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Effectiveness of Giving Crude Extract of Red Betel Leaf (*Piper crocatum*) on the Hematology and Histopathology Profile of Goldfish (*Cyprinus carpio*) Gills Infected with *Aeromonas hydrophila* Bacteria

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Abstract: The purpose of this study was to analyze the hematology and histopathology of the gills of carp (C. carpio) treated with active compounds of red betel leaf extract (P. crocatum). This research method used a completely randomized design (CRD). The treatments used in this study were doses of 75 mg/L, 150 mg/L, 225 mg/L, 300 mg/L, 375 mg/L, negative control (healthy fish), and positive control (positive control), (healthy fish), and positive control (chloramphenicol antibiotic). Results of hematology and histopathology studies the results of hematology and histopathology studies on the gills of carp (C. carpio) after being infected with A. hydropilla bacteria experienced stress, this can be seen from. decreased red blood cells, increased leukocytes, and damage to the gill tissue. Carp returned to normal after being treated with red betel leaf extract. The best dose obtained after treatment was a dose of 300 mg/L. The results of clinical symptom observations showed a change from abnormal to normal conditions in goldfish after being treated. The conclusion is that red betel leaf extract contains dominant compounds in the form of flavonoids and alkaloids which can be useful as a medicine for goldfish infected with A. hydropilla bacteria.

Keywords: *Aeromonas hydropilla;* Hematology; Histopathology; *P. crocatum*

Introduction

Indonesia, being among the nations with diverse regions dedicated to freshwater fish culture, cultivates a multitude of freshwater fish commodities to sustain the economy and fulfill the demands of the community. One such commodity is Cyprinus carpio goldfish. Changing times and an increase in consumer demand are positively correlated with the advancement of technology in the fisheries sector, including intensive cultivation (Yuatiati et al., 2015). As a result of the increasing market demand, each cultivator has increased the stocking density during cultivation. This is consistent with the disease prevalence increasing beyond what it was previously, indicating that disease continues to play a significant role despite the relative simplicity of carp farming, particularly intensive cultivation. One of these diseases is fungi-induced, posing a significant obstacle to the expansion of aquaculture. Gold fish (*C. carpio*) are susceptible to various pathogens, including *Motile Aeromonas Septicemia* (MAS), which is caused by *A. hydrophila* bacteria (Maisyaroh et al., 2018).

According to Wahjuningrum et al. (2013), this disease is characterized by the following symptoms: decreased appetite, wounds on the body's surface,

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bleeding in the gill organs, an enlarged abdomen due to fluid accumulation, peeling fish scales, and injured tail fins. Furthermore, surgical intervention on infected organisms may reveal internal organ edema and injury, including lymph nodes, liver tissue, and kidneys.

Gill organs are exceptionally susceptible to pathogen exposure (including bacteria, viruses, fungi, and parasites) and are exceedingly sensitive to physical and chemical changes in water. Failure to promptly address damage to these gills may result in oxygen deprivation and ultimately fatality for the fish (Prasetio et al., 2021).

According to the findings of Kusrini et al. (2019), *A. hydrophila* bacteria are capable of inducing mortality in fish organisms, with a mortality rate ranging from 80% to 100% within a span of 1-2 weeks. MAS disease epidemics have also been documented on the Javanese, Kalimantan, and Sumatra islands. Generally, chemical treatment (antibiotics) is employed to combat diseases caused by *A. hydrophila* bacteria.

However, Labh et al. (2014), argue that prolonged antibiotic use can negatively affect the environment and promote the development of bacterial resistance to these drugs. Anggraini et al. (2017) provide explanations for additional adverse effects associated with antibiotic usage, including the potential for residues to form within the organism, thereby posing a threat to human consumption products. This effect necessitates the development of alternative materials in the form of natural ingredients that can serve as antibiotic substitutes and possess multiple qualities, including antibacterial activity, affordability, safety for organisms, profitability for producers, and safety for carp consumers.

