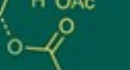
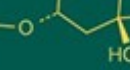
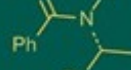
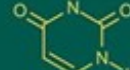


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Immunomodulatory effect of dietary *Murraya Koenigii* leaf extracts in *Anabas testudineus* challenged with *Aeromonas Hydrophila*

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ABSTRACT

The use of plant-derived bioactive compounds as natural feed additives in aquaculture has gained significant attention due to their potential to enhance fish health and disease resistance. This study focuses on analysing the potential of *Phyto* additives, specifically on *Murraya koenigii* leaf extracts, on cytokine immune gene expression, haematological parameters, and antioxidant enzymes of *Anabas testudineus*. The fish were divided into three groups, each weighing an average of 90.15 ± 6.2 grams. One group, acting as the control, was fed a diet without plant extract, while the other groups received diets containing 1% ALC. After 30 days of feeding, the fish were exposed to *Aeromonas hydrophila*. Blood and tissue samples from the liver, kidney, and spleen were collected at seven intervals over the next 21 days. TNF- α gene expression analysis revealed no change in the spleen, but significant upregulation in the kidney. IL-1 β did not show any change in the kidney or spleen, whereas IL-8, TGF- β , and IL-10 were significantly upregulated in the kidney and spleen in the treatment group. Blood parameters such as RBC, WBC, and HGB significantly increased. SOD and CAT were considerably more prevalent in the treatment groups than in control. By adding 1% ALC (*Murraya koenigii* extracts) to *Anabas testudineus* meals, immunological responses, antioxidant levels, and disease resistance against *Aeromonas hydrophila* were all markedly increased. Its potential is demonstrated by improved blood parameters and upregulated cytokines (IL-8, TGF- β , and IL-10). The long-term impacts and mechanisms of these plant extracts on immune regulation should be better understood through future research.

Keywords: Immune response, *Phyto* additives, Cytokine expression, Antioxidant enzymes, Disease resistance

1. Introduction

Over the last two decades, the role of fisheries and aquaculture in enhancing global food security and addressing nutritional needs has gained growing acknowledgement. The scale of aquaculture output and the geographic spread of cultivated species have surged significantly over the past two decades. Despite the wide variety of farmed aquaculture species a limited number of "staple" species prevail in aquaculture production and advancing the scale and efficiency of aquaculture is vital to meet the nutritional requirements of a rapidly growing global population, particularly in developing countries (2).

Aquaculture farmers often face significant economic losses due to interconnected factors such as species-specific vulnerabilities, suboptimal inputs, environmental stressors and managerial shortcomings (3). Many diseases in modern aquaculture result from intensified farming practices that disrupt the equilibrium between host immunity, pathogen virulence, and environmental stability (4) (5). Antibiotics are used in aquaculture as prophylactic or therapeutic measures or as feed additives (6) to control disease outbreaks. The routine prophylactic and therapeutic use of antimicrobials in aquaculture has been identified as the dominant factor driving antimicrobial resistance (AMR) development in aquatic ecosystems (7) Since no antibiotics are specifically developed for aquaculture, farmers must rely on veterinary medications approved for other livestock species (8).

As the aquaculture industry faces challenges such as disease outbreaks and antibiotic resistance, driving interest in sustainable alternatives like plant-derived additives (9). These plants contain natural compounds (secondary metabolites) that strengthen disease resistance, accelerate growth, and help fish cope with environmental stressors (10). A key ingredient in Indian cooking and herbal medicine, curry leaves (*Murraya koenigii* family Rutaceae) are valued for their unique fragrance and concentration of bioactive compounds. Rich in carbazole alkaloids (mahanimbine, koenimbine, girinimbine) with antioxidant/anticancer activity. Phenolic compounds such as quercetin, kaempferol, and gallic acid demonstrate anti-inflammatory effects, while essential oils like β -caryophyllene and α -pinene exhibit (11). The important phytochemicals present in this plant make it valuable for treating various diseases. In aquaculture, curry leaf extract has been linked to

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significantly altering blood parameters and increasing the resistance of gourami against Edwardsielliosis infection (12). Dietary inclusion of curry leaf extract in rohu elevated resistance against *Saprolegnia parasitica* infection with enhanced haematological values (13). While many plant-based additives have been thoroughly investigated for their immune-boosting effects in aquaculture, curry leaf extract remains underutilised as a phyto-genic supplement. Although experimental evidence suggests that plant-derived additives enhance fish immunity, the mechanistic pathways underlying these effects, including their roles in cytokine regulation and oxidative stress management, are inadequately characterised (14). Therefore, it is crucial to focus on discovering and utilising new medicinal plants as plants remain a vast, underexplored reservoir of unique chemical structures, offering innovative and affordable medications (15). In the present investigation, we assessed the immunomodulatory efficacy of dietary supplementation with *M. koenigii* (ALC) leaf extracts (1% w/w inclusion) in *A. testudineus* after 30-day dietary supplementation and subsequent bacterial challenge with *A. hydrophila*.

2. Materials and Methods

2.1 Experimental site

We conducted the study in the laboratory facilities of the Department of Aquatic Animal Health Management (AAHM) at Kerala University of Fisheries and Ocean Studies (KUFOS) in Panangad, Kochi, India. We sourced healthy climbing perch from a local fish farmer in Cherthala, Kochi, and transported them to the lab. Throughout the experiment, we carefully followed all ethical guidelines for animal care and handling approved by the Institutional Animal Ethics Committee (IAEC).

2.2 Extract and diet preparation

Fresh leaves of *M. koenigii* were collected from Ernakulam, Kochi. The leaves were washed with distilled water and shade-dried at room temperature. The dried leaves were finely ground into a powder using a kitchen grinder and sieved. The powder was stored in airtight containers at 4 °C. Aqueous extract was prepared by mixing 50 g of powdered leaves with 500 mL of distilled water in a conical flask. Using a magnetic stirrer, the mixture was gently agitated for two hours for even maceration. The mixture was then filtered through sterile filter paper to separate the liquid extract. The collected clear filtrate was concentrated by evaporating the water at 40 °C in a laboratory oven. (16). (Table 1 shows the ingredients list for diet preparation, and Table 2 shows the proximate composition of feed) (17). Commercial feed was ground, an appropriate quantity of ALC extract was mixed with water, and a dough mixture was made. The dough was pelletised to form pellets of 2 mm.

