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## Dietary supplementation with *Adiantum capillus-veneris* and *Ferula gummosa* enhanced antioxidant status and innate immune responses in Rainbow trout (*Oncorhynchus mykiss*)

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### Abstract

This study investigated the effects of dietary supplementation with *Adiantum capillus-veneris* and *Ferula gummosa* and a mixture of them on antioxidant status and innate immune responses in rainbow trout (*Oncorhynchus mykiss*). Fish with initial weight of  $20.2 \pm 0.01$  g were fed experimental diets containing plant powders individually and in combination for 8 weeks. Results indicated significant improvements in antioxidant enzyme activities including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). No significant difference was seen in malondialdehyde (MDA) levels in treatments ( $p > 0.05$ ). Innate immune indices namely lysozyme activity, complement activity (ACH50), as well as serum immunoglobulin M (IgM) were significantly enhanced, particularly in the combined treatment group ( $p < 0.05$ ). Administration a mixture of 0.5% *A. capillus-veneris* and 0.5% *F. gummosa* showed a synergistic effect between antioxidant-rich and immunostimulatory phytochemicals, highlighting their potential as natural alternatives to antibiotics in aquaculture.

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## Introduction

Rainbow trout (*Oncorhynchus mykiss*) has great economic value due to its high growth rate and delectable meat (Vidhya Hindu et al., 2019). The excessive use of antibiotics has elicited apprehensions pertaining to antimicrobial resistance and environmental impacts, thereby instigating the exploration of natural alternatives (Defoirdt et al., 2011; Vaseeharan and Thaya, 2014; Larsson and Flach, 2022). Phytocompounds have positive effects on the growth and health of fish, and disease resistance, are suitable alternatives to chemical compounds (Cappiello, 2020; Abasubong et al., 2023). Phytocompounds, including flavonoids, phenolics, and terpenoids, improve oxidative status and enhance innate immune responses in aquaculture (Sakai, 1999; Harikrishnan et al., 2011). Previous studies have demonstrated that phytochemicals increased lysozyme activity and immunoglobulin production in fish (Reverter et al., 2017; Dawood et al., 2018). The maidenhair fern (*Adiantum capillus-veneris*) has been used by people in several nations to treat a variety of medical conditions. It was frequently used in Iran's ancient medical practice named "Pare-siavashan," mostly for asthma, cough, as well as for use as a hair tonic. It has been used in India to treat digestive problems, fever, and respiratory conditions. It has applied for jaundice, kidney stones, spleen pain, and hair growth stimulation. Additionally, it was traditionally used to treat pertussis and coughing in Middle Eastern nations like Iraq. There is proof that *A. capillus-veneris* was employed as a diuretic and as a treatment for bronchitis, skin conditions, and nephritis in traditional Chinese medicine. The typical prescription covers problems of the skin, liver, and internal organ tumors in addition to respiratory signs. Additionally, it was once frequently used as a folk treatment of gastritis, bronchitis, nephritis, dermatitis, and cystitis, particularly in southern China. These diverse uses serve as an example of the herb's wide range of therapeutic applications across various cultural traditions (Schwartz, 2012; Dehdari and Hajimehdipoor, 2018; Akbar and Akbar, 2020; Qadir et al., 2025). It is known in case of substantial phenolic content and strong antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities properties. It has been reported to diminish oxidative stress and augment antioxidant enzyme activities in biological systems (Kanwal et al., 2018). However, its application in aquaculture remains limited. Similarly, *Ferula*

*gummosa* contains terpenoid-rich resins with antimicrobial and immunomodulatory properties (Iranshahy and Iranshahi, 2011; Boghrati and Iranshahi, 2019). Although evidence in fish is scarce, related species of *Ferula* have shown immune-stimulating effects in animal models. Given their complementary biological activities, antioxidant activities of *A. capillus-veneris* and immunostimulatory effects of *F. gummosa* their combined use may exert synergistic benefits on fish health. *Adiantum* is an important plant and has been used in treatments of a cough, bronchitis, colds, flu, pneumonia, extreme phlegm, tumors of the spleen, liver, gall stones, and other internal organs, and skin inflammatory ailments (Singh et al., 2008). Therefore, this study aimed to evaluate their effects on antioxidant defense and innate immune status in *O. mykiss*.