An additional option that may be employed to address bacterial fish diseases is the application of natural constituents. It is believed that red betel leaf (*Piper crocatum*) can be utilized to treat bacterial diseases. According to Afiff et al. (2017), the ethanol extract derived from red betel leaves exhibits antibacterial properties against both gram-negative and gram-positive microorganisms. The bacteria *Staphylococcus aureus* is susceptible to inhibition by red betel leaf extract. The average inhibition zone of red betel leaf extract is 0.70 ± 0.15 mm, indicating that the effective concentration is 80%. As reported by Jusri et al. (2024), an antibacterial solution derived from red betel leaf extract exhibited a 14.28 mm inhibition at a concentration of 250 mg/L against *A. hydropilla.*

The betel leaf is an exceptionally practical herbal plant. This is due to the fact that extract of red betel leaf contains active compounds that inhibit bacterial proliferation. Red betel leaf extract contains alkaloids, flavonoids, tannins, triterpenoids, and saponins as active constituents (Jusril et al., 2024). Phenomenic compounds, of which these flavonoid compounds are a component, have the following biological activities: anticancer, antiseptic, anti-inflammatory, antioxidant, antidiabetic, and antibacterial (Kenitasari et al., 2023).

It is deduced from the preceding description that red betel leaves comprise bioactive compounds with potential antibacterial properties. Therefore, it is imperative to undertake research on the antibacterial active compounds present in the leaf extract of red betel (*Piper crocatum*) and its potential use against A. hydrophila bacteria, as determined by hematology and histopathology of the gills of goldfish (C. carpio).

Method

Research Materials

The material used in the extraction activity is red betel leaves (*Piper crocatum*) obtained from Herbal Materia Medica, Batu, East Java. The solvent for maceration is ethanol with pro-analytical (PA) quality based on research by Hikmawanti et al. (2021), For medicinal activities, the materials used are carp seeds (*C. carpio*) with a size of 7 – 10 cm.

Research Methods

The exploratory methodology is the one that was used in this review. The exploratory strategy, as per Hastjarjo (2019), is research that includes controlling outer factors, controlling autonomous factors, and estimating the impact of free factors on the reliant variable. Trial research should contain components of a benchmark group, treatment gathering and treatment mediation.

Research Design

The design in this research was a Completely Randomized Design (CRD). RAL is a single factor design. The treatments used in this study were different doses, positive control, negative control and 3 repetitions (Rahmawati et al., 2020), with treatment doses of 75, 150, 225, 300, and 375 mg/L. negative control used healthy fish and positive control used the antibiotic chloramphenicol.

Research Procedures

Aeromonas Hydropilla Infection and Soaking of Red Belt Leaf Extract (P. crocatum)

The bacteria that had been prepared on the TSB media were infected directly into the goldfish live media according to the desired density until they showed signs of being infected. The treatment process for carp that has been infected with *A. hydropilla* bacteria is soaked in a

container filled with Red Belt leaf extract (*P. crocatum*) according to the treatment dose.

Hematological Observations

Hematological observations were made on normal fish, fish that had been infected with *A. hydropilla* bacteria and after rearing for 7 days after being given the addition of red betel leaf extract (*P. crocatum*). Fish blood was taken with a 1 ml syringe which had been mixed with EDTA anticoagulant and erythrocyte count was carried out, hemoglobin, leukocytes and leukocyte differential.

Histopathological Observations of Gills

According to Nugrahani et al. (2016), tissue harvesting of target organs was carried out on all test fish. The tissue that has been taken is cleaned using distilled water. After that, put it in a film bottle containing 10% formalin. Then, histopathological preparations were made

Fish Survival

The survival of carp (*C. carpio*) was observed after 7 days of post-treatment rearing. Where the number of dead and live fish was counted from the treatment, both the control treatment and the test treatment.

Research Supporting Parameters

Supporting parameters in this study were clinical symptoms (behaviour, appetite and visible damage to fish from the outside), namely by carrying out a series of observation activities on the object under study, in this case, goldfish to see signs/physical abnormalities that occur in fish. mas who is stricken with the disease, as well as behavioral disorders (behavior). Data collection techniques in this study were carried out by observation or direct observation of the objects studied in the field, and microscopic observations in the laboratory on sampled fish.

Data Analysis

The information got was examined measurably with information utilizing a totally randomized plan (CRD). Statistical Package for the Social Sciences (SPSS) 26.0 for Windows was used to process and analyze the data.