Table 1. Ingredient and composition of diet

Ingredient	g/Kg
Fish meal	320
Soyabean protein concentrate	60
Soyabean meal	250
Rice bran	80
Wheat meal	150
Fish oil	50
Vitamin - Mineral premix*	30
Wheat gluten	60

*Premix – for 500g (Growel Agrovet Private Limited, India) Vitamin B1: 100 mg, Vitamin B2: 30 mg, Vitamin B6: 40 mg, Vitamin B12 : 50 mcg, Vitamin E: 25 mg, Methionine: 2420 mg, Lysine: 500 mg Niacinamide: 500 mg, Biotin: 1 mg, Calcium Pantothenate: 60 mg, Choline Chloride: 540 mg Iron: 5 mg, Copper: 1.5 mg, Cobalt: 100 mcg, Manganese: 4.5 mg, Iodine: 5 mg, Magnesium: 8 mg, Zinc: 1 mg, Selenium: 2 mg

2.3 Experimental design

During the study, 200 healthy, uniform-sized *A. testudineus* weighing between (90.15 ± 6.2) grams each, underwent disinfection using a 1 ppm solution of KMnO₄. Fishes were acclimated and quarantined in 500 L FRP (fibreglass-reinforced plastic) tanks for a week. During this period, the fish were fed commercial feed twice daily until they were satiated. After the one-week quarantine, 15 fish were carefully moved to each 500L FRP tank (Triplicate) containing 150 L of water for each treatment group. Throughout the entire experiment, high-quality potable water was used, and the tanks were equipped with sponge filters and aeration systems. 30% of the tank water was refreshed daily, and key water quality indicators like pH, temperature, and dissolved oxygen levels were routinely monitored (18). fishes were fed twice daily, with the total feed given equivalent to 3% of their body weight for 30 days.

2.4 Primer designing and standardization of annealing temperature

Primer sequence TNF-α (Tumor Necrosis Factor α), IL-1 β (Interleukin 1 β), IL-8 (Interleukin 8), IL-10 (Interleukin 10), TGF- β (Tumour Growth Factor β) were designed from *Danio rerio* by reverse blasting using a primer designing tool primer-blast, and primers for *A. testudineus* were predicted and downloaded from NCBI (National Centre For Biotechnology Information) website and β actin acted as a reference gene (19). The temperature of primers for qPCR was determined using gradient PCR (BIO-RAD, USA). The PCR conditions were as follows: an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at a gradient temperature ranging from 57°C to 63°C for 40 seconds, extension at 72°C for 30 seconds, and a final termination step at 72°C for 5 minutes (Table 3 shows annealing temperature of primers). The PCR products were then checked on a 1.5% agarose gel to visualise the amplification bands.

Table 2. Proximate Analysis (%)

Nutrient	Percentage(%)
Crude Protein	43.5
Crude Fat	10.6
Ash	7.7

Table 3 shows the annealing temperature of primers (TNF-α, IL-1β, IL-8, IL-10, and TGF-β and β-actin)

Sr. no	Primer Name	Forward Primer Sequence (5'>3')	Reverse Primer Sequence (3'>5')	Size (bp)	Annealing temperature
1	IL-1 β	CCAGGTCAGACGACATGGAG	ACTTTGCGGTTGTTCATTGCC	127	58
2	TNF- α	CTCCAACGTAAGAGCAGCCA	GTAAGAGGCCGTTGTGTGGAA	151	58
3	IL10	AAACACAAAACGCACGCAGA	GAGCTGCACGGTGAAGTGTGA	185	58
4	TGF-β	ACTCGCAGGACTTCAAGACG	CGCTGCTCACCAGGTATCAT	145	58

(continued on next page)

Sr. no	Primer Name	Forward Primer Sequence (5'>3')	Reverse Primer Sequence (3'>5')	Size (bp)	Annealing temperature
5	IL 8	GCACTGCCGCTGCATTAAAA	GTCTAGGCAGACCTGTAGGC	142	58
6	β actin	TCGCGACCTCACAGACTACCT	TCACGCACGATTCCCTC	84	58

2.5 Hematological parameter

After the feeding trial, fish were anaesthetised (20) using clove oil. Blood samples were drawn from the caudal vein using sterile syringes and collected in EDTA-coated tubes at time intervals: 1, 2, 3, 5, 7, 14, and 21 days post-infection. The haematological parameters, including WBC (white blood corpuscles), RBC (red blood corpuscles), HGB (haemoglobin), and HCT (haematocrit), were analysed using the haematological analyser (Benespha, India).

2.6 Antioxidant parameter

For SOD (Superoxide Dismutase) analysis (21). Infected Liver tissue was homogenised in mortar and pestle with Sodium Bicarbonate and EDTA buffer solution. From that, 120 μ l of the solution was taken and mixed with 96 mm NBT (50 μ l). Then Triton X (10 μ l) was added to it. The reaction was started by adding 20 mm hydroxylamine hydrochloride (10 μ l). After 1.5 minutes, 10 μ l of the test sample was added. Initial reading is taken after 0.5 min (0-minute reading) (550 nm). The second reading was taken after 3 minutes (3-minute reading). A blank was simultaneously run along with the test. For Catalase (22), Liver tissue (75 mg) was homogenised with 1.5 ml phosphate buffer and then centrifuged at 5000 rpm for 5min. The supernatant (0.5 ml) was added to a mixture of 0.5 ml H_2O_2 (0.2M), 1 ml of phosphate buffer and 0.4 ml of distilled water. After 60 sec of incubation, 2 ml of dichromate /acetic acid reagent was added for termination. Tubes were then heated for 10min in a boiling water bath, and the colour developed was read at 610 nm in a spectrophotometer (Lab India).

2.7 Immune gene expression analysis

Tissue samples (kidney and spleen) were extracted from the *A. testudineus* at various time intervals: 1, 2, 3, 5, 7, 14, and 21 days post-infection from treatment and control groups. Total RNA isolation was performed using RNA isoplus reagent (Takara). The RNA samples were analysed using a Nanodrop spectrophotometer (Implen, Germany) to determine RNA concentration. To remove DNA contamination, the RNA samples were treated with DNase (Thermo Fisher Scientific). The RNA samples were reverse-transcribed into complementary DNA (cDNA) with the use of Takara's 1st-strand cDNA synthesis kit. Quantitative real-time polymerase chain reaction (qPCR) analysis for TNF- α , IL-1 β , IL-8, IL-10, and TGF- β was conducted on a Bio-Rad CFX96, with β -actin serving reference gene, with thermocycling conditions 95 °C 10 s, 40 cycles of 95 °C 10 s, 58 °C 10 s and 72 °C 20 s. Gene expression levels were calculated using the comparative threshold cycle ($2^{-\Delta\Delta C_q}$) (23). Statistical comparisons were made between the treatment groups and the corresponding control.

2.8 Challenge study

A. hydrophila was obtained from the laboratory facilities of the Department of Aquatic Animal Health Management (AAHM) at KUFOS. *A. hydrophila* was plated on an *Aeromonas* selective agar plate and incubated at room temperature. Then, a colony was suspended in tryptic soy broth (TSB) for a 24-hour incubation. The culture was then centrifuged at 11,000 x g for 10 minutes, washed, and resuspended in PBS (Phosphate-buffered saline) (24). The accuracy

of the density was also checked using a spectrophotometer at 600 nm, and the dose was standardised to a concentration of 6×10^8 CFU/mL in PBS. To confirm this concentration, a tenfold serial dilution method was used, and the bacteria were grown on an *Aeromonas* selective agar plate. The final count was calculated based on the number of visible colonies (25). A total of 30 fish were used for the study. Ten fish were injected with 0.2 ml of bacterial suspension concentration in duplicate using a 1 ml sterile disposable insulin syringe. Mortality was recorded every 24 hours for 96 hours, and all the dead fish were subjected to bacterial isolation.

