

*Research Article*

# Enhancing aquaculture performance and disease resistance in rainbow trout with Iranian herbal feed additives: A review

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## Keywords

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Rainbow trout,  
Antibiotics,  
Immunostimulant,  
Growth performance

## Abstract

In Iran, rainbow trout is the sole species of cold-water fish that is farmed, and it plays a crucial role in the aquaculture industry. However, challenges like poor environmental conditions, high stocking densities, and inadequate diets lead to stress and weakened immune systems, resulting in reduced growth and increased susceptibility to various diseases. Traditionally, antibiotics have been used to control infections, but their widespread use has resulted in drug residues, health risks for consumers, antibiotic-resistant strains, higher production costs, and environmental degradation. The growing demand for eco-friendly products has intensified the need to limit or ban antibiotic usage in aquaculture. Over the past two decades, interest in utilizing medicinal plants to prevent and treat aquatic diseases has increased. The herbs can help combat antibiotic resistance, eliminate drug residues, and maintain microbial balance in aquatic environments. In this review, we assess the effectiveness of native Iranian medicinal plants on growth, immunity, blood and serum and antioxidant indices, and resistance to microbial diseases in rainbow trout. We also explore how factors such as type of plant material, dosage, duration of use, and pathogen type influence treatment efficacy. Our findings indicate that diets enriched with these plants significantly improve growth, immunity, and disease survival, regardless of the variables considered. This study highlights that plant-based supplements derived from native Iranian herbs offer a versatile alternative for fish farmers, from small-scale to industrial operations.

## Article info

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## Introduction

Aquaculture plays an essential role in providing income and significantly contributes to food security and social development across numerous countries. Referring to the latest available published information, Iran produced over 200,000 tons of rainbow trout, accounting for more than 25% of the global production, according to the Food and Agriculture Organization (FAO) (D'Agaro *et al.*, 2022). To improve the productivity of the industry in Iran, current strategies focus on intensive and semi-intensive farming methods. However, these practices have led to environmental pollution and increased disease outbreaks, negatively impacting fish growth, weakening immune responses, and raising vulnerability to infections (Ramesh and Souissi., 2018; Zhou *et al.*, 2018). The use of antibiotics is a common strategy for controlling infectious diseases in aquaculture. However, this approach can result in serious consequences, such as the emergence of antibiotic-resistant pathogenic bacteria (AMR) in both fish and humans. Moreover, the reliance on antibiotics can increase production costs due to treatment expenses, disrupt the microbial balance in aquatic ecosystems, leave drug residues in fish, and pose health risks to consumers (Lulijwa *et al.*, 2020; Bondad-Reantaso *et al.*, 2022).

Several studies have indicated the development of antibiotic resistance in certain pathogenic bacteria in rainbow trout such as *Streptococcus iniae*, *Streptococcus agalactiae*, *Lactococcus garvieae* and *Yersinia ruckeri* in Iran (Zandi *et al.*, 2016; Faeed and Ramazani, 2018; Ghiasi *et al.*, 2021). In the same vein, antibiotic residues

in aquaculture fish meat pose a risk of systemic toxicity to consumers, potentially damaging the human gastrointestinal microbiota and leading to detrimental health effects (Monteiro and Andrade, 2018). Despite limited information on antibiotic residues in rainbow trout meat in Iran, several studies indicate that the levels of antibiotic residue in the meat have exceeded standard limits (Soltani *et al.*, 2014; Adel *et al.*, 2017; Rafati *et al.*, 2018). These issues can result in import refusals and negatively impact international trade. The increasing demand for eco-friendly, high-quality products highlights the urgent need to restrict or ban antibiotics in aquaculture (Lulijwa *et al.*, 2020; Bondad-Reantaso *et al.*, 2022). Despite efforts to prevent infectious diseases in farmed fish through improved water quality, increased investment in health management, enhanced biosecurity, and the implementation of disease prevention strategies (such as SPF breeding and fish vaccination), these measures have not significantly reduced the industry's reliance on antibiotics or the associated health risks for consumers (Caputo *et al.*, 2022). To effectively reduce the use of antibiotics in aquaculture disease prevention and treatment, a more holistic strategy is required (Lieke *et al.*, 2019).