Result and Discussion

Phase Research

Hematology

This study aims to determine the best dose of red betel leaf extract for the treatment of carp (*C. carpio*) after being infected with *A. hydropilla* bacteria with a bacterial density of 2.53 x 10^7 cells/ml according to the previous test results. This observation was carried out for 7 days with extract doses of 75 mg/L, 150 mg/L, 225 mg/L, 300 mg/L, and 375 mg/L.

Erythrocytes

Observations of the erythrocyte value of goldfish (*C. carpio*) were made during the study, namely before bacterial infection, after infection with *A. hydropila* bacteria, and after treatment using crude extract of red betel leaf (*P. crocatum*). The observation data can be seen in Table 1.

Table 1. Average Number of Goldfish Erythrocyte Cells Value During Research (x10^6)

Treatment (mg/L)	Healthy (x10^6)	Infected (x10^6)	P. Treatment (x10^6)
K-	1.72 ± 0.07	1.67 ± 0.04	1.67 ± 0.07
75	1.74 ± 0.04	0.85 ± 0.02	1.19 ± 0.05
150	1.70 ± 0.03	0.85 ± 0.04	1.26 ± 0.02
225	1.70 ± 0.02	0.87 ± 0.06	1.29 ± 0.01
300	1.71 ± 0.04	0.86 ± 0.03	1.39 ± 0.02
375	1.74 ± 0.05	0.87 ± 0.03	1.32 ± 0.02
K+	1.75 ± 0.05	0.86 ± 0.02	1.60 ± 0.03

Based on the results of this study, the average number of goldfish erythrocytes after treatment using red betel leaf extract showed significant differences between treatments ($P < 0.05$). The value of erythrocytes showed increased activity after treatment. The treatment process with red betel leaf extract at a dose of 300 mg/L has the highest mean value of the other treatments, but at a dose concentration of 375 mg/L decreased the mean value of erythrocytes caused by stress factors due to too high a dose used and can cause anemia. The number of erythrocytes decreased can be caused by other factors including the lack of nutrients that enter the fish body. These nutrients play a role in the formation of red blood cells in the body.

The decrease in the number of erythrocytes that occurs is thought to be caused by the pathogenicity of *A. hydropilla.* This bacterium produces a hemolysin enzyme that can lyse red blood cells, so that the number of red blood cells in blood vessels can be reduced (Maisyaroh et al., 2018). Reduced erythrocyte values in fish can also be caused by bleeding in organs that have not recovered and can damage external organs and cause injuries (Maharani et al., 2022).

Hematocrit

The results of research on the treatment of carp (*C. carpio*) infected by *A. hydropilla* bacteria and treated using red betel leaf extract that in the process of observing the hematocrit value of carp (*C. carpio*) carried out during the study, namely before bacterial infection, after infection with *A. hydropilla* bacteria and after

treatment using red betel leaf extract. Hematocrit value data during the study are presented in Table 2.

The results of the explanation of the table above, the average amount of goldfish hematocrit after treatment has shown a significant difference between treatments (P <0.05). The hematocrit values were different after treatment. When compared with post-infection, post-treatment hematocrit values began to increase towards normal levels of hematocrit values in goldfish (*C. carpio*) The occurrence of this effect, the best hematocrit value at a dose concentration of 300 mg/L extract with a range of hematocrit values of 27.47%. These conditions indicate that the dosing of red betel leaf extract significantly affects the hematocrit value. According to Putra (2015), that the hematocrit value of normal goldfish ranges from 24-40%. These hematocrit levels vary depending on nutritional factors, body size, sex, and age of the fish. According to Nugrahani et al. (2016), The increase in erythrocyte levels in the blood of goldfish is the effect of the activity of crude betel leaf extract.

