2025, Scienceline Publication
World's Veterinary Journal
World Vet J, 15(3): 612-621.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2025.wvj61
PII: S2322456825000061-15

Effectiveness of Guava Leaf Fraction on Phagocytosis Activity and Leukocyte Differentiation in Tilapia (*Oreochromis niloticus*) Infected with *Aeromonas hydrophila*

Lailatul Munawaroh Dewi Kusuma Wardani[1]*, Sri Andayani[2], and Yunita Maimunah[2]

[1] *Graduate Student of Aquaculture, Faculty of Fisheries and Marine Science, Brawijaya University, Jalan Veteran, Malang, 65145, Indonesia*
[2]*Aquaculture Department, Faculty of Fisheries and Marine Science, Brawijaya University, Jalan Veteran, Malang, 65145, Indonesia*
*Corresponding author's Email: lailatulmdk@gmail.com

ORIGINAL ARTICLE
Received: June 20, 2025
Revised: July 24, 2025
Accepted: August 26, 2025
Published: September 30, 2025

ABSTRACT

One obstacle to the sustainability of fisheries is the bacterial infection caused by *Aeromonas hydrophila (A. hydrophila)*. To overcome the sustainability issues posed by antibiotic use against *A. hydrophila* in fisheries, guava leaf (*Psidium guajava* L.) immunostimulants offer a promising natural alternative. The present study aimed to examine the impact of dynamic compounds derived from guava leaves on leukocyte differentiation and phagocytosis in tilapia infected with *A. hydrophila*. A completely randomized design was established, consisting of three treatment groups and two controls, with three replications. The groups included 150 ppm of guava leaf fraction (Group A), 125 ppm of guava leaf fraction (Group B), and 100 ppm of guava leaf fraction (Group C), which were injected into tilapia. The negative control group was infected with *A. hydrophila* bacteria without receiving the guava leaf fraction, and the positive control group was not infected with *A. hydrophila* bacteria and did not receive the guava leaf fraction. The dosage determination of the guava leaf fraction was conducted using the lethal concentration 50% test. The results demonstrated that Fraction 5 of guava leaf extract significantly enhanced the non-specific immune response of tilapia. The optimal dose of 150 ppm led to a substantial increase in lymphocyte counts and phagocytic activity following infection with *A. hydrophila*. Consequently, all treatment groups (100, 125, and 150 ppm) exhibited survival rates statistically equivalent to those of the positive control group, which was significantly higher than the 39% survival rate in the negative control group. Thus, guava leaf Fraction 5, particularly at 150 ppm, serves as an effective immunostimulant and natural antibiotic alternative for controlling A. hydrophila in tilapia.

Keywords: *Aeromonas hydrophila*, Guava leaf fraction, Immune response, Immunostimulant

INTRODUCTION

Tilapia (*Oreochromis niloticus*) is a key aquaculture commodity in Indonesia, widely cultivated due to its relatively low price, resulting in higher demand for domestic consumption. The farming of tilapia in Indonesia is increasing at a rate of 23.8% per year (Hadie et al., 2018). However, one of the obstacles to the development and sustainability of aquaculture, especially in tilapia, is bacterial infections (Saputra and Indaryanto, 2018). A significant concern is the infection caused by *Aeromonas hydrophila* (*A. hydrophila*), which frequently affects tilapia and can result in mortality rates of up to 85% in aquaculture (Tondi et al., 2023).

Aeromonas hydrophila is a bacterium that is widespread in fresh, brackish, and saline waters, and it can infect a variety of freshwater fish. Fish affected by *A. hydrophila* typically exhibit signs of abdominal swelling and bleeding on the outer surface or skin, as well as around the anus (Nahar et al., 2016). Infected fish may develop ulcers, red spots on the body surface, inflamed skin, decayed fins and tail, abdominal swelling, and bulging eyes (Arwin et al., 2016). Other clinical signs include detached scales from the body, a dark body color, a pale liver, skin bleeding, excessive mucus production, and tail damage (Pattipeiluhu et al., 2022).

Motile *Aeromonas hydrophila* (MAS) can result from several stress factors, including overcrowding in ponds, changes in environmental conditions, and inadequate water quality. Clinical signs of MAS include ulcers, bloating, fin irritation, and exposed scales (Tondi et al., 2023). Infected fish experience blood cell damage as a result of the immune response, and blood examination is used as an indicator of disease severity, highlighted by changes in leukocyte count values (Pattipeiluhu et al., 2022). The abundance of *A. hydrophila* tends to increase with declining water quality and stressed fish conditions (Saputra and Indaryanto, 2018). Furthermore, high levels of *A. hydrophila* in water can contribute to environmental pollution and toxicity (Hidayaturohman et al., 2021). While chemicals and antibiotics can control *A. hydrophila*, their use is frequently costly and may harm the environment and human health (Cao et al., 2012).

The control of *A. hydrophila* infections in fish often involves the use of several effective antibiotics, including enrofloxacin, ciprofloxacin, and fluoroquinolones. Enrofloxacin, in particular, is notable for its effectiveness in treating

To cite this paper: Wardani LMDK, Andayani S, and Maimunah Y (2025). Effectiveness of Guava Leaf Fraction on Phagocytosis Activity and Leukocyte Differentiation in Tilapia (*Oreochromis niloticus*) Infected with *Aeromonas hydrophila. World Vet. J.*, 15(3): 612-621. DOI: https://dx.doi.org/10.54203/scil.2025.wvj61

these infections. Additional antibiotics such as tetracycline, sulfonamides, trimethoprim, sulfamethoxazole, and ciprofloxacin can be employed to address skin infections caused by *A. hydrophila* (Perkasa and Dhewantara, 2019). However, the use of antibiotics in aquaculture is often inefficient, highlighting the need for alternative control measures. One promising approach is to enhance fish immunity through the use of immunostimulants derived from natural medicinal plants. These plants provide a safe and environmentally friendly alternative to traditional antibiotics. Guava leaves (*Psidium guajava* L.) are particularly noted for their potential as immunostimulants in fish, as they contain active compounds such as tannins, flavonoids, saponins, and phenolics, which can bolster defenses against diseases (Sine and Fallo, 2016; Amelia et al., 2021).