## Materials and Methods

### Dietary preparation

Fish were nourished a commercial feed (Faradaneh Company, Iran). Fish were randomly separated into four groups including in basal diet (T0), 0.1% *A. capillus-veneris* (T1), 0.1% *F. gummosa* (T2), and combination (0.5% + 0.5%) (T3). For this purpose, initially dried parts of both plants were ground into powder and incorporated into commercial feed. The dietary components were homogenized and mixed with water. The resulting dough was processed through a grinder (MG1400R, Pars Khazar, Iran), and subsequently shaped into pellets. Before being used in this investigation, these pellets were air-dried and then stored at -20°C.

### Rearing condition and experimental design

In this research 240 healthy *O. mykiss* with initial weight of  $20.2 \pm 0.01$  g were acquired from a farm in Mazandaran (Iran) and were taken to the laboratory (Amol veterinary university, Amol) and placed in four tanks, then the fish underwent a 14-day acclimatization period. Through this period, they were given a basal diet three times a day. After the acclimatization phase, fish were randomly assigned to 12 fiberglass tanks (1000 liter), and were supplied with continuous aeration. Over the course of eight weeks, the fish of four treatments were nourished (3% body weight) daily. The tanks were daily siphoned and were refilled with half a volume of fresh water.

Throughout the experimental period, water quality parameters, including temperature ( $12.2 \pm 2^\circ\text{C}$ ), pH ( $7.5 \pm 0.5$ ), and  $\text{O}_2$  ( $8 \pm 0.7$  mg/l) were routinely followed to ensure optimal conditions.

### Sampling

After eight weeks, five fish each tank were selected at random and given MS-222 to induce anaesthetization by the administration of 50 mg/l of MS-222 (Zhang *et al.*, 2020). Venipuncture was used to obtain blood samples from the caudal vein. These materials were then separated into tubes and centrifuged for 10 minutes at 1600 g. After that, supernatant was kept at  $-70^\circ\text{C}$  for further examination.

### Serum factors

Lysozyme (LYZ) activity was quantified by Ellis method (Ellis, 1990). Complement activity (ACH50), and serum immunoglobulin M (IgM, Eastbiopharm) Glutathione peroxidase (GPx, ZelBio), superoxide dismutase (SOD, ZelBio), catalase (CAT, ZelBio), and malondialdehyde (MDA, ZelBio) were examined using diagnostic reagent kits (Armobin *et al.*, 2023).