Table 2. Average Goldfish Hematocrit Value During Research (%)

Treatment (mg/L)	Healthy (%)	Infected (%)	P. Treatment (%)
K-	28.40± 0.10	28.57± 0.31	28.50 ± 0.20
75	28.42± 0.16	16.00 ± 0.17	17.70 ± 0.20
150	28.62± 0.26	15.87 ± 0.15	21.93 ± 0.15
225	28.50± 0.30	15.77 ± 0.15	24.90 ± 0.20
300	28.53± 0.15	15.93 ± 0.15	27.47 ± 0.15
375	28.53± 0.25	15.83 ± 0.21	25.93 ± 0.15
K+	28.61± 0.18	16.03 ± 0.31	27.47 ± 0.40

The results of hematocrit values decreased again at a dose of 375 mg/L due to stress factors due to too high a dose used. The decrease after the optimal dose is caused by fish experiencing stress, according to Shabirah et al. (2022), explained that when fish experience infection, hematocrit levels will decrease. Fish that have hematocrit values below 20% indicate that the fish are experiencing erythrocyte deficiency. The low number of erythrocytes and hematocrit is an indication that the fish is anemic.

Hemoglobin

Treatments carried out during the study of goldfish (*C. carpio*) that were infected by *A. hydrophila* bacteria which was then given red betel leaf extract (*P. crocatum*), gave different average results on the amount of hemoglobin in goldfish. Based on the results of the research that has been done, the average data on the amount of hemoglobin can be seen in Table 3.

Based on the table above, the highest increase in hemoglobin value in the 300 mg/L treatment was 6.84 g/dL. The average amount of hemoglobin of goldfish after treatment has shown a significant difference between treatments (P <0.05). From the table above, it can be seen that the extract concentration of 300 mg/L is the best treatment of red betel leaf extract (*P. crocatum*) to increase the hemoglobin value of goldfish (*C. carpio*) after treatment. The antibacterial properties of red betel leaves also play a role in repairing and activating damaged protein molecules and can help repair and reactivate damaged hemoglobin (Maryani et al., 2020).

Table 3. Average Hemoglobin Value of Goldfish (*C. carpio*) Value During Research

Treatment (mg/L)	Healthy (g/dL)	Infected (g/dL)	P. Treatment (g/dL)
K-	7.342± 0.62	7,338± 0.41	7.314±0.26
75	7.239± 0.58	4,220± 0.25	5,161±0.10
150	7.273±0 .28	4,242± 0.13	5.601±0.38
225	7.272± 0.79	4,309± 0.46	6,163±0.10
300	7.302± 0.83	4,286± 0.26	6.846±0.39
375	7.213± 0.43	4,353± 0.27	6.688±0.16
K+	7.342± 0.88	4,408± 0.34	7.133±0.40

However, at a dose of 375 mg/L, the hemoglobin value decreased to 6.68 g/dL, this was caused by stress factors due to the too high dose used and also caused anemia. As explained by Astuti et al. (2017) explained that when the value of erythrocytes in the blood decreases, there will be a decrease in the value of hemoglobin in the blood of fish. This can cause the availability of oxygen in the fish body to decrease (hypoxic conditions) so that it will cause a lack of energy which indicates anemia. According to Fallah et al. (2014), low hemoglobin levels are an indicator that fish are anemic. Anemic fish cannot absorb enough iron to form hemoglobin. In this condition, red blood cells are formed that contain small amounts of hemoglobin.

Leukocytes

The results showed that the treatment carried out during the study of goldfish (*C. carpio*) infected by A. hydrophila bacteria which was then given red betel leaf extract (*P. crocatum*) gave different average results for each treatment on the number of leukocytes in goldfish (*C. carpio*). Based on the results of research that has been done obtained data on the average number of leukocytes can be seen in Table 4.

Table 4. Average Leukocyte Value of Goldfish (*C. carpio*) Value During Research

Treatment (mg/L)	Healthy ($x10^4$)	Infected ($x10^4$)	P. Treatment ($x10^4$)
K-	9.63 ± 0.49	9.34 ± 0.47	9.31 ± 0.17
75	9.58 ± 0.20	14.46± 0.21	13.34± 0.09
150	9.65 ± 0.43	14.48± 0.11	12.34± 0.31
225	9.57 ± 0.81	14.46± 0.13	11.50± 0.25
300	9.54 ± 0.35	14.55± 0.28	10.23± 0.08