Immunostimulants serve to replace antibiotics and vaccines by activating or enhancing the components of the immune system in a non-specific manner. Immunostimulants have been shown to stimulate non-specific immune responses in fish, such as tilapia, improving the fish's defense against diseases (Sine and Fallo, 2016). The immune system includes B cells and T cells. B cells can differentiate into plasma cells that produce antibodies, while T cells play a crucial role in mediating immunity and regulating immune responses. T cells can become cytolytic T cells, which target and destroy pathogens (Setyowati and Astriana, 2020). Immunostimulants have been shown to increase T cell lymphocytes in numerous animals, including fish, enhancing immunity against intracellular bacteria and viruses (Setyowati and Astriana, 2020). Evidence suggested that compounds found in guava leaves can enhance the activity of immune cells such as macrophages, T cells, and B cells, leading to improved phagocytosis (Rochmasari, 2011). These leaves can boost the function of neutrophils, basophils, monocytes, lymphocytes, and eosinophils (Kullu et al., 2013). In healthy fish, lymphocytes typically constitute about 71.12% to 82.88% of total leukocytes, outnumbering neutrophils and monocytes. An increase in lymphocyte production is significant in enhancing the immune response, thereby improving the fish's resistance to disease and infection (Setyowati and Astriana, 2020).

The use of guava leaf fraction is expected to serve as an immunostimulant, enhancing the nonspecific immune system, as indicated by leukocyte differential and phagocytosis activity in tilapia infected with *A. hydrophila*. Based on the description provided, it can be inferred that one of the obstacles in tilapia aquaculture is infection by *A. hydrophila*. Therefore, the present study aimed to provide guava leaf fractions as immunostimulants in tilapia to prevent *A. hydrophila* bacterial infections in aquaculture.

MATERIALS AND METHODS

Ethical approval

The authors have designed the study in accordance with the ethical guidelines of Brawijaya University, Indonesia. The study was carried out in accordance with the applicable regulations for the master's program at the Faculty of Fisheries and Marine Science of Brawijaya University, Indonesia, as outlined in Rector's Regulation Number 427/PER/2012 concerning academic regulations.

Study area

The present study was conducted at the Laboratory of the Faculty of Fisheries and Marine Science, Brawijaya University, Malang, Indonesia, from September 2024 to February 2025. The animals used were tilapia strain Sultana, measuring 10 to 12 cm (± 18 grams/head), with a density of 18 fish per aquarium, sourced from Surowono, Kediri, Indonesia.

Parameters

The primary parameters measured were phagocytosis activity and leukocyte differentials in tilapia. Additional factors included the survival rate (%) and water quality, which covered temperature (°C), pH, and dissolved oxygen (DO, %).

Survival rate

The survival rate (SR) represents the percentage of organisms that remain alive at the end of a rearing period compared to those alive at the beginning. Daily data collection included recording the number of surviving tilapia fish both at the beginning and the end of the maintenance period. Subsequently, the data was calculated using the following formula.

Survival rate = Number of live tilapia at the beginning of rearing / Number of tilapia at the end of rearing (Fahrizal and Nasir, 2017).

Water quality

To measure the temperature, the thermometer was submerged at least 10 cm beneath the water's surface and kept in the water for five minutes. For pH measurement, a piece of litmus paper was placed in the water, and the resulting color change was matched against a pH chart to assess the pH level. To evaluate DO, the DO Meter's pen was immersed in the water, and the dissolved oxygen reading was automatically shown on the DO Meter's display (Nursobah et al., 2022).

Experimental design

A completely randomized design was employed, consisting of five treatment groups and three replicates. The negative control group consisted of tilapia that were not infected with *A. hydrophila* and were treated with a guava leaf fraction. The positive control group comprised tilapia infected with *A. hydrophila* and not administered the guava leaf fraction. Group A received the guava leaf fraction at a dose of 150 ppm. Group B received guava leaf fraction at a dose of 125 ppm, and Group C received guava leaf fraction at a dose of 100 ppm. On day 0, prior to the first counting day, the fish's body weight was recorded, blood was drawn, and the first injection of guava leaf fraction was given in all treatment groups except the negative control group. Injections were administered to the fish at the base of the pectoral fin or the tip of the dorsal fin, after which all groups (Group A, B, and C) were allowed to recover for 72 hours. Blood was drawn intramuscularly from the pectoral fins and dorsal fins into a small tube and examined under a microscope (ReHaZe, Indonesia) at 1000x magnification in the laboratory (Haditomo, 2017). On day three, blood was drawn from all groups for observation before infection, followed by the second injection of guava leaf fraction. On day six, blood was taken for observation before infection, after which the fish were infected with *A. hydrophila*. On days 9 and 12, blood was drawn for observation after *A. hydrophila* infection.

Lethal concentration 50% and lethal dose 50%

The toxicity profile of guava leaf fraction in freshwater fish has been documented to range from 250 ppm to 3250 ppm, demonstrating its potential utility in managing pathogenic bacterial infections in aquaculture (Rosidah and Afizia, 2012). A detailed examination of the lethal concentration 50% (LC_{50}) is essential in ascertaining the optimal dosage of the guava leaf fraction. In the relevant study, guava leaf fraction was administered intramuscularly at dosages of 125, 150, 175, 200, and 225 mg/kg of fish body weight. Results from the bacteriostatic inhibition assays indicated that the guava leaf fraction effectively suppressed the growth of *A. hydrophila*, with a significant effect observed at a concentration of 175 ppm. The fractions designated for further application were identified at concentrations of 150 ppm, 125 ppm, and 100 ppm.

To evaluate the lethal dosage 50% (LD_{50}), an experimental approach was utilized. Initially, pure cultures of *A. hydrophila*, at a concentration of 10^{10} cells/ml, were subjected to dilution, resulting in concentrations of 10^9 cells/ml, 10^8 cells/ml, 10^7 cells/ml, and 10^6 cells/ml in tryptone soy broth (TSB; Merck, Germany) for LD_{50} assessment (Sulastra and Khaerati, 2020). The incubation of these dilutions occurred over a 24-hour period at temperatures ranging from 27°C to 30°C (Telaumbanua et al., 2019). In the experimental setup, 18 tilapias were infected with *A. hydrophila* and kept in 10 liters of media water, following established treatment protocols, and monitored at four-hour intervals. Mortality rates among the fish were carefully documented over durations of 24 to 72 hours, with the initial occurrence of 50% mortality serving as the criterion for determining the LD_{50} threshold. The LC_{50} calculation used the previously mentioned dosages of guava leaf fraction administered through intramuscular injection. These dosages were based on inhibition tests that assessed their effects on *A. hydrophila* proliferation. Each test container contained 18 fish, with observations made throughout 24 to 72 hours. The first observation of 50% mortality was crucial for determining the LC_{50} dosage.