Over the past twenty years, medicinal herbs and their derivatives have garnered significant interest in aquaculture. Diets supplemented with these herbs have demonstrated benefits such as enhanced growth, improved feeding efficiency, and better hematological and serological parameters. They also boost immune responses, including lysozyme,

complement, and phagocytic activity, as well as total protein and immunoglobulin levels in both fish serum and mucus. Furthermore, these diets exhibit antioxidant properties and enhance resistance to various fish pathogens (Reverter *et al.*, 2021; Tadese *et al.*, 2021). Between 2004 and 2019, extensive research has been conducted on the clinical effects of orally administered medicinal plants on fish growth, immunity, and disease resistance. This field has experienced significant expansion, particularly from 2014 to 2019. The majority of these studies were carried out in low- and middle-income countries, with Iran playing a prominent role. Additionally, 20% of the research was conducted in high-income countries, highlighting a global interest in incorporating medicinal plants into aquaculture diets. The shift towards organic and sustainable food production in these countries has driven this interest, emphasizing environmentally friendly methods for disease prevention and treatment, and enhancing product value (Reverter *et al.*, 2021). Iran, with its distinctive climate and topography, hosts around 8,200 species of vascular plants. Among these, approximately 2,300 species (over 28%) are identified as medicinal or aromatic herbs. Notably, about 70% of these plants are concentrated in the provinces of Esfahan, Kerman, Fars, Tehran, Chaharmahal and Bakhtiari, East Azarbaijan, Lorestan, West Azarbaijan, Hamadan, and Mazandaran (Hassanpouraghdam *et al.*, 2022). Given that Lorestan, Chaharmahal Bakhtiari, and Mazandaran are major centers for rainbow trout production in Iran ([www.shilat.com](http://www.shilat.com)),

prioritizing research on the use of native medicinal plants in these regions could greatly assist farmers in managing fish health. This focus would ultimately lead to the production of higher-quality fish products. This review delves into the application of medicinal plants in rainbow trout farming industry in Iran, utilizing the latest data on the effects of native Iranian medicinal plants on rainbow trout growth, health, immunity, and resistance to bacterial infectious diseases. By exploring their potential mechanisms, this review aims to highlight the importance of these national resources in improving aquaculture and public health, thereby raising awareness among veterinary and medical researchers.

## Materials and methods

### *Search strategy*

A thorough review of English-language articles published between 2013 and 2023 was undertaken to identify studies assessing the impact of native Iranian herbs on growth performance, hemato-serological parameters, immunological indices, and resistance against bacterial pathogens of rainbow trout. Three search engines, namely Science Direct ([sciencedirect.com](http://sciencedirect.com)), Google Scholar ([scholar.google.com](http://scholar.google.com)), and PubMed ([pubmed.ncbi.nlm.nih.gov/advanced](http://pubmed.ncbi.nlm.nih.gov/advanced)) were utilized to search for relevant articles. The literature search was performed following keyword combination: (Iranian herb or Iranian medicinal plant) AND (rainbow trout OR growth OR immunostimulant OR hematological parameters OR serological parameters OR bacterial infection)

(Reverter *et al.*, 2021; Ghaednia *et al.*, 2024).

#### *Inclusion and exclusion criteria*

Articles were reviewed to ensure they met the following criteria: (i) reporting at least one or more parameters for both control and plant-enriched diets, including growth, hematological indices, serological measures, immunological factors, and resistance to pathogenic bacteria; (ii) providing means, replicates, and standard deviations/errors either numerically or graphically; and (iii) clearly identifying the type of extract, inclusion rate, and duration of treatment. (ii) providing mean, replicates, and standard deviation/error numerically or graphically, and (iii) clearly identifying the type of extract, inclusion rate and treatment duration. When a study examined multiple plant species or dosages, each was treated as a separate observation. When a study reported parameters at multiple time points, only the final time point was considered to minimize dependency between observations. For each observation, we extracted the following data: plant taxonomy (species and family), type of plant extract used (powder, aqueous, ethanol, methanol, essential oil and other), treatment duration (days), inclusion rate (percent plant kg<sup>-1</sup> feed), and type of pathogen used for the infection (only for the survival data, since for all other datasets, parameters were measured from healthy fish) (Reverter *et al.*, 2021; Ghaednia *et al.*, 2024).

#### *Data extraction*

The following experimental details were organized into an Excel spreadsheet: family of the herbs, growth, blood, immune, serum, and antioxidant indices, treatment duration, concentrations delivered, and type

of plant material. At first, all measured indices present (growth, blood, immune, serum, and antioxidant indices) in the selected studies were recorded in their respective sections. Then, the presence or absence of each index in each study was coded as 1 (present) or 0 (absent). Subsequently, in each study, the characteristics that demonstrated positive statistical changes were coded as 1, while those that remained unchanged were coded as 0 (Reverter *et al.*, 2021).