2.9 Statistical analysis of the result

One-way ANOVA was conducted using SPSS Version 26. The significance of the difference in immune gene expression, haematological and antioxidant status at various sampling times was assessed by one-way analysis of variance (ANOVA) with Duncan's Test ($\alpha = 0.05$).

3. Result

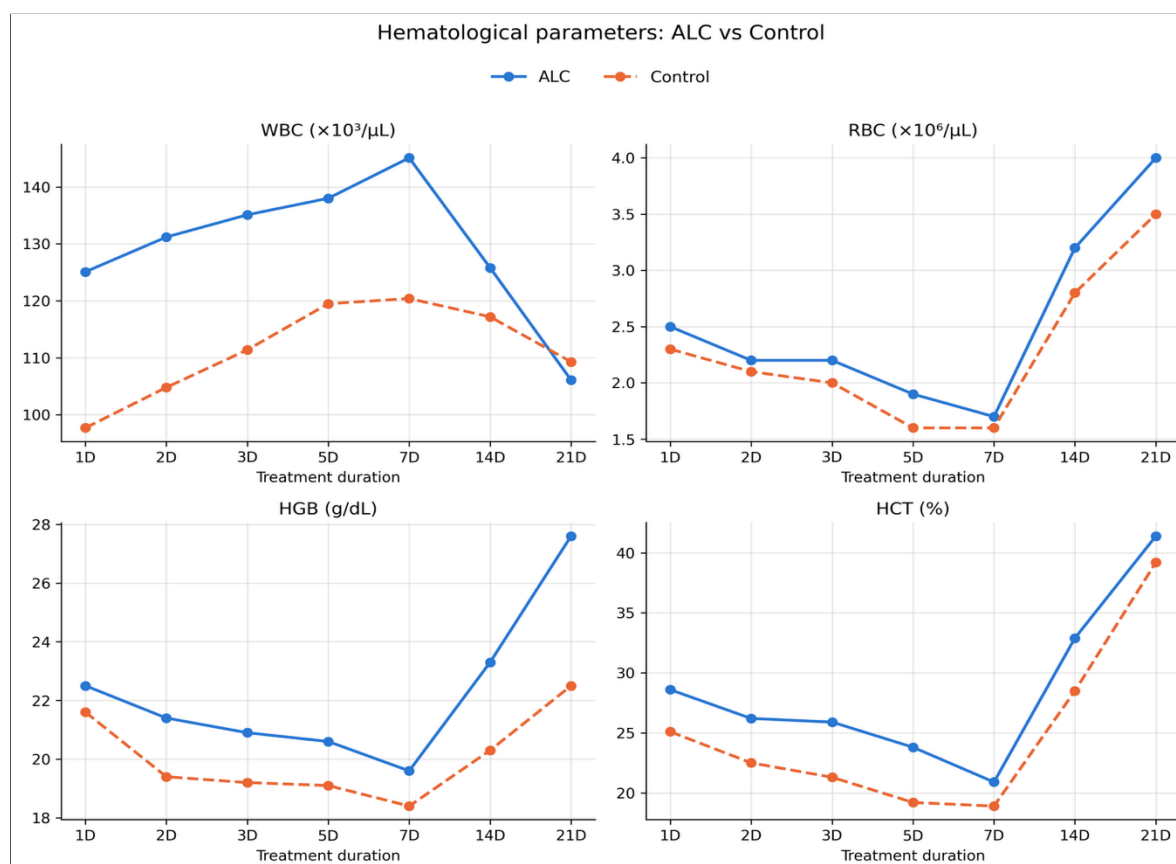
Bioactive compounds in plant extracts - such as alkaloids, terpenoids, tannins, saponins, glycosides, flavonoids, phenolics, steroids, and essential oils - reduce stress, promote growth, stimulate appetite, enhance vitality, strengthen immune systems, accelerate the maturation of cultured species, improve reproductive health, and combat pathogens in fish and shrimp aquaculture (27). Anti-nutrients, commonly present in plants, can exert both beneficial and harmful impacts on health. The primary antinutritional factors in curry leaves include flavonoids, glycosides, and tannins, though their concentrations remain within safe, acceptable limits. (28)

The 96-hour median lethal dose (LD_{50}) of *Aeromonas hydrophila* was determined using probit regression analysis. Mortality data recorded at three bacterial concentrations (6×10^6 , 6×10^7 , and 6×10^8 CFU/mL) were converted to probit values and regressed against the $\log_{10}$ -transformed dose. The relationship between dose and mortality response was found to be strongly linear, explaining approximately 97% of the variation in the data. Based on this regression, the concentration of *A. hydrophila* required to cause 50% mortality in *A. testudineus* within 96 hours was estimated at 6×10^8 CFU/mL.

3.1 Haematological parameter

Upon completion of the 30-day feeding experiment, the effects of dietary supplements of ALC indicated a significant difference in white blood corpuscles (WBC), red blood corpuscles (RBC), and haemoglobin content (HGB), as well as in the hematocrit value (HCT) among treatments ($P < 0.05$). The data is presented in Fig 1. The haematological parameters in the treatment groups were elevated compared to the control. A study reported that guava and neem supplementation improved WBC, RBC, haemoglobin, and hematocrit levels in Nile tilapia (29). Similarly, Nhu *et al.* (30) found that diets enriched with guava, amla and thong extracts increased immune cells in a dose-dependent manner in striped catfish. The curry leaf extract showed similar effects on the blood cells of *A. testudineus* as many other medicinal herbs.

Fig. 1 shows the haematological parameters of *A. testudineus* fed with a diet containing ALC for 30 days.



3.2 Antioxidant parameter

Dietary supplementation with ALC was observed to significantly influence the levels of antioxidant enzymes SOD and CAT in the liver across treatment groups ($P < 0.05$), as outlined in Fig 2. All evaluated

antioxidant markers demonstrated higher values in the supplemented groups relative to the control.