Thin-layer chromatography and column chromatography

Fractionation analysis was carried out using thin-layer chromatography (TLC; Merck, Germany) and column chromatography, following the method of Fasya et al. (2018) to purify fractions from guava leaves. After obtaining the fractions, non-specific immune response tests were performed on tilapia. Before focusing on the most promising fraction, LD_{50} and LC_{50} tests were conducted to ensure safety and effectiveness. As noted in Rusmiyati's study (2023), this fractionation process was designed to separate compound groups by their polarity, using n-hexane and ethyl acetate as solvents. For the TLC plates, strips were cut to be 1 cm wide and 10 cm tall, with a 0.5 cm margin at the top and a 1 cm margin at the bottom. The plates were then placed in an oven (Memmert, Germany) with a capacity of 55L, set to a temperature of 100°C to 120°C for approximately 15 minutes to evaporate any moisture. The guava leaf fraction was dissolved in a sufficient amount of ethanol. Approximately 5 µl of the dissolved fraction was then applied to the prepared TLC plate using a microsyringe. The plate was positioned upright in a 250 ml chamber containing 10 ml of eluent solvent and was covered with cling wrap to prevent evaporation. Once the eluent reached the upper limit, the TLC plate was carefully removed using tweezers. The staining agent used was ultraviolet (UV) light, which made the stain visible under near-UV (254 nm) and far-UV (366 nm) illumination.

Blood collection

Leukocyte differentiation was performed by taking anticoagulated fish blood with a capillary pipette and placing it on the tip of an object glass. The object glass was fixed with methanol for two minutes. Following this, the blood smear was stained with Giemsa solution (Larutan Giemsa 100ml Indo Reagen, Indonesia) and left for 20 minutes. The glasses were rinsed with distilled water and allowed to dry. Object glasses were observed using a microscope (ReHaZe, Indonesia) at 1000x magnification in the laboratory. Fish phagocytosis activity was initiated by treating fish blood with anticoagulants, then taking 50 µL, placing it into a microtiter plate, and adding 50 µL of *A. hydrophila* suspension in FBS (10^8 cells/mL). The blood was incubated at 27°C for 20 minutes (Haditomo, 2017).

Statistical analysis

The data obtained were then statistically analyzed using analysis of variance (ANOVA) with a significance level of 95% ($p > 0.05$). This analysis was followed by Duncan's test using the IBM SPSS program application version 25.

RESULTS AND DISCUSSION

Thin-layer chromatography analysis

The TLC was conducted to determine the optimal solvent ratio for the mobile phase eluent in the fractionation analysis of the guava leaf fraction. The primary objective of this analysis was to identify the most effective eluent for producing the guava leaf fraction. The results of the analysis indicated that the combination of n-hexane and ethyl acetate in a 6:4 ratio was the most effective at generating color on silica gel plates (Darmstadt, Germany) under UV light at 365 nm.

Column chromatography analysis

Column chromatography was conducted to yield pure fractions in substantial quantities. The solvent utilized in this process was a mixture of n-hexane and ethyl acetate in a 6:4 ratio, serving as the mobile phase, with silica gel powder functioning as the stationary phase. The outcomes were derived from the column chromatography test, using the plate that exhibited the most intense stain color. The present study successfully produced five distinct fractions. All fractions were tested on the same species of fish, tilapia, at concentrations of 150 ppm (Group A), 125 ppm (Group B), and 100 ppm (Group C). The fish in all groups were identical to precisely evaluate how these fractions affect non-specific immune responses, especially leukocyte activity.

Non-specific immune response test

The non-specific immune response test of tilapia against all guava leaf fractions was conducted to identify one candidate fraction from five types of fractions, based on the specific immune response (Leukocytes) of tilapia *in vivo*. The current results highlighted the best candidate fraction of guava leaves for enhancing tilapia leukocytes (Table 1).

The leukocyte count across all guava leaf fractions indicated that fraction five was significantly different ($p < 0.05$) from the leukocyte count measured on day seven, which was 26.31 ± 0.13 cells/mL, as observed in groups A, B, and C. the results for fraction five in groups A, B, and C demonstrated a significant increase, with a value of 25.46 ± 0.37 cells/ml on day three and 26.31 ± 0.13 cells/ml on day seven, marking it as the highest value among the other fractions. The rise in leukocyte values suggested that exposure to fraction five of guava leaves may enhance the body's ability to defend against foreign bodies (Lestari et al., 2017). Thus, it can be concluded that fraction five was the most promising candidate for assessing the non-specific immune response of tilapia at the LC_{50} and LD_{50} stages. On day three, the leukocyte test showed a mean value of 0.286 and a median value of 0.489 ($p < 0.05$) across all groups. By day seven, the leukocyte test revealed a mean value of 0.129 and a median value of 0.621 ($p < 0.05$).

Table 1. Tilapia leukocyte test results from five guava leaf fractions on days 3 and 7 after infection

Faction (25 ppm) \ Day (After infection)	Day 3	Day 7
Result of fraction 1	15.37 ± 0.17[b]	16.01 ± 0.45[b]
Result of fraction 2	16.36 ± 0.20[b]	17.21 ± 0.11[b]
Result of fraction 3	18.18 ± 0.06[b]	18.50 ± 0.34[b]
Result of fraction 4	20.40 ± 0.16[c]	21.42 ± 0.19[b]
Result of fraction 5	25.46 ± 0.37[d]	26.31 ± 0.13[c]
Control	11.28 ± 0.12[a]	11.40 ± 0.15[a]
Total	17.84 ± 4.53	18.47 ± 4.75

[a,b,c, and d] Mean the same superscript letters in the same column are not significantly different ($p > 0.05$).

The lethal concentration 50% test of fraction five of the guava leaf

The LC_{50} test for fraction five of guava leaves was conducted to determine the dose that kills 50% of the tilapia individuals exposed over a specified timeframe. The concentrations tested included 125 ppm, 150 ppm, 175 ppm, 200 ppm, and 225 ppm, as part of an assessment evaluating the inhibitory effects of guava leaf fraction on bacteria. These concentrations were used to determine the LC_{50} of fraction five of guava leaves in terms of tilapia mortality over a 72-hour period. The current results indicated an effective dosage of 175 ppm. Consequently, the dosage levels for subsequent divisions in the present study were 150 ppm, 125 ppm, and 100 ppm.

The lethal dosage 50% test of *Aeromonas hydrophila*

The LD_{50} test for *A. hydrophila* was conducted to determine the dose necessary to kill 50% of the tested tilapia population, serving as a preliminary assessment for groups A, B, and C. In the present study, the total bacterial densities used were 10^6 cells/mL, 10^7 cells/mL, and 10^8 cells/mL. The current findings indicated that the LD_{50} of *A. hydrophila* against tilapia was 10^7 cells/mL. As microbial doses rise, mortality rates also increase. Higher concentrations administered to the fish may lead to more significant infections, particularly as their immune systems become compromised (Susandi et al., 2017).