Treatment (mg/L)	Healthy ($x10^4$)	Infected ($x10^4$)	P. Treatment ($x10^4$)
375	9.55 ± 0.53	14.62± 0.36	10.41± 0.16
K+	9.80 ± 0.50	14.42± 0.11	9.51 ± 0.11

Based on the results of the treatment of red betel leaf extract (*P. crocatum*), there was a decrease in the best leukocyte value during the study, namely the concentration of 300 mg/L at $10.87x10^4$ cells/mm^3. The decrease in the number of leukocytes is thought to be due to the activity of red betel leaf extract which plays a role in the treatment process of goldfish after being infected with *A. hydropilla* bacteria, so that the number of leukocytes in the blood of fish returns to normal. according to Gerrine et al. (2023), explaining that the decrease in leukocyte count is thought to be due to the activity of red betel leaf extract which plays a role in the treatment process of carp after being infected with *A. hydropilla* bacteria.

However, the more extract used, the more it will be considered as a foreign body by fish leukocytes. This is indicated by the increase in leukocyte values again at 375 mg/L extract treatment concentration. So that in the fish body defense system, leukocytes will play a role to phagocytize the extract which is considered a foreign body. This shows that the more extracts used will not necessarily also help treatment efforts due to bacterial infections (Sumsanto et al., 2019). The active components contained in an extract are toxic to test animals if the concentration is too high. The use of natural ingredients with the right dose can stimulate the immune system and increase protection against infection (Awad et al., 2017).

Histopathology of Goldfish Gill Organs (C. carpio)

Histopathological observation of the gill organs in carp (*C. carpio*) can provide an overview of the condition of the fish's gills both before being infected with *A. hydrophila bacteria*, after being infected, and after treatment using crude extract of red betel leaves (*P. crocatum*).

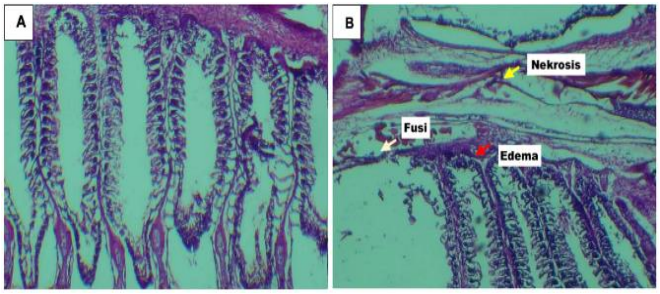

Figure 1. Condition of goldfish gills (*C. carpio*) without treatment (a) and infection with *A. hydropilla bacteria* (b) with 400x magnification

In light of the picture above with an amplification of multiple times, it shows the solid state of the goldfish (1a) showing that the gill organ tissue is still intact and does not show any significant damage. Meanwhile, the gills infected with *A. hydropilla bacteria* (1b) show a lot of damage to the gill organ tissue that occurs, such as fusion, edema and necrosis which, if left unchecked, will inhibit the fish's breathing and result in death.

From the results of observations, it was found that tissue damage to the gills of carp (*C. carpio*) was reduced after treatment with red betel leaf extract (*P. crocatum*), starting from doses of 75 mg/l, 150 mg/l, 225 mg/l, 300 mg/l, and 375 mg/l. The highest level of damage was found in treatment A, namely a dose of 75 mg/l, while the least level of damage was found in treatment E, a dose of 300 mg/l. This can be explained by the effect of treatment using red betel leaf extract (*P. crocatum*) which contains antimicrobials, which has succeeded in inhibiting and repairing tissue damage to the gill organs of goldfish.