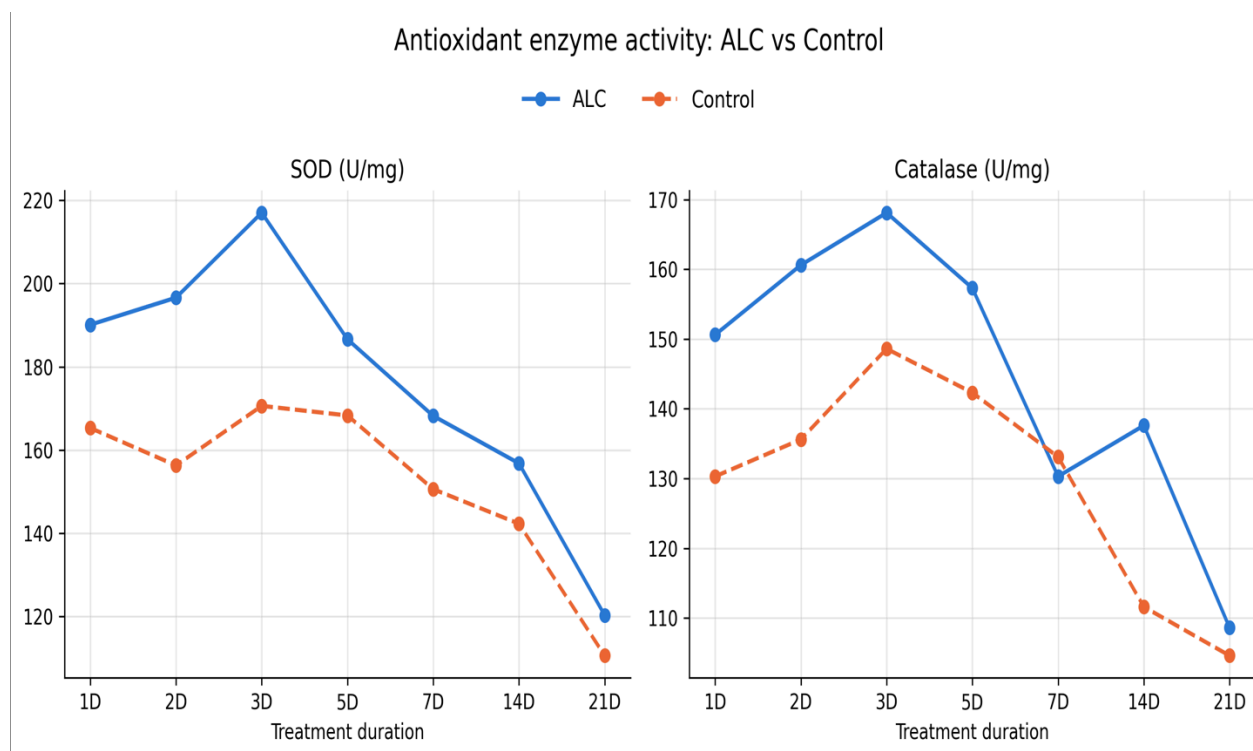


Figure 2. Antioxidant enzyme assay of *A. testudineus* fed with a diet containing ALC for 30 days.

3.3 Immune gene expression analysis

Following a 30-day feeding trial, the fish were challenged with *A. hydrophila*. Tissue samples (spleen, kidney) were collected at seven intervals over 21 days, starting 24 hours post-exposure. Quantitative

real-time PCR analysis of immune-related gene expression levels indicated variations in the spleen and kidney across treatment groups. Analysis of gene expression in *A. testudineus* showed significant alterations in both pro-inflammatory and anti-inflammatory genes, with significant changes among the different time points. TNF- α exhibited

an upregulation in the kidney ($P < 0.05$) in ALC-treated groups compared to the control (Fig. 3). However, there was no significant difference in gene expression in the spleen ($P > 0.05$) (Fig. 3), while IL-1 β showed no significant difference between control and treatment in either organ ($P > 0.05$) (Fig. 4). The chemokine IL-8 displayed a significantly higher upregulation in both the spleen and kidney ($P < 0.05$

) (Fig. 5) in ALC treatment compared to the control. The anti-inflammatory genes TGF- β (Fig. 6) and IL-10 (Fig. 7) were also significantly upregulated in both organs ($P < 0.05$) in treatment groups compared to controls. All genes showed significant differences at different time points from 1D to 21D ($P < 0.05$).

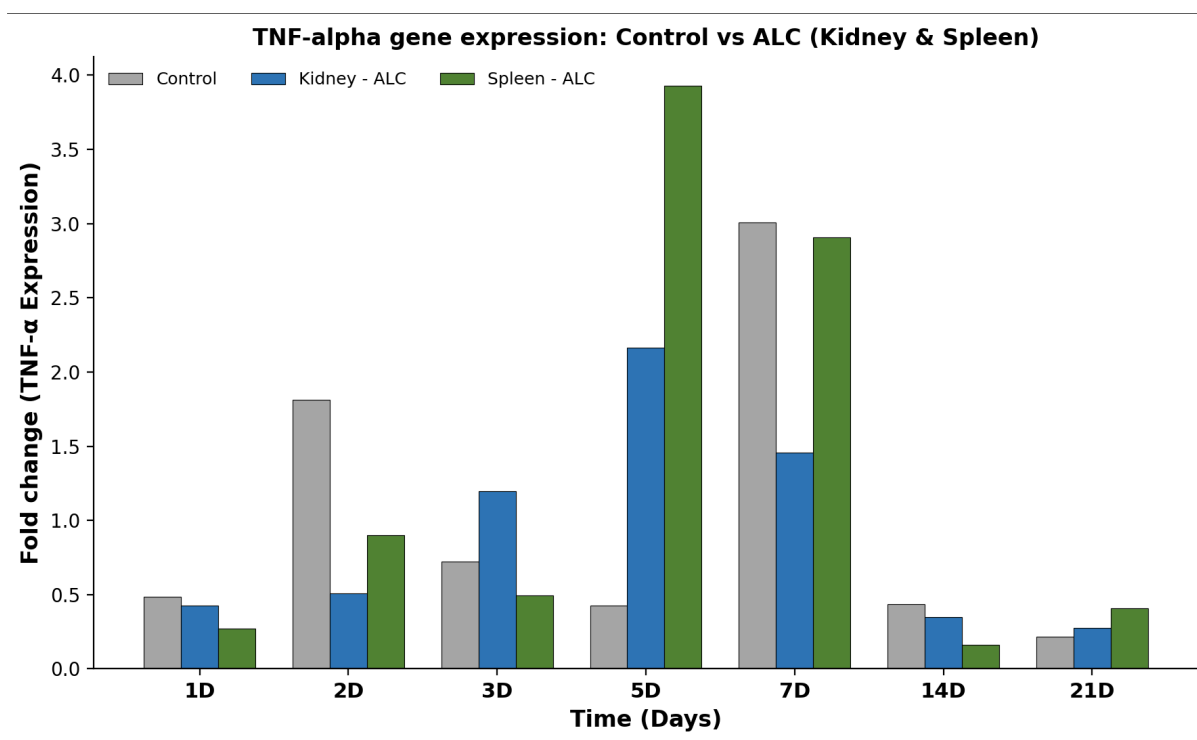


Figure 3. Fold change expression TNF- α gene in *A. testudineus* (kidney and spleen) after ALC administration and subsequent challenge with *A. hydrophila*