Leukocyte differential

The leukocyte differential values consisted of lymphocytes, monocytes, and neutrophils (Table 2). Lymphocytes are responsible for secreting antibodies in response to antigens, while neutrophils play a vital role in the immune response to

infections caused by pathogenic organisms and possess phagocytic capabilities. Monocytes contribute to fighting infections and removing dead cells from the fish's body tissues. The differentiation in leukocyte functions facilitates the elimination of infectious and toxic materials through phagocytosis (Ilmayati et al., 2015). According to Abdillah et al. (2022), lymphocytes are a key indicator in antibody formation. A decrease in lymphocyte count in fish leads to a reduction in antibody levels, whereas an increase in lymphocytes corresponds to an increase in antibodies. Observations conducted on days 9 and 12 following *A. hydrophila* infection revealed a significant increase in lymphocyte values ($p < 0.05$) across all groups, indicating that the fish had entered a phase of nonspecific immune response and had developed a more robust self-defense mechanism.

The analysis of leukocyte differentiation percentages across treatment doses of 150 ppm, 125 ppm, and 100 ppm, as well as the positive and negative control groups, revealed a significant increase ($p < 0.05$) in the percentage of lymphocytes in each treatment group. In groups A, B, and C, fluctuations in lymphocyte values accordingly influenced monocyte levels, though neutrophil values remained unaffected. Notably, the highest lymphocyte percentage before infection was recorded in Group A, ranging from 70.36% to 73.34%, followed by Group B, which ranged from 69.48% to 71.35%, and Group C, which exhibited values between 61.58% and 69.36%. Post-infection, lymphocyte values increased, with the highest percentage ($p < 0.05$) observed in Group B, ranging from 78.50% to 90.48%.

Before infection, monocyte values in Group A ranged from 30.23% to 28.40%, in Group B, fell from 31.32% to 29.40%, and in Group C, ranged from 32.45% to 31.31%. Following infection, the percentages of monocytes decreased from 24.47% to 12.32% in Group A, 21.35% to 8.20% in Group B, and 27.58% to 14.58% in Group C. The monocyte values recorded on the twelfth day of observation were significantly lower than those in the control groups ($p < 0.05$).

Table 2. The tilapia (*Oreochromis niloticus*) leukocyte differentiation value during the study from day 3 to day 12, before and after infection by *Aeromonas hydrophila*

Treatment (ppm)	**Day (Before infection by *Aeromonas hydrophila*)**		**Day (After infection by *Aeromonas hydrophila*)**	
	3	6	9	12
Lymphocytes				
0 (Negative control group)	61.35 ± 0.11^{a}	65.49 ± 0.14^{a}	65.56 ± 0.18^{a}	85.29 ± 0.15^{b}
150 ppm of guava leaf fraction (Group A)	70.36 ± 0.24^{c}	73.34 ± 0.23^{c}	75.32 ± 0.14^{b}	88.46 ± 0.11^{b}
125 ppm of guava leaf fraction (Group B)	69.48 ± 0.16^{b}	71.35 ± 0.98^{c}	78.50 ± 0.14^{c}	90.48 ± 0.03^{c}
100 ppm of guava leaf fraction (Group C)	61.58 ± 0.14^{a}	69.36 ± 0.20^{b}	72.38 ± 0.17^{b}	87.29 ± 0.17^{b}
0 (Positive control group)	64.29 ± 0.17^{a}	67.38 ± 0.27^{a}	67.44 ± 0.28^{a}	71.56 ± 0.08^{a}
Total	65.41 ± 3.97	69.38 ± 2.88	71.84 ± 4.97	84.62 ± 6.97
Monocytes				
0 (Negative control group)	35.33 ± 0.80^{c}	31.36 ± 0.10^{c}	31.32 ± 0.10^{d}	14.67 ± 0.13^{c}
150 ppm of guava leaf fraction (Group A)	30.23 ± 0.14^{a}	28.40 ± 0.20^{a}	24.47 ± 0.14^{b}	12.32 ± 0.20^{a}
125 ppm of guava leaf fraction (Group B)	31.32 ± 0.13^{b}	29.40 ± 0.13^{b}	21.35 ± 0.12^{a}	8.20 ± 080^{a}
100 ppm of guava leaf fraction (Group C)	32.45 ± 0.12^{a}	31.31 ± 0.22^{c}	27.58 ± 0.14^{c}	14.58 ± 0.10^{b}
0 (Positive control group)	34.19 ± 0.80^{c}	32.41 ± 0.75^{c}	43.17 ± 0.75^{e}	27.26 ± 0.14^{d}
Total	32.70 ± 1.92	30.57 ± 1.51	21.58 ± 7.82	15.40 ± 6.59
Neutrophil				
0 (Negative control group)	0.85 ± 0.03^{a}	0.56 ± 0.58^{a}	0.75 ± 0.03^{a}	0.78 ± 0.04^{a}
150 ppm of guava leaf fraction (Group A)	1.46 ± 0.22^{a}	0.84 ± 0.04^{c}	1.65 ± 0.07^{c}	0.96 ± 0.03^{d}
125 ppm of guava leaf fraction (Group B)	1.11 ± 0.07^{a}	0.77 ± 0.05^{b}	0.95 ± 0.03^{b}	0.88 ± 0.04^{b}
100 ppm of guava leaf fraction (Group C)	0.89 ± 0.02^{a}	0.57 ± 0.05^{b}	0.85 ± 0.03^{b}	$0.83 \pm 0,03^{b}$
0 (Positive control group)	0.61 ± 0.03^{a}	0.34 ± 0.03^{a}	0.64 ± 0.04^{a}	0.92 ± 0.03^{c}
Total	0.98 ± 0.31	0.62 ± 0.18	0.97 ± 0.36	0.87 ± 0.07

The numbers in the same column followed by the same superscript letters are not significantly different ($P < 0.05$). [a,b,c,d, and e] Mean the same superscript letters in the same column are not significantly different ($p < 0.05$).