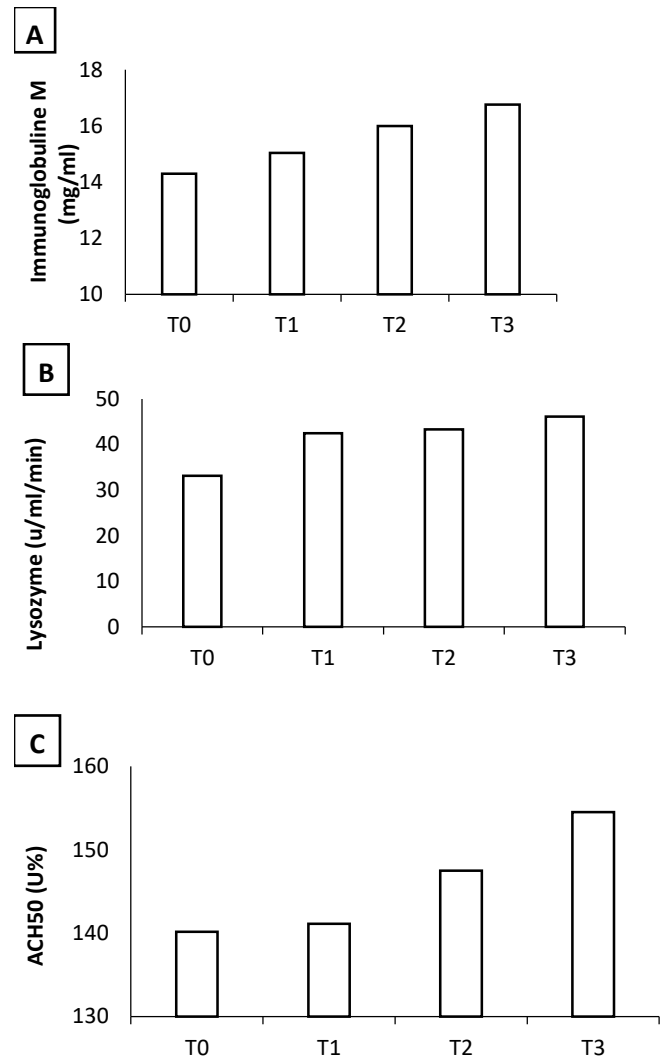
### Statistical analysis

Version 22 of the SPSS was used to analyze all the data at a 95% confidence level. First, the Kolmogorov-Smirnov was used to determine the data was normal, Levene's test was used to determine whether the variance was homogeneous. Duncan's multiple range test was used to evaluate differences between treatments, and ANOVA was used.

## Results

### Immune response

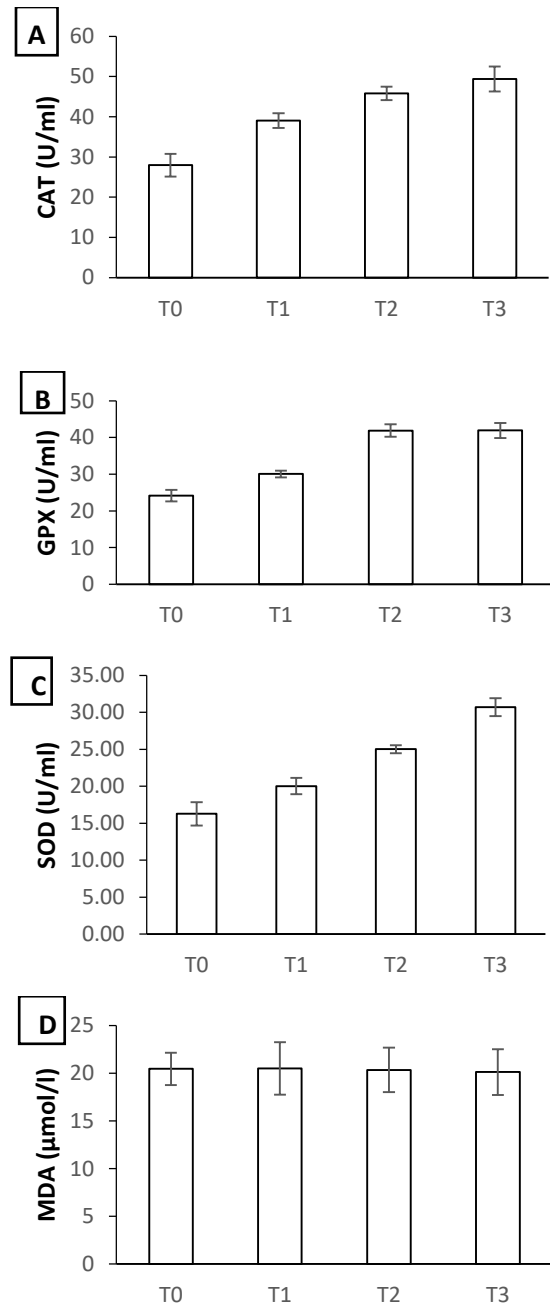
Lysozyme activity, ACH50, and IgM levels were noticeably higher in fish receiving *A. capillus-veneris* (T1) and *F. gummosa* (T2) in comparison to the T0. The combined treatment group (T3) exhibited the highest immune response values across all measured parameters (Fig. 1, A, B, and C,  $p < 0.05$ ).



**Fig. 1.** Serum immune parameters of fish fed with experimental diet. The mean  $\pm$  SD was used to express data. (A): immunoglobulin M, (B): lysozyme, (C): ACH50.

### Antioxidant response

CAT, SOD, and GPX were among the serum antioxidant enzymes that significantly increased in the experimental groups as compared to the control group. (Fig. 2, A, B and C,  $p < 0.05$ ). No significant difference was detected in oxidative stress marker (MDA) levels between treatments (Fig. 2, D,  $p > 0.05$ ).



**Fig. 2.** Antioxidant enzymes of fish fed with experimental diet. The mean  $\pm$  SD was used to express data. (A): CAT, (B): GPX, (C): SOD, (D): MDA.