## **Results**

### *Plant use and reporting*

Following a comprehensive review of the literature, we identified and selected 51 articles published between 2013 and 2023. These studies investigated the *in vivo* effects of dietary Iranian medicinal plant supplements on growth, hemato-serological parameters, immune system response, antioxidant activity, and disease resistance in rainbow trout. Research on the *in vivo* effects of Iranian herbs supplementation in rainbow trout has significantly increased over the past decade, with more than half of the publications (over 60%) emerging in the last five years (2019 – 2023) (Fig. 1). The reviewed literature explored the effects of 38 Iranian herb species from 23 different families, with the most represented families being *Lamiaceae*, followed by *Apiaceae*, *Asphodelaceae* and *Asteraceae* (Fig. 2). Our findings indicate that over half (52%) of the studies utilized plants harvested from their natural habitats, while 30% sourced whole plants from local markets, and 18% obtained commercial preparations, including powders, extracts, and essential oils.

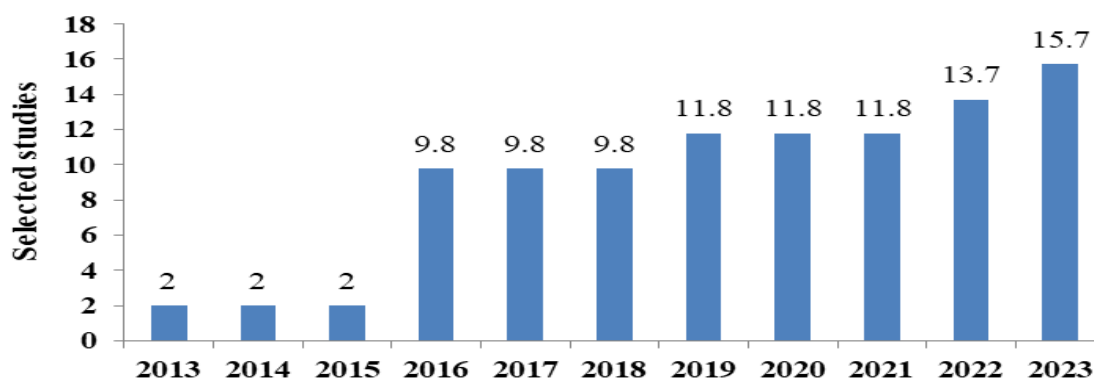


Figure 1: The percent of selected articles on Iranian herbs in the diets of rainbow trout by year.

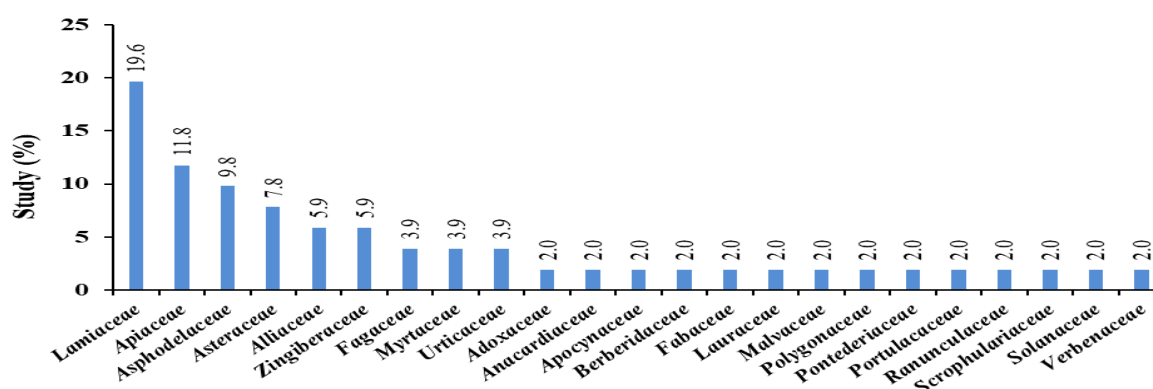


Figure 2: Percentage frequency of Iranian medicinal plant families included in the diet of rainbow trout.

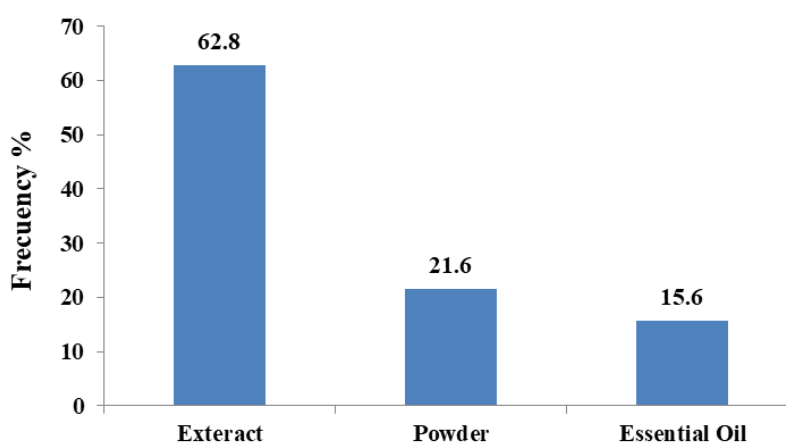
#### *Utilization of medicinal plants: Types, dosages, and duration*