Neutrophil values, both before and after infection, were influenced by the treatment dosages applied and demonstrated a direct correlation with increasing dosage levels. Notably, the percentage of neutrophil values was significantly lower ($p < 0.05$) in comparison to lymphocytes and monocytes, a finding likely attributable to the fundamental role of neutrophils as the initial line of defense against pathogenic invaders (Ilmayati et al., 2015). Lymphocytes are specialized cells that facilitate the production of antibodies and serve as effector cells in response to antigenic challenges. Conversely, neutrophils play a crucial role in the immune response against pathogenic organisms, exhibiting critical phagocytic capabilities. Similarly, macrophages function comparably to neutrophils, utilizing phagocytosis to eliminate foreign entities that infiltrate the organism. The efficiency of phagocytic activity in these cells depends on the type of material ingested (Wulandari et al., 2018). Materials that are easily digested by lysosomal enzymes, which include different digestive and bactericidal compounds, are quickly broken down and neutralized (Ilmayati et al., 2015).

The monocyte values recorded on day 12 of the observation period were significantly lower ($p < 0.05$) compared to control values. The neutrophil counts obtained before and after infection demonstrated a direct proportionality to the sequential increases in treatment dosage ($p < 0.05$). Observational results recorded on days 9 and 12 post-infection indicated a significant increase ($p < 0.05$) in lymphocyte values, suggesting that the subjects had entered a phase of nonspecific immune response characterized by enhanced self-protective mechanisms. The percentage of monocytes in the treatment group before infection ranged from 29.40% to 32.45%, which subsequently diminished post-infection, falling within the range of 8.20% to 27.58% ($p < 0.05$). This reduction was likely due to damage and the depletion of monocyte cells. The observed percentage of neutrophils remained lower ($p < 0.05$) than that of both lymphocytes and monocytes, reinforcing the understanding of neutrophils as the primary defense against foreign agents. Furthermore, the guava leaf fractions were found to influence leukocyte values, as evidenced by significant increases ($p < 0.05$) on days 3, 6, 9, and 12. It is noteworthy that guava leaf fractions influenced both monocyte and neutrophil levels in groups A, B, and C, despite a decrease in their values from day 3 to day 12 ($p < 0.05$).

Based on the results of the leukocyte differential, dosing with guava leaf fraction significantly affected the leukocyte differential value, where the average dose of fraction treatment increased the number of lymphocytes during maintenance and became the first line of defense against antigens that enter the fish's body. According to Siagian et al. (2014), lymphocytes act as antibody producers in fighting antigens that enter the fish's body, monocytes function as macrophages, and neutrophils function as phagocytes.

Phagocytic activity

Phagocytosis is a cellular process through which foreign substances entering a fish's body are engulfed and subsequently broken down. This mechanism initiates with the identification and attachment of these foreign particles, which are then internalized and digested (Prakarsa et al., 2024). Macrophages are the primary cells responsible for phagocytosis, given their superior ability to engulf compared to other phagocytic cells. Once a macrophage engulfs foreign material, its membrane seals around the particles, transporting them into the cytoplasm. Here, the particles are enclosed within phagocytic vacuoles for digestion (Wulandari et al., 2018). Enhanced phagocytic cell activity in fish blood indicates increased immunity in fish (Utami et al., 2013; Table 3).

The results of the phagocytosis activity assessment indicated that the administration of fraction five of guava leaves at doses of 150, 125, and 100 ppm resulted in a significantly higher phagocytosis activity index ($p < 0.05$) compared to the control group that did not receive fraction five of guava leaves. This enhancement was attributed to the capacity of fraction five of guava leaves to better recognize related antigens entering the tilapia's body. Based on these findings, it is evident that increasing the doses of guava leaves had a significant impact on the percentage of phagocytosis activity in tilapia, which facilitated regression calculations to explore the relationship between dosing and phagocytosis activity (Figure 1).

On days 9 and 12, the calculations indicated a quadratic relationship, with R^2 values of 0.9998 on day 9 and 0.9990 on day 12, which led us to conclude that the measurements taken had a significant impact on the phagocytosis activity of tilapia ($p < 0.05$) at levels of 98% and 99%. The decreases noted on days 6 and 9 were linked to the highest dosage of 150 ppm, whereas the peak on day 12 corresponded to a dosage of 100 ppm. On day 12, phagocytosis activity indicated that fish treated with the guava leaf extract were healthy, as their immune defense began to build against the incoming antigen three days after infection. This increase in phagocytosis activity demonstrated an improvement in the fish's immunity (Ilimu and Saparuddin 2023).

Table 3. The values of phagocytosis activity of tilapia (*Oreochromis niloticus*) during the study from day 6 to day 12

Treatment (ppm)	Day (After infection by *Aeromonas hydrophila*)		
	6	9	12
0 (Negative control group)	20.30 ± 0.73^{b}	31.35 ± 0.50^{b}	31.16 ± 0.52^{a}
150 ppm of guava leaf fraction (Group A)	63.52 ± 1.16^{e}	69.40 ± 0.90^{e}	43.36 ± 0.41^{c}
125 ppm of guava leaf fraction (Group B)	57.53 ± 0.07^{d}	66.23 ± 0.95^{d}	58.28 ± 0.06^{d}
100 ppm of guava leaf fraction (Group C)	39.38 ± 0.14^{c}	56.36 ± 0.61^{c}	59.20 ± 0.08^{d}
0 (Positive control group)	18.26 ± 0.06^{a}	24.17 ± 0.80^{a}	37.52 ± 0.06^{b}
Total	39.80 ± 19.21	49.50 ± 19.05	45.90 ± 11.56

The numbers in the same column followed by the same superscript letters are not significantly different ($P < 0.05$). $^{a,b,c,d, \text{ and } e}$ Mean the same superscript letters in the same column are not significantly different ($p < 0.05$).