### Synergistic effects

The combination group demonstrated superior antioxidant and immune responses compared to single-plant treatments, indicating a synergistic interaction between the two phytochemical additives, *A. capillus-veneris* and *F. gummosa*.

## Discussion

Fish's natural defenses against infections may be strengthened by using dietary medicinal herbs as immunostimulants and growth promoters in the aquaculture sector (Haghighi and Sharif Rohani, 2013). Previous researches showed that natural antioxidants, spatially plant-derived compounds, are important for the ability in combating oxidative damage. *A. capillus-veneris* has exceptional antioxidant potential (Roy et al., 2019; Boukada et al., 2022; Qadir et al., 2025).

Plant-based flavonoids have stimulated antioxidant enzymes, including SOD, CAT, and GSH, while decrease MDA as a marker of oxidative stress, in vertebrates (Jiang et al., 2011). Furthermore, *A. capillus-veneris* hydroalcoholic extract was tested to be effective in decreasing oxidative stress markers and enhancing the antioxidant ability (Ahmadpouri et al., 2020).

The present study demonstrated that supplementation with *A. capillus-veneris* and *F. gummosa* significantly enhanced antioxidant defense and innate immune responses. The increased SOD, CAT, GPx in fish fed *A. capillus-veneris* can be attributed to its high flavonoid and phenolic content, which effectively neutralizes ROS. Results are in line with earlier studies showing the antioxidant potential of medicinal plants (Dawood et al., 2018). *F. gummosa* significantly enhanced immune parameters, including lysozyme and complement activity. This immunostimulatory effect may be due to terpenoid compounds that activate macrophages and other immune cells, as previously suggested for related species (Harikrishnan et al., 2011; Iranshahy and Iranshahi, 2011). Importantly, the combined treatment resulted in the highest immune and antioxidant responses, suggesting a synergistic interaction between antioxidant and immunostimulatory pathways. Such synergy likely improves physiological resilience by simultaneously reducing oxidative stress and enhancing immune readiness. Similar synergistic effects have been reported in studies involving multiple herbal additives, where multi-target mechanisms improved fish health more effectively than single compounds (Reverter et al., 2017). These findings imply that combining antioxidant-rich and immunostimulatory medicinal plants is a promising strategy to enhance fish immunity without antibiotics.

Dietary inclusion of *A. capillus-veneris* and *F.*

*gummosa* improves antioxidant capacity and innate immune responses in *O. mykiss*. The combined supplementation exhibited synergistic effects, making it a promising natural immunostimulant strategy in aquaculture.

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## Conflict of Interest

The authors do not have any potential conflict of interest to declare.