The assessment of the studies indicated that over 62% utilized plant extracts (with solvents like ethanol, methanol, acetone, and water), followed by powdered plants at 19.6% and essential oils at 15.6% (Fig. 3). The percentage of different forms of plant ingredients included in the diet of rainbow trout ranged from 0.001 to 10%. The most frequently used doses of various forms of medicinal plants in the studies were: extract: 0.1 and 1%, powder: 1 and 2%, and essential oil: 0.02, 0.04, and 0.06%. In the evaluation of the literature, the duration of diets containing Iranian medicinal plants varied from a minimum of 21 days to a maximum of 70 days. The results revealed

that more than 60% of the studies were conducted over a 56-day period.

#### *Efficacy of Iranian plant-enriched diets on growth performance in rainbow trout*

After data screening, we identified six growth performance metrics: final weight (FW), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), condition factor (CF), and protein efficiency ratio (PER). The most frequently used indices were ranked as FW, SGR, FCR, WG, PER, and CF. 50-60% of studies showed significant positive effects on FW, SGR, FCR and WG respectively, while PER, and CF demonstrated less responsiveness (40% or less). (Fig. 4a).



**Figure 3: Percentage frequency of articles on each form of plant matter.**

#### *Efficacy of Iranian plant-enriched diets on hematology parameters in rainbow trout*

Researchers investigated various hematological indices, including total red blood cell (RBC) and white blood cell (WBC) counts, lymphocytes (Lym), neutrophils (Neu), monocytes (Mon), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Over 70% of studies measured WBC levels, more than 60% assessed RBC, Hb, and Hct, and over 45% evaluated Lym, Neu, and Mon percentages. Significant effects were noted for WBC and RBC (over 72%), Hct and Hb (over 59%), and Neu and Lym (over 40%), while MCH, MCV, and MCHC effects were minor, each under 20%. (Fig. 4b).

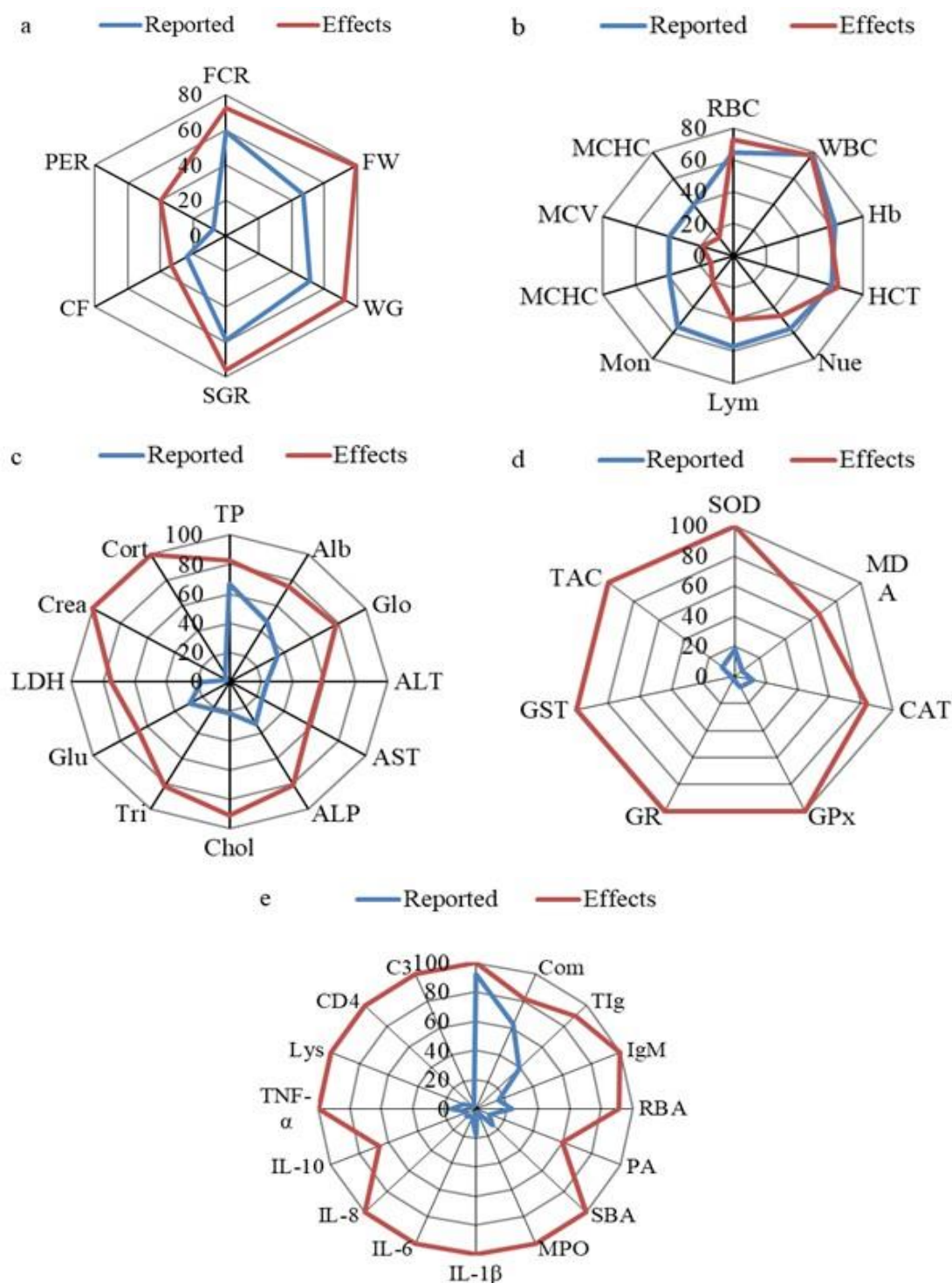
#### *Efficacy of Iranian plant-enriched diets on biochemical parameters in rainbow trout*