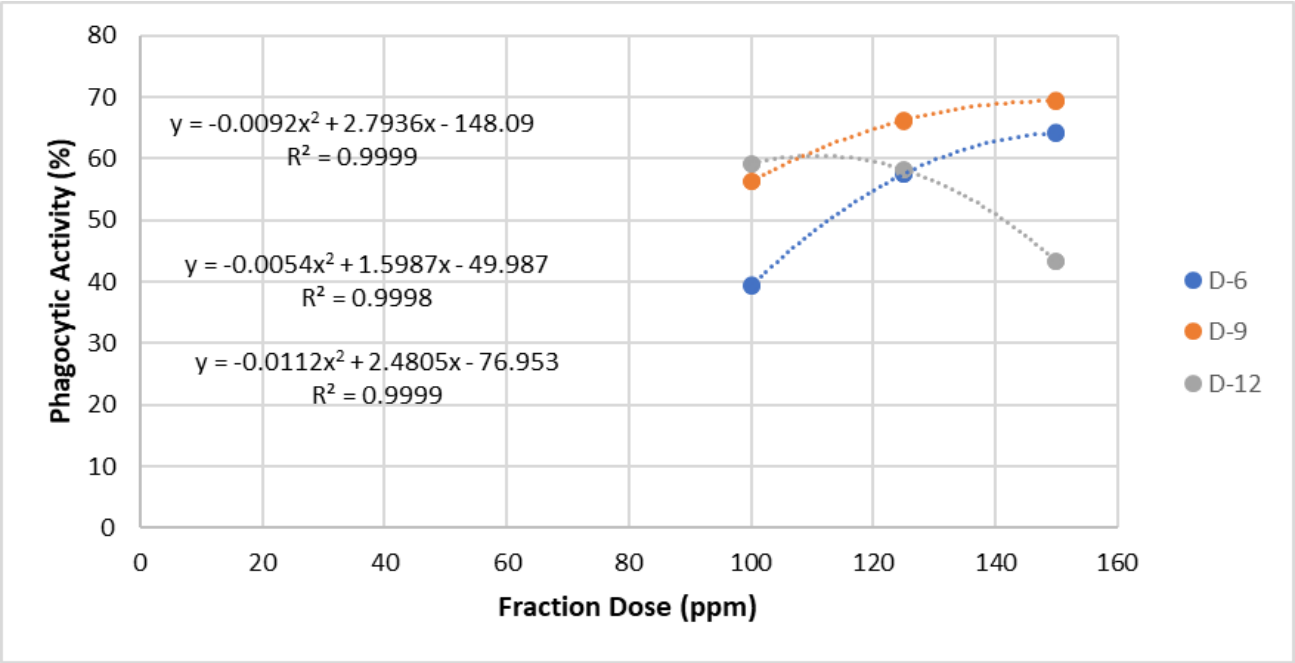

Figure 1. The dose-response relationship of guava leaf fraction five with total phagocytosis activity in tilapia from day 6 to 12.

Survival rate

The SR refers to the percentage of biota that remain alive after a specified period (Niode et al., 2017). According to Kuswanto et al. (2023), a survival rate over 50% is considered suitable, while rates from 30% to 50% are moderate, and those below 30% are regarded as poor. The survival rate is influenced by different biotic factors, including competition, parasites, age, predators, density, and human handling. In contrast, abiotic factors pertain to the physical and chemical characteristics of water. Key factors affecting fish survival include stocking density, feeding practices, disease prevalence, and water quality, which encompasses temperature, ammonia and nitrite levels, DO, and pH (Arzad et al., 2019). To ensure optimal survival rates and fish growth, it is crucial to provide food that meets the fish's nutritional needs. Feed lacking the necessary nutritional content for fish seed life is believed to compromise the physiological condition of the fish seeds. Insufficient feed nutrients can slow growth and negatively impact the survival of cultured biota (Sutedja et al., 2019). The survival rate during maintenance is illustrated in Figure 2. According to the SR graph, groups A, B, and C did not produce a pathogenic effect ($p > 0.05$), as the treatment values were nearly identical to those observed in the negative control group. In contrast, the positive control group achieved an infection rate of 39%. Thus, it can be concluded that tilapia treated with fraction five of guava leaf and infected with *A. hydrophila* experienced a higher infection rate ($p > 0.05$) compared to those treated with other fractions.

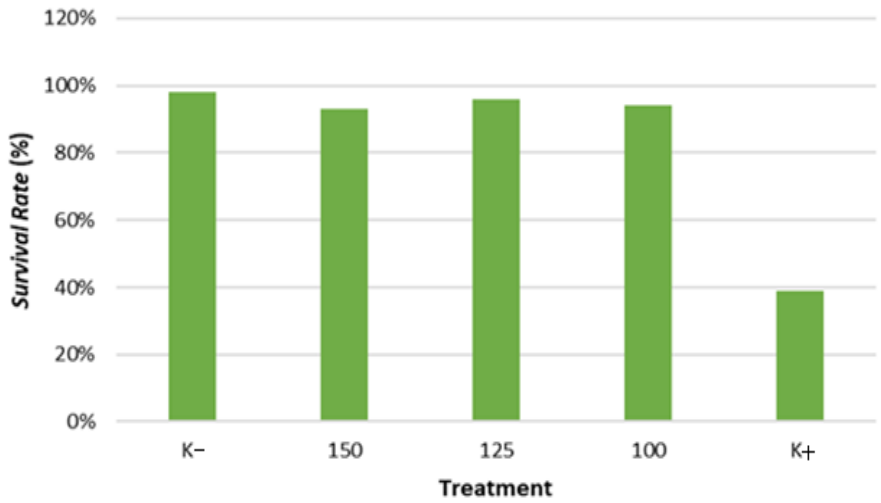

Figure 2. The survival rate of Tilapia (Oreochromis niloticus) during the 12-day study. K-: Negative control group, 150 ppm of guava leaf fraction (Group A), 125 ppm of guava leaf fraction (Group B), 100 ppm of guava leaf fraction (Group C), K+: Positive control group.

Water quality observations

Optimal water quality promotes a thriving environment for life, whereas poor water quality leads to the opposite effect. When water quality is not ideal, it can result in inadequate development (Scabra and Setyowati, 2019). During the

present study, water quality measurements were conducted, including temperature, pH, and DO, in the morning and evening for approximately one hour. Water changes were performed every three days, replacing up to 30% of the aquarium's volume. Data collected over a 12-day observation period indicated that the water quality parameters remained within optimal ranges, suggesting no adverse effects on the physiological condition of the tilapia. The results of the water quality measurements are presented in Table 4.

Table 4. Water quality parameter data during maintenance

Parameter	During maintenance	Standard range*
Temperature	25°C-27°C	25°C-30°C
pH	7-7.5	7-8
dissolved oxygen (DO)	6.5 mg/l-7 mg/l	5 mg/l-7 mg/l

***** Source of standard range: Indriati and Hafiludin (2022)

CONCLUSION

The present study identified an optimal guava leaf fraction dose of 175 ppm, with experimental doses at 150 ppm, 125 ppm, and 100 ppm. The LC_{50} calculations used doses of 125, 150, 175, 200, and 225 mg/kg fish body weight, based on inhibition tests against *Aeromonas hydrophila*. Eighteen fish per container were monitored for 24 to 72 hours, and the LC_{50} was determined from the initial mortality rates. The ideal dosage for enhancing non-specific immune responses in tilapia was found to be 150 ppm, which raised lymphocyte counts post-treatment, unlike the control group, which experienced a decline. By day three post-infection, treated fish demonstrated increased phagocytosis activity, indicating a strengthened immune response. Future research can investigate the long-term effects of guava leaf fractions on different fish species and aquatic environments.

DECLARATIONS

Acknowledgments

The authors would like to thank Professor Sri Andayani and Dr. Yunita Maimunah for their guidance in the present study and all parties involved in its implementation.

