and when the extract dosage is sufficient to have a severe impact on the organism. There was increased degeneration, congestion, and necrosis at treatment levels of 650 mg/L and 700 mg/L. This shows that the effectiveness of the extract has reached its peak at the optimal dose, and doses higher than the optimal dose can be toxic, causing more severe kidney tissue damage. At high doses, secondary metabolites contained in the crude extract of *M. koenigii* leaves can be toxic to gourami fish. Rand et al. (2015) explained that as the dose of extract given increases, tissue cell damage also increases.

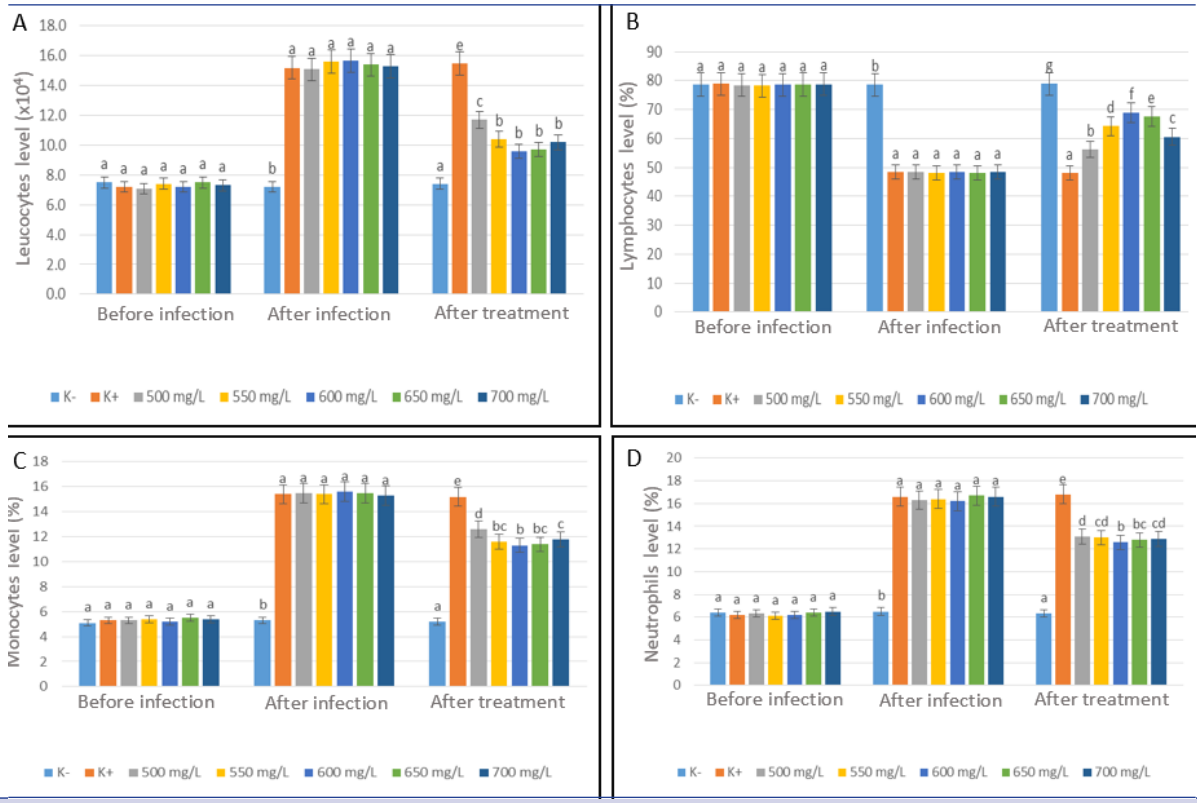

Figure 5. The value of leukocytes (A), lymphocytes (B), monocytes (C), and neutrophils (D) from gourami fish in this study. (K+) Normal (non-infected), (K-) Infected fish (not-treated).

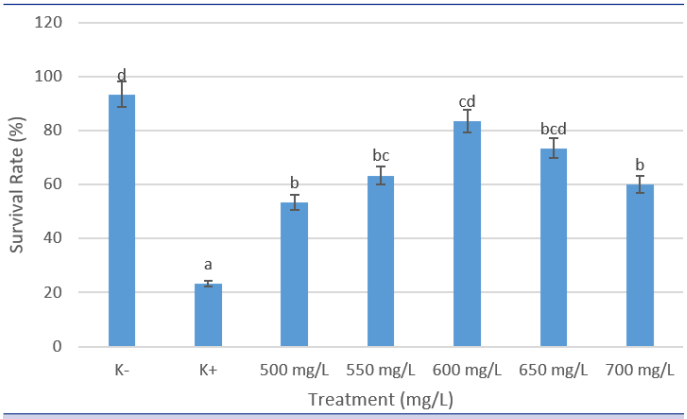

Figure 6. Survival Rate of Gourami Fish After Treatment.

CONCLUSION

In summary, the result of this study revealed that *M. koenigii* leaves are rich in alkaloids, terpenoids, saponins, flavonoids, and tannins so it provides an opportunity for usage as a natural product for the treatment of bacterial infections in fishes. The most effective dose for combating edwardsiellosis in gourami fish is 600 mg/L. This dose significantly influenced various hematological parameters, including hematocrit, hemoglobin, erythrocytes, leukocytes, lymphocytes, monocytes, and neutrophils. Additionally, it had a notable impact on histopathological aspects, with scores indicating mild degeneration, congestion, and necrosis.

To further advance the application of *M. koenigii* extract, additional research and experimentation should be conducted directly within aquaculture activities. This would help to better understand its potential benefits and feasibility in practical aquaculture settings.

Conflict of Interest: The authors declare no conflict of interest.

Ethics Committee Approval: This research does not harm the experimental animals used.

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