Key biochemical parameters identified include serum total protein (TP), globulin (Glo), albumin (Alb), glucose (Glu), cholesterol (Chol), triglycerides (Tri), creatinine (Crea), cortisol (Cort), alanine aminotransferase (ALT), aspartate

aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH). Analysis of the contributions of various biochemical indices indicated that TP (66.7%), Alb (47.1%), and Glo (35.3%) stand out as the most critical factors for assessment in these investigations. The most pronounced effects were on Chol, TP, Tri, and ALP (over 81%), with significant impacts also observed on Glo, Alb, LDH (over 74%), Glu (nearly 75%), ALT and AST (over 58%). While studies on Cort and Crea are limited, medicinal plants have shown nearly 100% beneficial effects on these parameters (Fig. 4c).

#### *Efficacy of diets on rainbow trout antioxidant parameters*

Results showed that antioxidant indices were less emphasized compared to others. However, diets enriched with herbs significantly improved these indices, with superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), and total antioxidant capacity (TAC) increasing by up to 100%. Meanwhile, the influence of malondialdehyde (MDA) and catalase was 66.7% and 83.3%, respectively (Fig. 4d).



**Figure 4:** The reported growth performance (a), hematological parameters (b), biochemical parameters (c), antioxidant indices (d) and immunological parameters (e) of rainbow trout, and their comparative responsiveness following the use of Iranian herbs.

#### *Efficacy of diets on rainbow trout immunological parameters*

The review identified key sero-immunological indices for assessing

rainbow trout, including lysozyme (Lys), complement (Com), total immunoglobulin (TIg), IgM levels, serum antibacterial activity (SBA), and myeloperoxidase



(MPO) levels. It also included respiratory burst activity (RBA), phagocytosis activity (PA), and the expression of various cytokine-associated genes (e.g., IL-1 $\beta$  GR, IL-6 GR, IL-8 GR, IL-10 GR, TNF- $\alpha$  GR, Lys GR, C3 GR, CD4 GR). Lyso (92.2%), Com (62.7%), TIg (39.2%), and RBA (23.5%) were identified as the most critical factors. IgM and SBA were also significant at 15.7%. Gene expressions varied from 15.7% to 2%. The analysis of data showed significant improvements in all studies, particularly in Lys, IgM, SBA, MPO, and interleukin gene expressions (except IL-10), with Com, RBA, and TIg improving in over 80% of studies (Fig. 4e). Regarding bacterial resistance, tree pathogens were tested: *Yersinia ruckeri* (31% of studies), *Streptococcus iniae* (13.7%), and *Aeromonas hydrophila* (4%). Fish receiving medicinal plants showed significantly higher survival rates than the control group across all studies.

## Discussion

Herbal medicines, traditionally used for health in humans and animals, are gaining popularity in fish aquaculture as a preferred alternative to antibiotics. They are effective, low in toxicity, have minimal side effects, target diverse pathways, and pose a lower risk of drug resistance. Recent reviews emphasize their role in enhancing growth, hemato-immunological parameters, and controlling fish diseases in aquaculture (Zhang *et al.*, 2022).