Authors' contributions

Lailatul Munawaroh Dewi Kusuma Wardani contributed to the data analysis and manuscript write-up. Sri Andayani and Yunita Maimunah contributed to writing the manuscript, as well as supervising the study. All authors read and approved the final edition of the manuscript.

Competing interests

The authors declared no conflict of interest.

Funding

This study did not receive any financial support.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors.

Availability of data and materials

The data to support the present study's findings are available upon reasonable request to the corresponding author.

REFERENCES

Abdillah MR, Lamid M, and Budi DS (2022). Effect of transfer factor formula on the increase of lymphocytes and survival rate of koi carp (*Cyprinus carpio*) infected with *Aeromonas hydrophila*. Journal of Basic Medical Veterinary, 11(1): 31-36. DOI: https://www.doi.org/10.20473/jbmv.v11i1.36158

Amelia R, Harpeni E, and Fidyandini HP (2021). Use of guava leaf extract (*Psidium guajava* Linnaeus) as an immunostimulant of carp (*Cyprinus carpio L.*) infected with Motile *Aeromonas septicemia*. Journal of Aquatropica Asia, 6(20): 48-59. DOI: https://www.doi.org/10.33019/aquatropica.v6i2.2564

Arzad M, Ratna R, and Fahrizal A (2019). Effect of stocking density on growth of tilapia (*Oreochromis niloticus*) in an aquaponic system. Median: Jurnal Ilmu Ilmu Eksakta, 11(2): 39-47. DOI: http://www.doi.org/md.v11i2.503

Arwin M, Ijong FG, and Tumbol R (2016). Characteristics of *Aeromonas hydrophila* isolated from tilapia (*Oreochromis niloticus*). Aquatic Science & Management, 4(2): 52-55. DOI: https://www.doi.org/10.35800/jasm.4.2.2016.14450

Cao H, He S, Wang H, Hou S, Lu L, and Yang X (2012). Bdellovibrios, potential biocontrol bacteria against pathogenic *Aeromonas hydrophila*. Veterinary Microbiology, 154(1): 413-41. DOI: https://www.doi.org/10.1016/j.vetmic.2011.07.032

Fahrizal A and Nasir M (2017). Effect of probiotic addition with different doses in feed on growth and feed conversion ratio (FCR) of tilapia (*Oreochromis niloticus*). Journal of Exact Sciences, 9(1): 69-80. DOI: https://www.doi.org/10.33506/md.v9i1.310

Fasya AG, Tyas AP, Mubarokah FA, Ningsih R, and Masjid AD (2018). Variation of column diameter and sample-silica ratio on isolation of steroids and triterpenoids of red alga *Eucheuma cottonii* by wet column chromatography. ALCHEMY: Journal of Chemistry, 6(2): 57-64. DOI: https://www.doi.org/10.18860/al.v6i2.7015

Hadie LE, Kusnendar E, Priono B, Dewi RRSPS, and Hadie W (2018). Production strategies and policies in competitive tilapia farming. Indonesian Journal of Fisheries Policy, 10(2): 75-85. DOI: http://www.doi.org/10.15578/jkpi.10.2.2018.75-85

Haditomo AHC (2017). Feeding garlic extract as immunostimulant on survival and blood profile of catfish (*Pangasius* sp.). Journal of Aquaculture Management and Technology, 6(3): 234-241. Available at: https://ejournal3.undip.ac.id/index.php/jamt/article/view/20440

Hidayaturohman F, Widyorini N, and Jati OE (2021). Analysis of the abundance of *Aeromonas hydrophila* bacteria in the waters of Rawa Pening, Kebondowo village, Semarang. Jurnal Pasir Laut, 5(1): 1-8. DOI: https://www.doi.org/10.14710/jpl.2021.31894

Ilimu E and Saparuddin S (2023). Improved immune response of common carp (*Cyprinus carpio*) by administration of mahang syrup (*Macaranga involucrate*) leaf extract. Arowana: Scientific Journal of the Water Study Program, 5(1): 51-56. DOI: https://www.doi.org/10.51179/jipsbp.v5i1.1898

Ilmayati MM, Syawal H, and Adelina A (2015). Differentiation of leukocytes of Nile tilapia (*Oreochromis niloticus*) with feed consist of noni fruit flour (*Morinda citrifolia L*). Online Journal of Students of the Faculty of Fisheries and Marine Science, Riau University, 1(1): 10-14. Available at: https://www.neliti.com/publications/201271/differentiation-of-leukocytes-of-nile-tilapia-oreochromis-niloticus-with-feed-co#cite

Indriati PA and Hafiludin H (2022). Water quality management in tilapia breeding (*Oreochromis niloticus*) at the Teja Timur Pamekasan fish seed center. Juvenil: Marine and Fisheries Scientific Journal, 3(2): 27-31. DOI: https://www.doi.org/10.21107/juvenil.v3i2.15812

Jati CW (2023). Effectiveness of *Aeromonas salmonicida* inactivated vaccine on total leukocytes and phagocytosis activity in Koi fish (*Cyprinus carpio*). Journal of Lemuru, 5(2): 313-319. DOI: https://www.doi.org/10.36526/jl.v5i2.2890

Kullu AR, Tabassum W, and Sinha MP (2013). Effect of *Psidium guajava* aqueous extracts on haematological profile and serum lipid variables of albino rat. The Bioscan, 8(2): 743-746. Available at: https://www.semanticscholar.org/paper/EFFECTS-OF-PSIDIUM-GUAJAVA-AQUEOUS-EXTRACTS-ON-AND-Kullu-Tabassum/0021ce35c63643dc82ab2ef0ba889d24be648b38

Kuswanto JKJ, Koprawir M, and Nugraha AF (2023). Smart fish farm tilapia farming uses an integrated NodeMCU based on the Internet of things. Computer Science Research and Its Development Journal, 15(2): 144-155. Available at: https://csridjournal.potensi-utama.org/index.php/CSRIDjournal/article/view/35

Lestari J, Syawal H, and Riauwaty M (2021). Differentiation of leukocytes of Siamese crayfish (*Pangasionodon hypophthalmus*) fed contains fermented herbs. Journal of Ruaya, 9(1): 1-9. Available at: https://ojs.unud.ac.id/index.php/itepa/article/view/67758/37556

Nahar S, Rahman MM, Ahmad GU, and Faruk MAR (2016). Isolation, identification, and characterization of *Aeromonas hydrophila* from juvenile farmed pangasius (*Pangasianodon hypophthalmus*). International Journal of Fisheries and Aquatic Studies, 4(4): 52-60. Available at: https://www.fisheriesjournal.com/archives/2016/vol4issue4/PartA/4-3-32-237.pdf