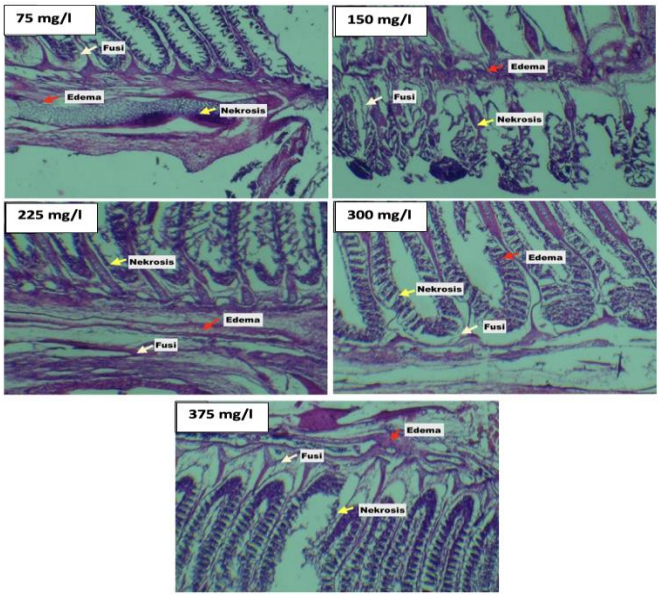

Figure 2. Condition of goldfish (*C. carpio)* gills after treatment with 400x magnification, (a) dose 75 mg/ L , (b) dose 150 mg/ L , (c) dose 225 mg/ L , (d) dose 300 mg/ L , and (e) dose 375 mg/ L

The results of this study additionally demonstrate the various forms of tissue injury observed in the gills of goldfish (C. carpio), namely Edema, Fusion, and Necrosis. Discussion of data analysis regarding this form of damage will continue below.

Table 5. Post-Treatment Analysis of Gill Organ Damage Infected with *A. hydropilla* Bacteria

Treatment (mg/L)	Edema (%)	Fusion (%)	Necrosis (%)
K-	0.53±0.23	0.53±0.11	0.53±0.11
75 mg/L	2.53± 0.23	2.40± 0.34	2.53± 0.30
150 mg/L	2.27± 0.30	2.13± 0.11	2.27± 0.23
225 mg/L	1.93± 0.23	1.93± 0.11	2.00±0.20
300 mg/L	1.40± 0.20	1.67± 0.23	1.47± 0.11
375 mg/L	1.53± 0.11	1.73± 0.50	1.67± 0.23
K+	0.73±0.11	0.73±0.11	0.73±0.11

Edema

Result the treatment involving the administration of red betel leaf extract (*P. crocatum*) during the investigation revealed that goldfish (*C. carpio*) infected with A. hydropilla exhibited varying average scores for gill damage manifested as edema, as shown in Table 7. The findings indicate that the damage to gill tissue, particularly edema, differed significantly between the drugs (P<0.05). The findings of the research demonstrated that the application of red betel leaf extract (*P. crocatum*) effectively diminished the extent of histopathological gill damage in goldfish infected with A. hydropila microorganisms. Specifically, the treatment group treated at a concentration of 300 mg/L experienced the smallest reduction in edema damage by 1.40%.

Nonetheless, as treatment progressed, the concentration rose to 375 mg/L. This suggests that the administered extract has achieved its maximum efficacy in managing goldfish that are afflicted with A. hydropilla. According to Priyanka et al. (2020), the activity of an active compound is contingent upon its ability to reach its intended site of action, which necessitates blood absorption from the digestive tract.

Fusion

The research results presented in Table 8 indicate that there were significant variations in the extent of injury inflicted on gill tissue, particularly when considering the combination of medications (P<0.05). The findings of the research demonstrated that the application of red betel leaf extract (*P. crocatum*) effectively diminished the histopathological gill damage value in goldfish infected with A. hydropilla bacteria. Notably, the treatment portion at a concentration of 300 mg/L exhibited the most minimal value, amounting to a combined loss of 1.67%.

It appeared that the average fusion injury increased in the 375 mg/L treatment. This suggests that the administered extract has achieved its maximum efficacy in managing goldfish that are afflicted with *A. hydropila*. Consequently, exceeding the optimal dose will result in more pronounced harm to the gill tissue. This aligns with the findings of Castilhos et al. (2018), who discovered that a number of secondary metabolite compounds exhibit toxicity as a result of their function as a self-defense mechanism. Greater harm to tissue cells was indicated by the higher dose of extract administered; in the treatment group, the greater damage was indicated by the higher dose. Nevertheless, no test animals perished as a result of the treatment.