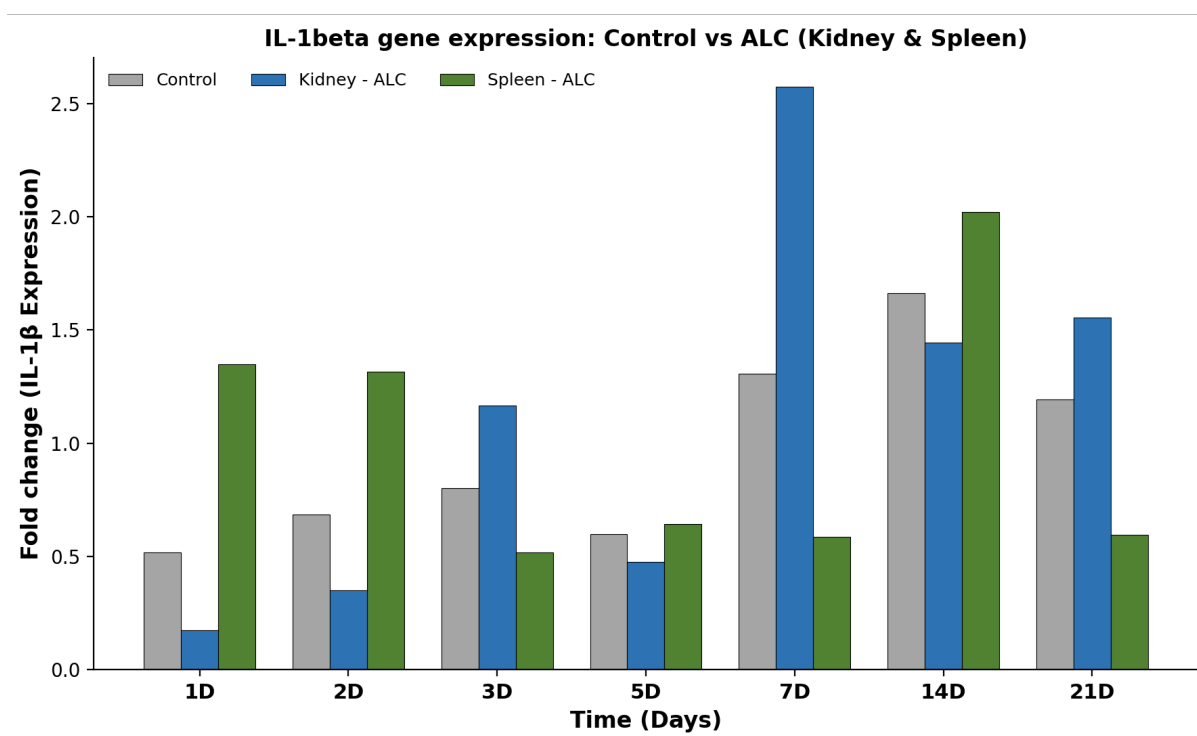


Figure 4. Fold change expression of the IL-1 β gene in *A. testudineus* (kidney and spleen) after ALC administration and subsequent challenge with *A. hydrophila*

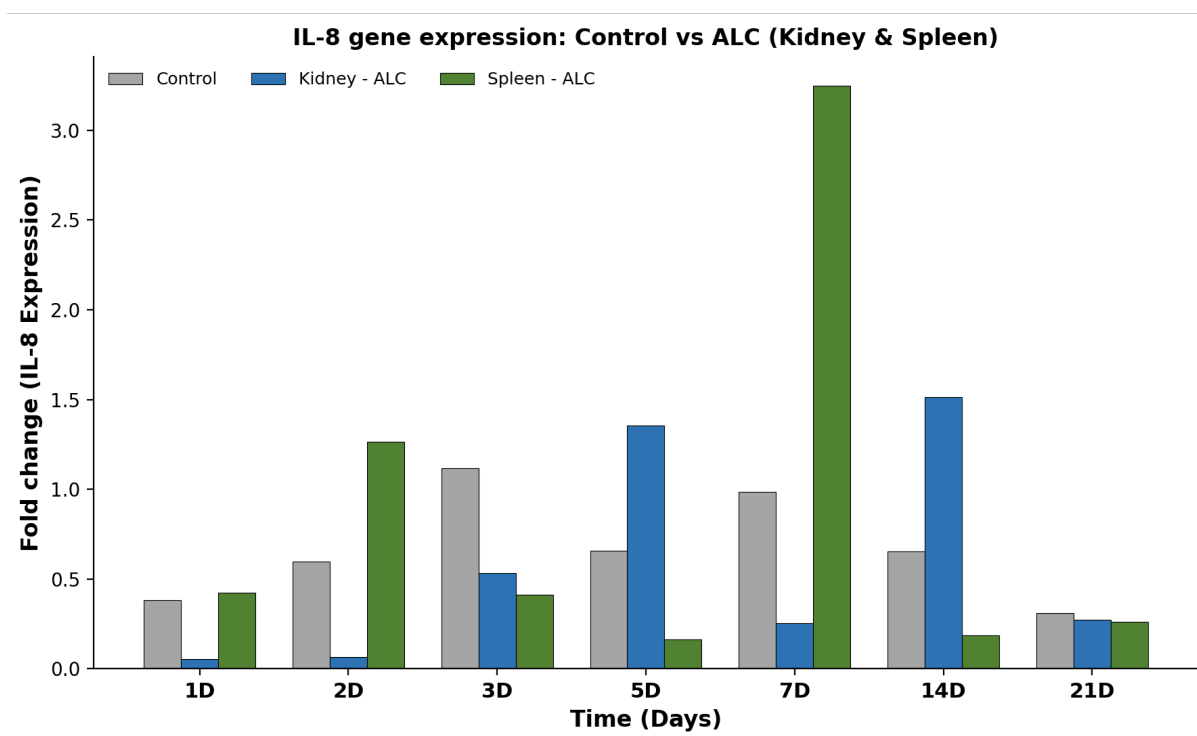


Figure 5. Fold change expression of the IL-8 gene in *A. testudineus* (kidney and spleen) after ALC administration and subsequent challenge with *A. hydrophila*