Niode AR, Nasriani N, and Irdja AM (2017). Growth and survival of tilapia (*Oreochromis niloticus*) fry on different artificial feeds. Akademika, 6(2): 28-33. DOI: https://www.doi.org/10.31851/jipbp.v14i2.3492

Nursobah N, Salmon S, Lailiyah S, and Sari SW. (2022). Prototype of telemetry system for water temperature and pH of freshwater fish farming pond (Tilapia) based on Internet of things (IoT). Sebatik, 26(2): 788-797. DOI: https://www.doi.org/10.46984/sebatik.v26i2.2053

Pattipeiluhu SM, Laimeheriwa BM, and Lekatompessy (2022). *Aeromonas hydrophila* infection and its impact on blood parameters of tilapia (*Oreochromis niloticus*). Journal of Fisheries and Marine Research, 6(3): 6-13. DOI: https://www.doi.org/10.21776/ub.jfmr.2022.006.03.2

Prakarsa C, Bodhi W, and Datu OS (2024). Testing the phagositosis activity of macrofag extract of gedi plant *Abelmoschus manihot* (l) as an immunomodulator. Pharmacon, 13(1): 419-430. DOI: https://www.doi.org/10.35799/pha.13.2024.49698

Rusmiyati N, Permatasari DAI, and Khasanah IN (2023). Test the antioxidant activity of the extract and fraction of n-hexane, ethyl acetate, and Australian guava bark juice (*Psidium guajava* l.) with the DPPH method (1, 1-Diphenyl-2-Pikrylhydrazil). Detector: Jurnal Inovasi Riset Ilmu Kesehatan, 1(4): 183-206. DOI: https://www.doi.org/10.55606/detector.v1i4.2553

Rochmasari Y (2011). Isolation study and molecular structure determination of chemical compounds in the neutral fraction of Australian Guava leaves (*Psidium guajava L.*). Universitas Indonesia, Depok, pp. 1-51. Available at: https://lib.ui.ac.id/detail.jsp?id=20289119

Rosidah and Afizia WM (2012). Potential of guava leaf extract as an antibacterial to overcome *Aeromonas hydrophila* bacterial attack in carp fish (*Osphronemus gouramy Lacepede*). Jurnal Akuatika, 3(1): 19-27. Available at: https://jurnal.unpad.ac.id/akuatika/article/view/473

Setyowati DN and Astriana BH (2020). Blood profile of White Snapper infected with *Vibrio* sp. bacteria by giving *Aloe vera*. Unram Fisheries Journal, 10(1): 55-69. DOI: https://www.doi.org/10.29303/jp.v10i1.175

Saputra I and Indaryanto FR (2018). Identification of *Aeromonas hydrophila* bacteria in fish commodities trafficked to Sumatra Island through the Merak-Banten crossing port. Journal of Fisheries and Marine Affairs, 8(2): 155-162. DOI: http://www.doi.org/10.33512/jpk.v8i2.6646

Scabra AR and Setyowati DNA (2019). Improving water quality for freshwater fish farmers in Gegerung Village, West Lombok Regency. Journal of Abdi Insani, 6(2): 267-275. Available at: https://abdiinsani.unram.ac.id/index.php/jurnal/article/view/243/131

Siagian JRU, Lukistyowati I, and Riauwaty M (2014). Differentiation of leukocytes catfish (*Mystus nemurus*) fed solutions containing curcuma (*Curcuma xanthorrhiza*). Online Journal of Students of the Faculty of Fisheries and Marine Science, Riau University, 1(2): 1-13. Available at: https://media.neliti.com/media/publications/185978-ID-none.pdf

Sine Y and Fallo G (2016). Antibacterial activity test of ketapang leaf extract (*Terminalia catappa L.*) and guava leaf (*Psidium guajava L.*) on the growth of *Aeromonas hydrophila* bacteria. Journal of Biology Education, 1(1): 9-11.

Susandi F, Mulyana M, and Rosmawati R (2017). Increased immunity of carp fry (*Osphronemus Gouramy Lac.*) against *Aeromonas hydrophila* using rosella (*Hibiscus Sabdariffa L.*). Journal of Mina Sains, 3(2): 1-13. DOI: https://www.doi.org/10.30997/jms.v3i2.889

Sulastra CS and Khaerati K (2020). Acute toxicity and lethal dose (LD_{50}) of ethanol extract of purple banggai uwi (*Dioscorea alata L.*) in white rats (*Rattus norvegicus*). Scientific Journal of Medicamento, 6(1): 10-14. DOI: https://www.doi.org/10.36733/medicamento.v6i1.715

Sutedja GP, Arthanaa IW, and Dewia APWK (2019). Potential development of Gourami fish farming (*Osphronemus goramy*) in floating net cages of Lake Batur Kintamani, Bali. Current Trends in Aquatic Science, 2(2): 40-47. Available at: https://ojs.unud.ac.id/index.php/ctas/article/view/49956

Telaumbanua S, Lukistyowati I, and Syawal H (2019). Sensitivity of harumanis mango seed (*Mangifera indica l*) solution toward *Aeromonas hydrophila*. Journal of Fisheries and Marine, 24(1): 24-31. Available at: https://www.neliti.com/publications/297796/sensitivity-of-harumanis-mango-seed-mangifera-indica-l-solution-toward-aeromonas#cite

Tondi DB, Wijayanti NPP, and Pebriani DAA (2023). Optimization of vitamin C on growth and survival rate of tilapia (*Oreochromis niloticus*) infected by *Aeromonas hydrophila*. Jurnal Biologi Udayana, 27(2): 173-182. DOI: https://www.doi.org/10.24843/JBIOUNUD.2023.v27.i02.p05

Utami DT, Prayitno SB, Hastuti S, and Santika A (2013). Overview of hematological parameters in tilapia (*Oreochromis niloticus*) given Streptococcus iniae DNA vaccine at different doses. Journal of Aquaculture Management and Technology, 2(4): 7-20. Available at: https://ejournal3.undip.ac.id/index.php/jamt/article/view/4803

Wulandari S, Jumadi R, and Rahmawati FF (2018). Effectiveness of cinnamon plant leaf powder (*Cinnamomum burmanii*) on leukocyte differential and phagocytic activity of tilapia (*Oreochoromis niloticus*) infected with *Streptococcus agalactiae*. Jurnal Perikanan Pantura, 1(1): 40-49. Available at: https://journal.umg.ac.id/index.php/jpp/article/view/293