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Oral administration of terpenoids and phenol fraction of *Padina gymnospora* stimulates the nonspecific immune response and expression of immune genes, and protects the common carp (*Cyprinus carpio*) from experimental *Aeromonas hydrophila* infection

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Highlights

- Terpenoids & phenol fraction boost carp's immune response - oral administration study.
- *Padina gymnospora* extract protects carp from Aeromonas hydrophila infection.
- Immune gene expression upregulated by terpenoids & phenol fraction in carp.
- Potential immunomodulatory agent for aquaculture: *Padina gymnospora* extract.

Abstract

Common carp (*Cyprinus carpio*), a valuable aquaculture species susceptible to various infections, requires effective immune enhancement strategies. This study investigates the immunomodulatory effects of orally administered terpenoids and phenol fraction (TPF) from Padina *gymnospora* in *C. carpio*, focusing on stimulation of nonspecific immune response, immune gene expression, and protection against experimental infection. *P. gymnospora* is a brown seaweed species known for its bioactive compounds and medicinal properties. TPF was extracted using the Harborne fractionation method, and the presence of terpenoids and phenol compounds was confirmed by qualitative analysis and high-performance thin layer chromatography (HPTLC). TPF was administered orally in different doses to carp. Nonspecific immune responses were evaluated by measuring cellular ROS, RNI, and peroxidase production. The expression of immune genes (lysozyme and interleukin-1β) was assessed by reverse transcriptase PCR. Furthermore, the protective efficacy of TPF was determined by infecting carp with a virulent pathogen, Aeromonas hydrophila, and monitoring mortality rates and disease symptoms. The results demonstrate that oral TPF administration significantly enhances nonspecific immune responses, with increased ROS, RNI, and peroxidase production, indicating improved immune function.

Expression levels of lysozyme and interleukin-1β were upregulated, suggesting immune system activation. Moreover, TPF exhibited significant protection against experimental infection, with lower mortality rates compared to the control group. These findings highlight TPF's potential as an effective immunostimulatory agent, enhancing immune responses and providing infection protection in carp. In conclusion, oral TPF administration stimulates nonspecific immune responses, modulates immune gene expression, and confers protection against experimental infection in carp, displaying its potential for enhancing immune responses and disease resistance in aquaculture species, and contributing to sustainable fish health management.

Introduction

The production of common carp through aquaculture plays a significant role in meeting the growing demand for fish protein worldwide. It provides a valuable source of nutritious food, contributes to rural livelihoods and economic development, and reduces pressure on wild fish stocks. Common carp aquaculture offers several advantages, including high growth potential, efficient feed conversion, and adaptability to a wide range of environmental conditions. It is a popular species due to its omnivorous feeding habit, which allows it to utilize both natural food sources and formulated feeds, reducing production costs. Overall, aquaculture carp contributes to 18% of total fish produced [1] of which common carp amounts for 9% of total finfish production [2]. Ongoing research and technological advancements in common carp aquaculture aim to further enhance production efficiency, disease resistance, and environmental sustainability, ensuring a sustainable and reliable source of fish for both domestic consumption and international markets [3].

Padina gymnospora is a brown seaweed species that holds significant interest due to its diverse bioactive compounds and medicinal properties. It is a Phaeophycean macroalga and is commonly found in tropical and subtropical coastal regions, particularly in the Indo-Pacific region. This macroalgal species exhibits a distinctive morphology with flattened thalli and a fan-like appearance, making it easily recognizable. *P. gymnospora* has gained attention in scientific research and traditional medicine practices due to its rich content of bioactive compounds, including terpenoids, phenols, polysaccharides, sterols, and flavonoids [4]. These compounds possess various biological activities such as antioxidant, antimicrobial [5], antitumor [6], and immunostimulatory properties [7]. As a result, *P. gymnospora* has been extensively studied for its potential therapeutic applications in treating ailments and promoting human health [8].