Necrosis

The research results in table 5 indicate that providing red betel leaf extract (*P. crocatum*) as a treatment method resulted in substantial variations in the occurrence of gill tissue damage, namely necrosis, among the different treatments (P<0.05). The investigation revealed that the application of red betel leaf extract (*P. crocatum*) effectively decreased the extent of histological gill damage in goldfish infected with *A. hydropilla* bacteria. The lowest level of damage, specifically necrosis damage of 1.53%, was seen at a treatment dose of 300 mg/L.

When goldfish were exposed to a concentration of 375 mg/L, there was a noticeable increase in tissue damage to their gills, with an average damage level of 1.67%. The reason for this is that the given extract has reached the perfect stage of goldfish aftercare, when it has become infected with A. hydropilla.

This indicates that the extract given has reached its optimal point in treating goldfish (*C.carpio*) after being infected with A.hydropila. So a dose that is higher than the optimum dose will cause more severe damage to gill tissue (Rand et al., 2015).

Clinical Symptom Analysis

Clinical symptoms of *A. hydropilla infection* in fish include bleeding at the base of the fins, reddish lesions on the top of the head accompanied by ulceration, exophthalmia, abdominal distension, anorexia, and swimming in circles. According to Dontriska et al. (2014), the symptoms that appear when fish are exposed to bacteria are that the body becomes dark, the fish's swimming ability begins to decrease, often reaching the surface of the water because its gills are damaged, causing difficulty breathing. You can also see that the stomach is slightly bloated or swollen. If it is too severe, the fins can be damaged and the gills will turn whitish. The eyes also protrude slightly. The aftereffects of noticing the clinical side effects of goldfish (*C. carpio*) in the wake of being tainted with *A. hydropilla* microscopic organisms are introduced in Figure 3.

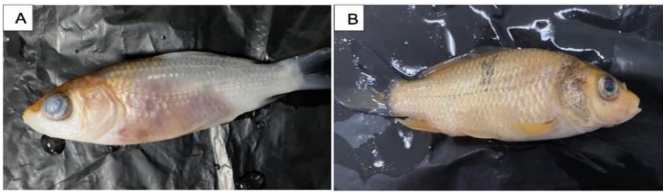

Figure 3. Clinical symptoms of goldfish (*C. carpio*) infected with *A. hydrophila*, (a) swollen eyes, pale body color, and inflammation of the body and (b) swollen eyes and peeling scales

The observations depicted in the image elucidate the many alterations in the clinical symptoms of goldfish (*C. carpio*) during treatment, ultimately leading to their restoration of good health. Additionally, the use of natural plants, namely red betel leaves, for therapy is backed by scientific evidence. These leaves are rich in antibacterial substances such as flavonoids, alkaloids, tannins, and saponins. According to Kurniawan et al. (2022), active antibacterial chemicals enter the fish's body by diffusion, which enhances the fish's immune system and helps combat pathogenic bacteria in ill fish. This condition accelerates the process of healing in the injured areas of the fish's body. According to Jusril et al. (2024), explaining that the effect of treatment using red betel leaf extract (*P. crocatum*) which contains antimicrobials, managed to repair tissue damage to the gill organs of goldfish and can return to health.

Figure 4. Condition of goldfish after treatment with red betel leaf extract (P. crocatum)

Survival Rate

Based on the table above, the survival results for goldfish (*C. carpio*) show that the highest mean survival value was obtained in the 300 mg/L treatment at 80% and was close to normal control at 90.00%, while the lowest mean value was in the Positive Control treatment at 23.33%. The percentage of survival at the best dose showed significantly different results to the Negative Control, so that in application in cultivation, apart from giving treatment with the primary dose, it is necessary to give repeated treatment with a booster dose as a booster to increase the percentage of survival.