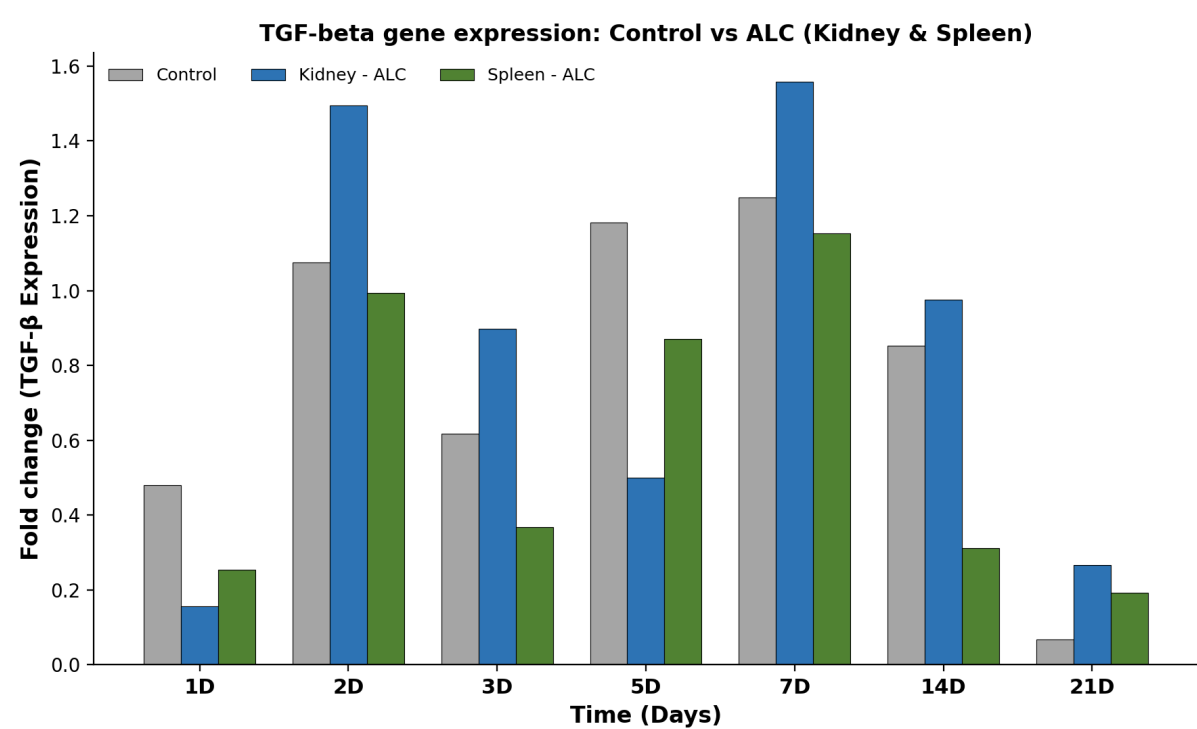


Figure 6. Fold change expression of the TGF- β gene in *A. testudineus* (kidney and spleen) after ALC administration and subsequent challenge with *A. hydrophila*

4. Discussion

Dietary supplementation with 1% *Murraya koenigii* leaf extract (ALC) produced measurable improvements in immune gene expression, haematological status, and antioxidant capacity in *Anabas testudineus* following *Aeromonas hydrophila* challenge. To our knowledge, this is the first study to characterise this specific cytokine panel across both kidney and spleen in climbing perch fed curry leaf extract, extending earlier reports of *M. koenigii*'s disease-resistance benefits in gourami challenged with *Edwardsiella tarda* (12) and its haemato-protective effects in *Labeo rohita* challenged with *A. hydrophila* (31).

4.1 Mechanistic basis of ALC's immunomodulatory activity

The protective effects observed here are consistent with the known pharmacology of *M. koenigii*. The plant is a rich source of carbazole alkaloids, and several of these — including murrayakonine A–D, isolated specifically from *M. koenigii* leaf and stem extracts — have been experimentally shown to inhibit TNF- α and IL-6 release in a dose-dependent manner in mammalian immune cell models (32). Separately, oral administration of *M. koenigii* leaf extract has been shown to enhance both humoral and cell-mediated immune responses in rodent models, supporting a genuine immunostimulatory rather than purely antioxidant mode of action (33). These mechanistic find

ings from mammalian systems provide plausible biological grounding for the cytokine modulation observed in the present fish study.

4.2 LD₅₀ determination

The 96-hour LD₅₀ of *A. hydrophila* for *A. testudineus*, determined by probit regression of dose–mortality data across three bacterial concentrations (6×10^6 – 6×10^8 CFU/mL), was estimated at 6×10^8

CFU/mL ($R^2 = 0.97$). This value was used to standardize the challenge dose for the subsequent immune and haematological assessments, ensuring a physiologically meaningful and reproducible infection pressure across treatment groups.

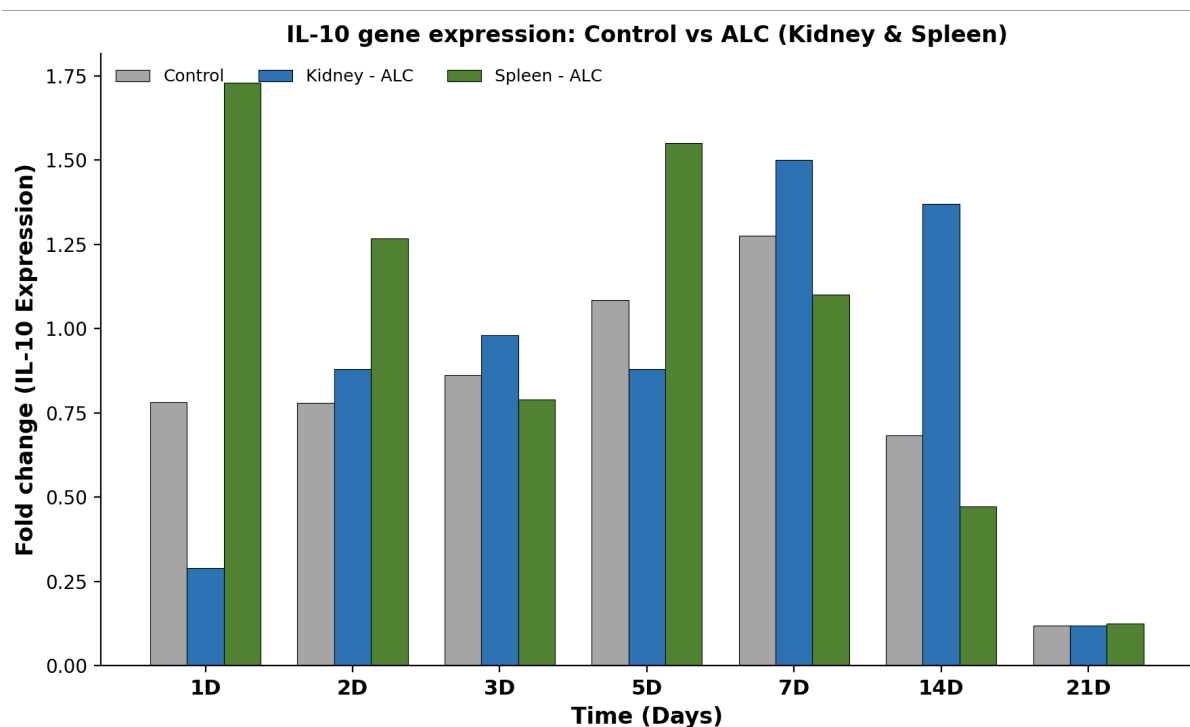


Figure 7. Fold change expression of the IL-10 gene in *A. testudineus* (kidney and spleen) after ALC administration and subsequent challenge with *A. hydrophila*

4.3 Haematological response

RBC, HGB, and HCT all declined from Day 1, reached their lowest values around Day 7, and recovered progressively through Day 21 in both treatment groups — a trajectory consistent with the "anaemia of infection" commonly reported following *Aeromonas* challenge, attributed to haemolysis, haemorrhagic blood loss, and stress-suppressed erythropoiesis. ALC-fed fish showed a significantly shallower decline in all three parameters relative to control, indicating that the extract mitigated, rather than prevented, this infection-associated anaemia. Conversely, WBC rose from Day 1, peaked around Day 5–7, and declined toward baseline by Day 21, with ALC-fed fish maintaining significantly higher counts than control throughout — consistent with enhanced leukocyte mobilization during the acute infection phase.