Notably, research on isolated compounds from this bioactive seaweed is limited but diverse in its findings with crude extracts/fractions. Conventionally, like other seaweeds, *P. gymnospora* is considered a rich source of essential micronutrients including iron, calcium, and iodine [9]. Nowadays, it is garnering more attention as an alternate source for bioactive compounds. Previously, we demonstrated its polysaccharide fraction as a potent immunostimulant in carp, offering protection against experimental infection [7]. Lipids from *P. gymnospora* displayed strong antioxidant properties [10]. The use of a hot water decoction, akin to making tea and rich in xanthophylls, including β-carotene, demonstrated antioxidant, anticancer, and anti-hyaluronidase properties [11]. Preliminary findings from one study revealed that crude extracts containing terpenoids from *P. gymnospora* inhibited the digestive enzyme α-glucosidase [12], while extracts with flavonoids and cardiac glycosides were found to inhibit acetylcholinesterase and butyrylcholinesterase enzymes in a separate study [13]. Numerous studies have demonstrated the anti-cancer activity, both in vitro and in vivo, of various extracts from *P. gymnospora*, each containing a diverse range of bioactive compounds.

The present study was aimed to investigate the immunostimulatory properties of the terpenoid and phenolic fraction of *P. gymnospora* (TPF) by orally administering it to carp, *Cyprinus carpio*. The extent of immunostimulatory potential of TPF was evaluated through nonspecific cellular assays, analysis of immune gene expression, and assessment of protection against experimental *Aeromonas hydrophila* infection. By exploring the immunomodulatory potential of TPF, its effects on the immune response of carp and its potential as an immunostimulant were assessed.

Section snippets

Fish and maintenance

A total of 1000 *Cyprinus carpio* individuals weighing 100±5g was obtained from a local fish farmer in Madurai, India. The fish were acclimated in large 500-litre fibre reinforced plastic (FRP) tanks for a period of 2–3 weeks. During acclimation, optimal fish rearing conditions

were maintained, including appropriate stock density (4g/L), feeding regime (two times daily at ad libitum), and water quality parameters such as pH (7.3±0.3), dissolved oxygen (DO) levels (3.2–3.5mg/L), and water…

Qualitative analysis and HPTLC

The qualitative analysis of phytochemical constituents in *P. gymnospora* fractions confirmed the presence of terpenoids through the Salkowski's test and phenolic compounds through the $FeCl_3$ test, as evidenced by the respective colour changes. The TLC plates of TPF revealed distinct bands when examined under UV light at both 254nm and 366nm. A detailed examination at 254nm showed the presence of four well-defined peaks, each corresponding to terpenoids, and these peaks were consistently…

Discussion

Many compounds extracted from seaweeds especially the terpenoids and phenolics have biological and medicinal applications [[21], [22], [23]]. In this study, TPF was extracted using the Harborne fractionation method, and the presence of terpenoids and phenol compounds was confirmed through qualitative analysis and HPTLC. The study aimed to evaluate the effects of TPF on the immune system by administering different doses orally to carp.

The assessment of nonspecific immune responses included…

Conclusion

In conclusion, this study demonstrates that oral administration of TPF significantly enhances the nonspecific immune responses, modulates immune gene expression, and provides protection against experimental infection in common carp. The use of TPF, extracted using the Harborne fractionation method, leads to an increase in leukocyte count, specifically in granulocytes and monocytes, indicating enhanced immune function. The TPF, effectively stimulates the production of ROS and peroxidase,…

Declaration of competing interest

The authors declare that they have no competing interests.…

CRediT authorship contribution statement

Sekaran Kalaivani Priyadarshini: Investigation, Writing – original draft. **Monica Murugesan:** Investigation. **R. Dinakaran Michael:** Conceptualization, Funding acquisition, Resources, Supervision. **Parasuraman Aiya Subramani:** Data curation, Software, Visualization, Writing – original draft, Writing – review & editing. **Priyatharsini Rajendran:** Conceptualization, Investigation, Writing – original draft, Supervision.…

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT to improve language and readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the publication.…

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