More than 50% of studies on Iranian herbs in rainbow trout diets were conducted in the last five years (2019-2023). Reverter *et al.* (2021) noted that from 2004 to 2019, 137 global articles examined the effects of

medicinal plants on fish growth, immunity, and disease resistance, with over half published between 2014 and 2019. This underscores the growing global interest in using medicinal plants in aquaculture.

Our review indicated that medicinal plants from families *Lamiaceae*, *Apiaceae*, *Asphodelaceae*, *Asteraceae* and *Alliaceae* were the most commonly used in the studies. This is probably linked to the abundance of plant species within these families, as studies have shown that native Iranian medicinal plants used in human medicine predominantly belong to four families: *Lamiaceae* (84 spp.), *Apiaceae* (37 spp.), *Asteraceae* (7 spp.), and *Alliaceae* (2 spp.) (Hassanpouraghdam *et al.*, 2022). It appears that the use of these plants in rainbow trout is directly related to the information available on their use in human consumption.

The results indicate that over half (52%) of the studies utilized plants harvested from their natural habitats. This finding highlights the value of local ethnobotanical knowledge and suggests that using medicinal plants aligns with an ecosystem-based approach to aquaculture (EAA), integrating aquaculture into its economic and biophysical contexts (Aubin *et al.*, 2019). Thirty percent of the plants were bought whole at local markets whereas 18% obtained commercial preparations. It appears that the lack of information on the effects of Iranian herbs on rainbow trout has led researchers to initially use cheaper sources of these plants to gather preliminary data. When results are validated and the research aims to evaluate more parameters, more expensive sources are utilized.



The findings show that over 62% of studies used plant extracts, while fewer than 22% utilized powdered plants. The efficacy of medicinal plants in fish is linked to bioactive compounds like polyphenols, polysaccharides, and flavonoids, which are more accessible in extract form (Zhu, 2020; Zhang *et al.*, 2022). Although powdered plants are cheaper, they often contain indigestible and antinutritional compounds that may reduce efficacy (Lech and Reigh, 2012). This may explain the preference for extracts over powders.

The dosages in the reviewed literature varied by material. Studies using powdered plant material had the highest dosages (1% and 2%), followed by extracts (0.5% and 1%) and the lowest for essential oils (0.02%, 0.04%, and 0.06%). Extraction increases the concentration of bioactive compounds compared to powder, especially with essential oils (Pogorzelska-Nowicka *et al.*, 2024). Thus, it is logical for dosages to vary based on the plant form used.

Treatment duration is crucial for efficacy and economic considerations. Over 60% of studies were conducted over 56 days, focusing on optimizing treatment duration for plant-enriched diets to enhance fish immunity and disease survival (Vazirzadeh *et al.*, 2017; Soltanian *et al.*, 2019; Rufchaei *et al.*, 2020; Ghiasi *et al.*, 2023). Increases in indices like WBC, lys, and TP were noted after 28 days in rainbow trout fed medicinal plants, while biochemical changes in AST, Chol, and growth performance were significant after 56 days. However, some studies found no significant differences in fish survival rates after being fed medicinal plants for 28 to 70

days (Mehrabi and Firouzbakhsh, 2020; Sarvi Moghanlou *et al.*, 2018; Zargar *et al.*, 2019; Zeilab Sendijani *et al.*, 2020; Hosseini Shekarabi *et al.*, 2021; Darvishi *et al.*, 2022). This suggests that the time needed for medicinal plants to enhance immunity is shorter than for other indices.

Our findings show that a herb-enriched diet enhances growth parameters in rainbow trout, notably in FW, SGR, WG, and FCR. Proposed mechanisms include improved diet palatability, increased digestive enzyme secretion, enhanced gut microbiota, and the presence of beneficial nutrients and bioactive compounds (Shalvei *et al.*, 2016; Mohamadi Saei *et al.*, 2016; Alishahi *et al.*, 2017; Mansouri Taei *et al.*, 2017; Saeidi asl *et al.*, 2017; Ramezanzadeh *et al.*, 2019; Naderi Farsani *et al.*, 2019; Zargar *et al.*, 2019; Adel *et al.*, 2020; Zeilab Sendijani *et al.*, 2020; Mohammadalikhani *et al.*, 2020; Firouzbakhsh *et al.*, 2021; Fattahi *et al.*, 2022; Hosseini Shekarabi *et al.*, 2022; Chekav *et al.*, 2023). In several studies, histological sections taken from the intestines of rainbow trout indicated that the use of medicinal plants resulted in an increase in the height of intestinal microvilli. The researchers concluded that this increase in microvilli height enhanced nutrient absorption, which subsequently improved growth indices. (Heidarieh *et al.*, 2013; Adineh *et al.*, 2020). Additionally, herbs have been shown to boost digestive enzyme levels, including amylase, protease, and lipase, which are key to improving growth parameters (Rashidian *et al.*, 2018; Rashidian *et al.*, 2020; Oroji *et al.*, 2021; Rashidian *et al.*, 2022).