4.4 Antioxidant response

SOD and catalase activities followed a comparable rise-and-decline pattern in both groups, peaking around Day 3 before gradually decreasing through Day 21, with ALC-fed fish showing significantly higher activity at every timepoint. This is consistent with the well-documented antioxidant properties of *M. koenigii*'s phenolic and flavonoid constituents, which likely helped offset oxidative stress generated by the respiratory burst of activated phagocytes during infection.

4.5 Cytokine gene expression

TNF- α : In the kidney, ALC-fed fish showed an early peak at Day 5, while control fish showed a delayed peak at Day 7 of comparable magnitude. This shifted kinetic pattern — earlier activation and earlier resolution in the protected group versus delayed, prolonged activation in the unprotected group — mirrors findings in silver catfish,

where TNF- α and IL-1 β expression rose sharply by 24 h post-infection in protected (vaccinated) fish and returned to baseline by 72 h, while unprotected fish showed a slower resolution (34). No significant TNF- α difference was observed in the spleen, suggesting the kidney — the primary haematopoietic and phagocytic organ in teleosts — was the principal site of this cytokine's regulation by ALC.

IL-1 β : No significant difference was observed between ALC and control in either kidney or spleen. This is not itself unusual — IL-1 β responses in fish are frequently organ- and species-specific; for instance, thermal-stressed common carp showed dramatic IL-1 β induction in head kidney but comparatively modest change in spleen following *A. hydrophila* challenge (35), illustrating that IL-1 β regulation can diverge substantially between lymphoid organs and does not always track other pro-inflammatory markers.

IL-8: Significant upregulation was observed in both kidney and spleen of ALC-fed fish relative to control. As the principal neutrophil-recruiting chemokine in teleosts, elevated IL-8 in both organs suggests ALC enhanced systemic phagocyte mobilization to the site of infection, consistent with reports that IL-8 expression in salmonids rises rapidly and is sustained longer than co-induced cytokines such as IL-1 β following bacterial challenge (36).

TGF- β and IL-10: Both anti-inflammatory cytokines were significantly upregulated in ALC-fed fish in both organs, peaking around Day 7 — shortly after the pro-inflammatory TNF- α and IL-8 peaks. This sequencing (pro-inflammatory response followed by anti-inflammatory resolution) is consistent with a well-regulated, self-limiting immune response rather than indiscriminate immune activation, and parallels findings in vaccinated red tilapia, where TGF- β expression rose significantly in spleen and head kidney of protected fish during the acute post-challenge window before returning toward baseline (37).

4.6 Integration with survival outcomes

Collectively, the cytokine, haematological, and antioxidant data describe a coherent physiological narrative: ALC-fed fish mounted a faster, better-regulated innate immune response — characterized by earlier pro-inflammatory cytokine peaks, stronger chemokine-mediated phagocyte recruitment, timely *anti*-inflammatory resolution, reduced infection-associated anaemia, and enhanced antioxidant defence — compared to control fish, which showed delayed and less-resolved inflammatory kinetics. This is consistent with the broader principle, demonstrated in *Puntius sarana* surviving experimental *A. hydrophila* infection, that early and well-coordinated innate immune gene expression is closely associated with favourable infection outcomes (38).

5. Conclusion

This study demonstrates that dietary supplementation with 1% *Murraya koenigii* leaf extract (ALC) confers measurable immunomodulatory, haemato-protective, and antioxidant benefits in *Anabas testudineus* challenged with *A. hydrophila*. ALC-fed fish exhibited an earlier and better-resolved TNF- α response in the kidney, significant upregulation of IL-8, TGF- β , and IL-10 in both kidney and spleen, reduced infection-associated anaemia, enhanced leukocyte mobilisation, and elevated SOD and catalase activity relative to control fish. Together, these findings indicate that curry leaf extract strengthens both innate immune competence and antioxidant defence during bacterial challenge, supporting its potential as a low-cost, sustainable dietary immunostimulant for climbing perch aquaculture. Further work — including dose-optimisation trials, isolation of the specific bioactive fractions responsible for these effects, and finer time-resolved sampling of the acute-phase cytokine response — is warranted to translate these findings into practical aquaculture application