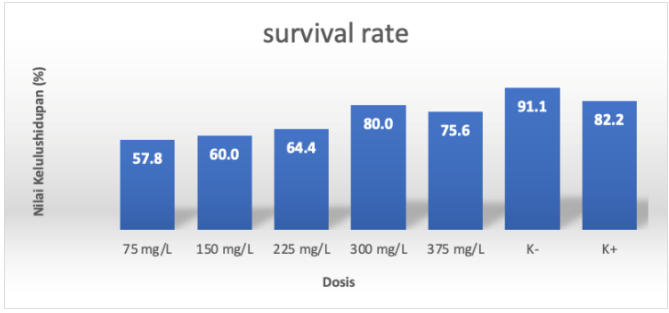

Figure 5. Survival rate of goldfish (*C. carpio*)

Test fish endurance is contrasting the quantity of test fish that are alive toward the finish of the review with the quantity of test fish supplied toward the beginning of the review (Gusman et al., 2014). Endurance is impacted by two elements, specifically inward factors and outside variables of the fish. Outside factors incorporate abiotic conditions (water quality), contest between species, expanding the quantity of fish populaces in a similar space (fish thickness component), and taking care of during treatment. Inner variables comprise old enough, the capacity of the fish to adjust to its current circumstance and the state of being of the fish. Fish thickness can likewise impact mortality (Hikmawanti et al., 2021).

Water Quality Observation

Water quality measurements during the study included temperature, pH, DO in each rearing media container. The water quality of the rearing media is the main key to changes in fish conditions. During the study, water quality measurements were taken twice a day in the morning and evening. The average results of water quality measurements during the study are presented in Table 6.

Table 6. Water Quality Parameters for Carp (*C. carpio*) Rearing

Parameter	Observation result	Optimal Range
Temperature (°C)	25-27	25-32 (Sihite et al., 2020)
pH	6.50-7.60	6.5-8.5 (Sihite et al., 2020)
Dissolved Oxygen (DO)	5.0-6.10	5 -7 (Nasir et al., 2016)

According to the findings of the research, the water quality of the maintenance media over the course of seven days of observation was satisfactory in that it did not compromise the physiological health of the fish. The water quality during the maintenance period of carp (*C. carpio*) remains within the optimal range, as indicated by the analysis results. Sihite et al. (2020) identify the optimal temperature range of 25-32 °C for carp maintenance (*C. carpio*), which includes the optimal

range for carp growth, within the maintenance media temperature range of 25-27 °C. The temperature of the water has a significant impact on the growth rate, metabolism, appetite, and oxygen solubility of fish. The findings indicated that the pH of the water fluctuated between 6.5-7.6 throughout the carp rearing period (C. carpio). The optimal pH range for carp growth, according to Dontriska et al. (2014), is between 6.5 and 8.5; therefore, the pH range is optimal during the maintenance period. The productivity of waters is influenced by the degree of acidity (pH) of the water, which becomes the primary determinant in determining whether a body of water is at its optimal quality.

Dissolved oxygen (DO) levels in the maintenance of carp (*C. carpio*) have been observed to range between 5.0 and 6.07 mg/l. According to Nasir et al. (2016), this condition is still deemed ideal, as the ideal concentration of dissolved oxygen for carp (*C. carpio*) falls within the range of 3-5 mg/l. Proper maintenance of fish maintenance media is imperative to prevent the induction of stress in fish, which can increase their vulnerability to diseases.

Conclusion

Based on the research results, the optimal dose of red betel leaf extract (P. crocatum) ranges from 288 mg/l – 451.57 mg/l and is bactericidal. The use of red betel leaf extract (P. crocatum) as a form of treatment for carp (C. carpio) after being infected with A. hydrophila has a significant effect on hematology (erythrocytes, hemoglobin, hematocrit, leukocytes, lymphocytes, monocytes, and neutrophils) and histopathology of the carp gills.

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Conceptualization, Pratama, M. J. A., Fadjar, M. And Paricahya, A. F.;methodology, Pratama, M. J. A.; and Paricahya, A. F.; validation, Paricahya, A. F.;. and Fadjar, M.; formal analysis, Pratama, M. J. A.;investigation, Pratama, M. J. A., Fadjar, M.; resources, Pratama, M. J. A., Fadjar, M and Paricahya, A. F.;data curation, Pratama, M. J. A.; writing—original draft preparation, Pratama, M. J. A.; writing—review and editing, visualization, Pratama, M. J. A.;supervision, Prajitno, A. and Fadjar, M.; project administration, Pratama, M. J. A., Fadjar, M, and Paricahya, A. F.; funding acquisition, Pratama, M. J. A., Fadjar, M.

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The authors declare no conflict of interest.

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