

Immunostimulatory Response of Supplement Feed in Fish against *Aeromonas hydrophila*

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Abstract : Introduction: Fish is an important protein source for humans and has great economic value. Fish cultures are affected due to various anthropogenic activities that lead to bacterial and viral infections. *Aeromonas hydrophila* is a fish pathogenic bacterium that causes several aquaculture outbreaks throughout the world and leads to huge mortalities. In this study, plants of no commercial value were used to investigate their immunostimulatory, antioxidant, anti-inflammatory, anti-bacterial, and disease resistance potential in fish against *Aeromonas hydrophila*, through fish feed fortification. Methods: The plant was dried at room temperature in the shade, dissolved in methanol, and analysed for biological compounds through GC-MS/MS. DPPH, FRAP, Phenolic, and flavonoids were estimated following standardized protocols. In silico molecular docking was also performed to validate its broad-spectrum activities based on binding affinity with specific proteins. Fish were divided into four groups (n=6; total 30 in a group): Group 1, non-challenged fish (fed on a non-supplemented diet); Group 2, fish challenged with bacteria (fed on a non-supplemented diet); Group 3 and 4, fish challenged with bacteria (*A. hydrophila*) and fed on plant supplemented feed at 2.5% and 5%. Blood was collected from the fish on 0, 7th, 14th, 21st, and 28th days. Serum was separated for glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase assay (ALP), lysozyme activity assay, superoxide dismutase assay (SOD), lipid peroxidation assay (LPO) and molecular parameters (including cytokine levels) were estimated through ELISA. The phagocytic activity of macrophages from the spleen and head kidney, along with quantitative analysis of immune-related genes, were analysed in different tissue samples. The digestive enzymes (Pepsin, Trypsin, and Chymotrypsin) were also measured to evaluate the effect of plant-supplemented feed on freshwater fish. Results and Discussion: GC-MS/MS analysis of a methanolic extract of plant validated the presence of key compounds having antioxidant, anti-inflammatory, anti-bacterial, anti-inflammatory, and immunomodulatory activities along with disease resistance properties. From biochemical investigations like ABTS, DPPH, and FRAP, the amount of total flavonoids, phenols, and promising binding affinities towards different proteins in molecular docking analysis helped us to realize the potential of this plant that can be used for investigation in the supplemented feed of fish. Measurement liver function tests, ALPs, oxidation-antioxidant enzyme concentrations, and immunoglobulin concentrations in the experimental groups (3 and 4) showed significant improvement as compared to the positive control group. The histopathological evaluation of the liver, spleen, and head kidney supports the biochemical findings. The isolated macrophages from the group fed on supplemented feed showed a higher percentage of phagocytosis and a phagocytic index, indicating an enhanced cell-mediated immune response. Significant improvements in digestive enzymes were also observed in fish fed on supplemented feed, even after weekly challenges with bacteria. Hence, the plant-fortified feed can be recommended as a regular feed to enhance fish immunity and disease resistance against the *Aeromonas hydrophila* infection after confirmation from the field trial.

Keywords : immunostimulation, antipathogen, plant fortified feed, macrophages, GC-MS/MS, in silico molecular docking

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