References

- Food and Agriculture Organization. The state of world fisheries and aquaculture 2022: Towards blue transformation. FAO; 2022.
- Gjedrem T, Robinson N, Rye M. The importance of selective breeding in aquaculture to meet future demands for animal protein. *Aquaculture*. 2012;350-353:117-129.
- Ruttenberg BI, Lee PT, Smith JA. Risk factors for aquaculture losses in small-scale systems. *Aquaculture Reports*. 2021;21:100815.
- Snieszko SF. The effects of environmental stress on outbreaks of infectious diseases of fish. *Journal of Fish Biology*. 1974;6(2):197-208.
- Stentiford GD, Shrimpton J, Williams B. Sustainable aquaculture requires disease prevention strategies. *Nature Reviews Microbiology*. 2020;18:267-268.
- Subasinghe RP, Arthur JR, Phillips MJ. Antibiotic use in aquaculture: Practices and risks. *Reviews in Aquaculture*. 2009;1(1):87-105.
- Reverter M, Sarter S, Caruso D, Avarre JC, Combe M, Pepey E, Pouyau L, Vega-Heredia S, de Vardal H, Gozlan RE. Aquaculture at the nexus of emerging antimicrobial resistance. *Nature Reviews Microbiology*. 2020;18(5):267-278.
- Santos L, Ramos F. Antimicrobial resistance in aquaculture: Current knowledge and alternatives to tackle the problem. *International Journal of Antimicrobial Agents*. 2018;52(2):135-143.
- Dawood MAO, Koshio S, Esteban MA. Beneficial roles of feed additives as immunostimulants in aquaculture: A review. *Reviews in Aquaculture*. 2020;12(1):219-239.
- Kuralkar P, Kuralkar SV. Role of herbal products in animal production — An updated review. *Journal of Ethnopharmacology*. 2021;278:114246.
- Mishra D, Khare P, Singh DK, Luqman S. Essential oil composition and antibacterial activity of *Murraya koenigii* leaves. *Industrial Crops and Products*. 2013;42:25-30.
- Syaifurrisal A, Suciyo S, Santanumurti MB, Prajitno A, Fadjar M, Riyadi FM, Fauziyyah AI, Pariawan A, Abualreesh MH. Effect of curry leaf (*Murraya koenigii*) as edwardsiellosis treatment on gourami fish (*Osphronemus goramy*). *Aquatic Sciences and Engineering*. 2024;39(3):179-188.
- Debnath C. Evaluating the dietary effects of curry and/or marigold leaf extracts on physiological and immunological responses of *Labeo rohita* and its resistance against *Saprolegnia parasitica* infection. *Fish Physiology and Biochemistry*. 2025;51(2):68.
- Giri SS, Sen SS, Chi C, Kim HJ, Yun S, Park SC, Sukumaran V. Effect of guava leaves on the growth performance and cytokine gene expression of *Labeo rohita* and its susceptibility to *Aeromonas hydrophila* infection. *Fish & Shellfish Immunology*. 2015;46(2):217-224.
- Cowan MM. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*. 1999;14:564-582.
- Awodele O, Oreagba IA, Odoma S, da Silva JA, Osunkalu VO. Toxicological evaluation of the aqueous leaf extract of *Moringa oleifera* Lam. (Moringaceae). *Journal of Ethnopharmacology*. 2012;139(2):330-336.
- Association of Official Analytical Chemists. Official methods of analysis (16th ed.). AOAC International; 1995.
- American Public Health Association, American Water Works Association, Water Environment Federation. Standard methods for the examination of water and wastewater (20th ed., pp. 413-426). American Public Health Association; 1998.
- Ahammad AS, Asaduzzaman M, Ahmed MB, Akter S, Islam MS, Haque MM, Ceylan H, Wong LL. Muscle cellularity, growth performance and growth-related gene expression of juvenile climbing perch *Anabas testudineus* in response to different eggs incubation temperature. *Journal of Thermal Biology*. 2021;96:102830.
- Hamackova J, Kouril J, Kozak P, Stupka Z. Clove oil as an anaesthetic for different freshwater fish species. *Bulgarian Journal of Agricultural Science*. 2006;12(2):185.
- Madesh M, Balasubramanian KA. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. *Indian Journal of Biochemistry & Biophysics*. 1998;35(3):184-188.
- Sinha AK. Colorimetric assay of catalase. *Analytical Biochemistry*. 1972;47(2):389-394.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods*. 2001;25(4):402-408.
- Shahriar F, Ibasina AA, Uddin N, Munny FJ, Hussain MA, Jang WJ, Lee JM, Lee EW, Hasan MT, Kawsar MA. Effects of commercial probiotics on the growth, hematology, immunity, and resistance to *Aeromonas hydrophila* challenge in climbing perch, *Anabas testudineus*. *Aquaculture Research*. 2024;3901035.
- Green LH, Goldman E, editors. Practical handbook of microbiology. CRC Press; 2021.
- Chakraborty SB, Hancz C. Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture. *Reviews in Aquaculture*. 2011;3(3):103-119.
- Citarasu T. Herbal biomedicines: A new opportunity for aquaculture industry. *Aquaculture International*. 2010;18(3):403-414.
- Ifemeje JC, Egbuna C, Eziokwudiaso JO, Ezebuo FC. Determination of the anti-nutrient composition of *Ocimum gratissimum*, *Corchorus olitorius*, *Murraya koenigii* Spreng and *Cucurbita maxima*. *International Journal of Innovation and Scientific Research*. 2014;3(2):127-133.

29. Abarike ED, Dandi SO, Ampofo-Yeboah A. A blend of guava, bitter, and neem leaf extracts improves haematology and resistance to co-infection of *Streptococcus agalactiae* and *Aeromonas jandaie* but not liver health in Nile tilapia. *Fish and Shellfish Immunology Reports*. 2022;3:100066.
30. Nhu TQ, Hang BT, Hue BT, Quetin-Leclercq J, Scippo ML, Phuong NT, Kestemont P. Plant extract-based diets differently modulate immune responses and resistance to bacterial infection in striped catfish (*Pangasianodon hypophthalmus*). *Fish & Shellfish Immunology*. 2019;92:913–924.
31. Debbarma S, Chrisolite B, Sivasankar P, Kumar PS, Kumar VS, Daniel N, Paulraj MK. Investigating the immunomodulatory and haemato-protective roles of curry leaf ethanolic extract (*Murraya koenigii*) in *Labeo rohita* fingerlings infected with *Aeromonas hydrophila*. *Fish Physiology and Biochemistry*. 2025;51(5):171.
32. Nalli Y, Khajuria V, Gupta S, Arora P, Riyaz-Ul-Hassan S, Ahmed Z, Ali A. Four new carbazole alkaloids from *Murraya koenigii* that display *anti-inflammatory* and *anti-microbial* activities. *Organic & Biomolecular Chemistry*. 2016;14(12):3322–3332.
33. Kaur I, Bhatia S, Bhati Y, Sharma V, Mediratta PK, Bhattacharya SK. Augmented primary humoral immune response and decreased cell-mediated immunity by *Murraya koenigii* in rats. *Journal of Basic and Clinical Physiology and Pharmacology*. 2014;25(2):211–215.
34. de Figueiredo Soveral L, de Almeida PA, Kreutz Y, Ribeiro VA, Frandoloso R, Kreutz LC. Modulation of expression of proinflammatory genes and humoral immune response following immunisation or infection with *Aeromonas hydrophila* in silver catfish (*Rhamdia quelen*). *Fish and Shellfish Immunology Reports*. 2022;3:100053.
35. Shahi N, Ardó L, Fazekas G, Góczy E, Kumar S, Révész N, Jeney Z. Immunogene expression in head kidney and spleen of common carp (*Cyprinus carpio* L.) following thermal stress and challenge with Gram-negative bacterium *Aeromonas hydrophila*. *Aquaculture International*. 2018;26(3):727–741.
36. Fast MD, Johnson SC, Jones SRM. Differential expression of the pro-inflammatory cytokines IL-1 β -1, TNF α -1 and IL-8 in vaccinated pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon juveniles. *Fish & Shellfish Immunology*. 2007;22(4):403–407.
37. Monir MS, Yusoff MSM, Zamri-Saad M, Amal MNA, Mohamad A, Azzam-Sayuti M, Ina-Salwany MY. Effect of an oral bivalent vaccine on immune response and immune gene profiling in vaccinated red tilapia (*Oreochromis* spp.) during infections with *Streptococcus iniae* and *Aeromonas hydrophila*. *Biology*. 2022;11(9):1268.
38. Das A, Sahoo PK, Mohanty BR, Jena JK. Pathophysiology of experimental *Aeromonas hydrophila* infection in *Puntius sarana*: Early changes in blood and aspects of the innate immune-related gene expression in survivors. *Veterinary Immunology and Immunopathology*. 2011;142(3–4):207–218.

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