Blood is a vital indicator of health and physiological condition in vertebrates, including fish, with oxygen-carrying capacity reflecting metabolism and overall health (Gabriel *et al.*, 2019). Diets enriched with herbs significantly impact blood parameters such as RBC, WBC, Hb, and Hct, attributed to vitamins (B, C, A, E) and minerals (iron, magnesium, calcium) present in these plants (Adel *et al.*, 2016; Alishahi *et al.*, 2017; Saeidi asl *et al.*, 2017; Ramezanzadeh *et al.*, 2019; Heydari *et al.*, 2020; Rashidian *et al.*, 2020; Mehrabi and Firouzbakhsh, 2020). Additionally, bioactive compounds like polyphenols, flavonoids, and saponins promote erythropoiesis and enhance blood cell longevity, further improving blood parameters and overall performance (Ramezanzadeh *et al.*, 2019; Gharaei *et al.*, 2020; Darvishi *et al.*, 2022; Ghafarifarsani *et al.*, 2021a, b).

Blood serum contains various elements important for assessing fish health. The biochemical parameters such as TP, consisting of Alb and Glo, indicate proper metabolic and immune function when elevated. Enzymes like AST, ALT, LDH, and ALP are indicators of liver health and metabolism (Manera, 2021). The results clearly demonstrate the beneficial effects of herbs on biochemical indices, including increased TP, Alb, and Glo, along with decreased ALT, AST, LDH, and ALP. The primary mechanism suggested for this outcome is the presence of antioxidant compounds, especially polyphenols and flavonoids (Mohamadi Saei *et al.*, 2016; Alishahi *et al.*, 2017; Mansouri Taei *et al.*, 2017; Adel *et al.*, 2020; Rashidian *et al.*, 2020; Gholamhosseini *et al.*, 2020;

Mohammadalikhani *et al.*, 2020; Firouzbakhsh *et al.*, 2021; Hosseini Shekarabi *et al.*, 2022; Rashidian *et al.*, 2022; Yousefi *et al.*, 2022; Chekav, *et al.*, 2023; Ghiasi *et al.*, 2023). Assessing Glu and Cort levels is crucial as they indicate fish stress. Variations in Chol and Tri reflect metabolic status and liver health (Manera, 2021). The study found that a diet enriched with medicinal plants significantly reduced Glu, Cort, Chol, and Tri. The reduction in Glu is due to decreased Cort (anti-stress) and increased cell wall permeability to insulin (Rashidian *et al.*, 2018; Fattahi *et al.*, 2022; Rashidian *et al.*, 2022). The reduction in Chol and Tri is due to bioactive compounds like carvacrol and thymol inhibiting fat synthesis enzymes (Saeidi Asl *et al.*, 2017; Ghiasi *et al.*, 2023).

Antioxidant enzymes such as SOD, GST, GPx, and CAT play a crucial role in scavenging reactive oxygen species (ROS) in fish. These enzymes protect body tissues from oxidative stress damage caused by infectious agents and the immune system (Ameur *et al.*, 2012). Although antioxidant enzyme levels have been evaluated in less than 20% of studies compared to other indicators, their impact has been observed to range from 66% to 100% (Soltanian *et al.*, 2019; Rufchaei *et al.*, 2020; Adineh *et al.*, 2020; Hoseinifar *et al.*, 2020; Gholamhosseini *et al.*, 2020; Taheri Mirghaed *et al.*, 2020; Yousefi *et al.*, 2022). Research by Charde *et al.* (2011) has demonstrated that flavonoids and terpenoids are vital antioxidant compounds in plants, significantly boosting antioxidant enzymes to combat free radicals.