## References

- Abasubong, KP; Gabriel, NN and Adjoumani, JY** (2023). Ferulic acid as feed additives in aquaculture: A review on growth, immune response, and antioxidant status of finfish. *Emerg. Sustain. Aqua. Innov. Africa*, 251-272.
- Ahmadpouri, J; Chahardahcharic, SV and Setorki, M.** (2020). The effect of *Adiantum Capillus- veneris* L. hydroalcoholic extract on the oxidative stress rate of mice's blood and brain in the depression model caused by acute immobilization stress. *Pharm. Biomed. Res.*, 6: 3803.
- Akbar, S and Akbar, S** (2020). *Adiantum Venustum* G. Don. / *Adiantum Capillus veneris* L. (Pteridaceae). In *Handbook of 200 Medicinal Plants: A Comprehensive Review of Their Traditional Medical Uses and Scientific Justifications*, Springer, PP: 103–107.
- Armobin, K; Ahmadifar, E; Adineh, H; Samani, MN; Kalhor, N; Yilmaz, S; Hoseinifar, SH and Van Doan, H** (2023). Quercetin application for common carp (*Cyprinus carpio*): I. Effects on growth performance, humoral immunity, antioxidant status, immune-related genes, and resistance against heat stress. *Aqu. Nutr.*, 1: 1168262.
- Boghrati, Z and Iranshahi, M** (2019). *Ferula* species: A rich source of antimicrobial compounds. *J. Herbal Med.*, 16: 100244.
- Boukada, F; Sitayeb, S; Khadem, H; Meddah, B and Soltani, FZ** (2022). Chemical composition, antioxidant and antibacterial activity of *Adiantum Capillus- Veneris* L. extract from Algeria. *Kragujevac J. Sci.*, 44: 4091.
- Cappiello, F; Loffredo, MR and Del Plato, C** (2020). The reevaluation of plant- derived terpenes to fight antibiotic- resistant infections. *Antibiotics*, 9: 325.
- Defoirdt, T; Sorgeloos, P and Bossier, P** (2011). Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Curr. Opin. Microbiol.*, 14: 251-258.
- Dehdari, S and Hajimehdipoor, H** (2018). Medicinal properties of *AdiantumCapillus- Veneris* Linn. in traditional medicine and modern phytotherapy: A review article. *Iran. J. Public Health*, 47: 188–197.
- Ellis, AE** (1990). Lysozyme assays. *Techniq. Fish Immunol.*, 1: 101-103.
- Haghighi, M and Rohani, MS** (2013). The effects of powdered ginger (*Zingiber officinale*) on the haematological and immunological parameters of rainbow trout *Oncorhynchus mykiss*. *J. Med. Plant Herbal Ther. Res.*, 1: 8-12.
- Harikrishnan, R; Kim, MC; Kim, JS; Balasundaram, C and Heo, MS** (2011). Protective effect of herbal and probiotics enriched diet on haematological and immunity status of *Oplegnathus fasciatus* (Temminck & Schlegel) against *Edwardsiella tarda*. *Fish Shellfish Immunol.*, 30: 886-893.
- Iranshahy, M and Iranshahi, M** (2011). Traditional uses, phytochemistry and pharmacology of asafoetida (*Ferula assa-foetida* oleo-gum-resin)-A review. *J. Ethnopharmacol.*, 134: 1-10.
- Jiang, MZ; Yan, H; Wen, Y and Li, XM** (2011). *In vitro* and *In vivo* studies of antioxidant activities of flavonoids from *Adiantum capillus- veneris* L. *African J. Pharm. Pharmacol.*, 5: 2079–2085.
- Kanwal, Q; Qadir, A and Munir, B** (2018). Healing potential of *Adiantum capillus-veneris* L. plant extract on bisphenol A-induced hepatic toxicity in male albino rats. *Environ. Sci. Pollution Res.*, 25: 11884-11892.
- Larsson, DG and Flach, CF** (2022). Antibiotic resistance in the environment. *Nature Rev. Microbiol.*, 20: 257-269.
- Dawood, MAO; Koshio, S and Esteban, MA** (2018). Beneficial roles of feed additives as immunostimulants in aquaculture: a review. *Rev. Aqua.*, 10: 950-974.
- Qadir, SA; Awlqadr, FH; Qadir, MH; Qadir, AM; Faraj, AM; Salih, SA and Hosseini, SMN** (2025). *Adiantum capillus-veneris*: A comprehensive review of its medicinal properties, bioactive compounds, and advanced extraction techniques. *Food Sci. Nutr.*, 13: e71118.
- Reverter, M; Tapissier-Bontemps, N; Sasal, P and Saulnier, D** (2017). Use of medicinal plants in aquaculture. Diagnosis and control of diseases of fish and shellfish, 223-261.
- Sakai, M** (1999). Current research status of fish immunostimulants. *Aqua.*, 172: 63-92.
- Schwartz, M** (2012). *Adiantum Capillus- Veneris* L.: ethnobotany, etymology, and Iranian cultural history. *Bull. Asia Institute*, 26: 97–101.
- Vaseeharan, B and Thaya, R** (2014). Medicinal plant derivatives as immunostimulants: an alternative to chemotherapeutics and antibiotics in aquaculture. *Aqua. Int.*, 22: 1079-1091.
- Vidhya Hindu, S; Chandrasekaran, N; Mukherjee, A and Thomas, J** (2019). A review on the impact of seaweed polysaccharide on the growth of probiotic bacteria and its application in aquaculture. *Aqua. Int.*, 27: 227-238.

**Zhang, L; Zhang, P; Xia, C; Cheng, Y; Guo, X and Li, Y**  
(2020).Effects of malic acid and citric acid on growth performance, antioxidant capacity, haematology and immune response of *Carassius auratus gibelio*. Aqua. Res., 51: 2766-2776.