The immune system in bony fish, similar to higher vertebrates, includes innate and

adaptive immunity. Early on, they rely on non-specific immunity, but as they grow, adaptive immunity becomes important, though less efficient than in mammals. Thus, bony fish mainly depend on innate immunity (Sahoo *et al.*, 2021). Key non-specific immune system mechanisms include physical barriers (scales, mucous, epithelial cells), cellular components (neutrophils, macrophages, lymphocytes), and humoral factors (transferrin, lectins, lysozyme, antimicrobial peptides, cytokines, natural antibodies, complement, reactive oxygen species) (Mokhtar *et al.*, 2023). The study revealed that the consumption of Iranian herbs led to significant improvements in most immune indices in rainbow trout. Moreover, these indices exhibited changes more rapidly compared to other parameters. The key immune indices assessed, which demonstrated significant responsiveness, included the activities of Lys, Com and RBA (Adel *et al.*, 2016; Alishahi *et al.*, 2017; Haghighi *et al.*, 2017; Adel *et al.*, 2020; Mehrabi and Firouzbakhsh, 2020; Hoseinifar *et al.*, 2020; Chekav, *et al.*, 2023; Fattahi *et al.*, 2022; Firouzbakhsh *et al.*, 2021; Hosseini Shekarabi *et al.*, 2021; Gholamhosseini *et al.*, 2020; Hosseini Shekarabi *et al.*, 2022; Pourmoghimi *et al.*, 2015; Naderi Farsani *et al.*, 2019; Oroji *et al.*, 2021; Ghiasi *et al.*, 2023). While the precise mechanism by which medicinal plants enhance Lys activity remains unclear, the presence of certain organosulfur compounds (such as allicin) is believed to play a significant role due to their potent antimicrobial properties (Hosseini Shekarabi *et al.*, 2022). Moreover, the presence of alkaloid in grass

carp and blunt-nosed fish has significantly increased the levels of C3 and C4 (Zhang *et al.*, 2022). Bony fish have three immunoglobulin isotypes: IgM, IgD, and IgT. Exposure to antigens or immune stimulants activates T cells and stimulates B cells, increasing their serum levels (Srivastava and Pandey, 2015). WBC are the sole producers of immunoglobulins. The observed increase in WBC with the administration of Iranian herbs suggests that this rise is the primary reason for the elevated immunoglobulin levels. Additionally, the consumption of these plants enhances the gene expression of various cytokines, Lys and IgM, further contributing to the increase. According to the results, medicinal plants enhanced the gene expression of various cytokines, Lys and IgM, with the exception of IL-10 (Hoseinifar *et al.*, 2020; Hosseini Shekarabi *et al.*, 2021; Heydari *et al.*, 2020; Taheri Mirghaied *et al.*, 2020; Gharaei *et al.*, 2020; Vazirzadeh *et al.*, 2017; Yousefi *et al.*, 2022). While the exact mechanisms are not fully understood, it appears that the active compounds in these plants enhance the expression of cytokine and Lys genes. This enhancement boosts the innate immune activities, increasing the resistance of rainbow trout to infectious agents and making them more resilient to pathogenic bacteria. Research findings indicate that Iranian medicinal plants significantly enhance the resistance of rainbow trout against pathogenic bacteria such as *Y. ruckeri* (Adel *et al.*, 2016; Saeidi asl *et al.*, 2017; Adel *et al.*, 2020; Soltanian *et al.*, 2019; Naderi Farsani *et al.*, 2019; Firouzbakhsh *et al.*, 2021; Oroji *et al.*, 2021; Chekav *et al.*, 2023; Ghiasi *et al.*,

2023), *S. iniae* (Rashidian *et al.*, 2020; Hosseini Shekarabi *et al.*, 2021; Rashidian *et al.*, 2022) and *A. hydrophila* (Zargar *et al.*, 2019).

## Conclusions

Interest in using plants as growth and immunity-boosting feed supplements has surged in research related to the rainbow trout farming industry in Iran over the past decade. This trend is expected to continue due to challenges like antibiotic resistance in fish pathogens, residues in meat, and their adverse effects on public health. Since these plants are collected from their natural habitats and align with rainbow trout farming centers, they can benefit local farmers by increasing income, reducing reliance on external products, and preserving traditional knowledge. However, sustainability studies for repeated collection and industrial-scale production are needed. Plant-enriched diets, irrespective of dose, duration, and plant type, significantly enhanced growth performance, blood, serum, and immunity parameters, as well as antioxidant activity and resistance to pathogenic bacteria in rainbow trout. The results indicated that using Iranian medicinal plants can enhance immunity indices within 28 days, eliminating the need for prolonged use to reduce costs. Since all forms used (extract, powder, and essence) have proven effective, extracts should be given priority. This is due to their ease of use, the presence of suitable active compounds, low dosage requirements, and the simplicity of the technology involved. To improve the comparability and reproducibility of various experiments, studies should

provide more comprehensive details about the plant materials used. Additionally, there is a need for increased applied research and better structuring to translate basic research findings, such as those from laboratory experiments, into practical applications in rainbow trout farms. These farms often face more variable conditions and unique challenges.

## Conflicts of interest

All authors declare that they have no conflict of interest.

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