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Research Article

Terpenes and terpenoids: Innovative approaches in aquaculture health management

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Abstract

Aquaculture plays a key role in providing nutrition and livelihood security. Rapid intensification has been adopted in aquaculture to increase fish production, often leading to a stressful environment resulting in poor growth, compromised immune functions, and increased susceptibility to infections in fish. The use of synthetic chemicals, hormones, and antibiotics for growth promotion and disease control has resulted in the development of antimicrobial resistance and raised concerns about public health. There is a critical need to explore environmentally friendly alternatives for sustainable aquaculture. Plants contain a wide range of chemical substances and have shown their beneficial effects on fish growth, immunity, and health. Terpenes and terpenoids are among such natural products with a wide array of antimicrobial, growth-promoting, antioxidant, immune-stimulating, and anti-inflammatory effects in human and higher vertebrates. Terpenes are a broad class of natural secondary metabolites found in plants that have the potential to enhance immune systems, combat microbes, and strengthen antioxidant defences, thereby promoting aquatic animal health. While studies have explored the positive effects of compounds such as terpenes, phenolic compounds, saponins, and alkaloids in aquaculture, the utilization of terpenes and their derivatives remains underexplored. This review seeks to consolidate and emphasize the advantageous effects of terpenes and their derivatives on fish health, potentially expanding their use in aquaculture.

Introduction

Aquaculture currently accounts for 57% of global aquatic animal production intended for human consumption (FAO, 2024). The Asian aquaculture sector has grown, diversified, and performed commendably during the last few decades. Chemicals are essential components for successful farming and have been used in several sectors for decades (Faruk *et al.,* 2008). Aquatic drugs are required for the various phases of aquaculture viz., maintaining the health of aquatic animals, preparing ponds, maintaining water and soil quality, formulation of feed, enhancing natural productivity, manipulating fish reproduction, transporting live fish, promoting growth, processing and increasing the final product value (GESAMP, 1997; Sepehrfar *et al.,* 2023). Chemotherapeutic agents, like antibiotics and disinfectants have been used to maintain fish health, control diseases, and increase production for the last few decades. Sodium chloride, potassium permanganate, formalin, malachite green, methylene blue, glutaraldehyde, and hydrogen peroxide are frequently used for the prevention of fish disease (Sumon *et al.,* 2020). The effectiveness of these chemicals varies and can be hazardous to the aquatic environment, fish, and humans (Ogawa, 2015; Morales-Serna *et al.,* 2019). The usage of these chemotherapeutic agents leads to the development of antimicrobial resistance in fish. Furthermore, antibiotics have been used for more than 50 years in aquaculture to treat the bacterial infections in fish (Shamsuzzaman and Biswas, 2012). Recent studies have shown the consequences of using antibiotics and the development of antimicrobial resistance (AMR) in aquaculture (Rahman *et al.,* 2009). Increase in AMR not only aids in the development of novel resistance mechanisms but may have a deleterious effect on the efficacy of current antibiotics, resulting in therapeutic failure. Consequently, it's crucial to develop new practical approaches based on naturally occurring substances such as phytochemicals and essential oils (EOs) that may prevent the formation of AMR and serve as eco-friendly alternatives (Moo *et al.,* 2019). EOs are hydrophobic concentrated liquids synthesized from aromatic plants, having a strong odour and substantial antimicrobial properties. Some plants produce these oils naturally as secondary metabolites, which attract pollinators or serve for the prevention of pathogens or predators. They contain different volatile molecules such as terpenes and terpenoids, phenols and aliphatic compounds. They are used for sedative, antimicrobial, antioxidant, analgesic, spasmolytic properties, and also for local anaesthetic remedies. Terpenes are one of the largest classes of EOs that have an enormous structural diversity, which ensures a wide variety of biological properties, having anti-parasitic activity (Mafud *et al.,* 2016). Nevertheless, the reports on the evaluation of antiparasitic efficacies of terpenes on fish parasites are scanty. Terpenoids, the derivatives of terpenes, also represent a large group of phytochemicals that have a promising antimicrobial activity (Barbieri *et al.,* 2017).

Use of such natural alternatives to treat microbial diseases in aquaculture is crucial

(Adel *et al.,* 2020). Terpenes and terpenoids, known for their antioxidant and antimicrobial properties, represent promising therapeutic sources in fish farming. However, existing literature on this subject is limited. While various phytocompounds have been documented, terpenes and terpenoids are often only mentioned as a subset among them. Moreover, earlier studies lack conclusive evidence regarding the bioavailability and efficacy of these compounds in combating fish diseases, thus hindering their exclusive use as substitutes for chemotherapeutics. This paper aims to offer a comprehensive review of terpenes and terpenoids as cutting-edge alternatives to conventional chemical therapies in the aquaculture sector, with the goal of advancing their adoption for fostering sustainable and eco-conscious disease management practices in aquaculture.

Current trends in aquatic animal health management

Aquaculture provides the basic source of livelihood, trade and recreation for people, thus contributing significantly to the global economy (FAO, 2016, 2022). Artificial stressful conditions in intensive aquaculture systems impart immense stress making farmed fish more susceptible to diseases than wild (Hoque *et al.,* 2022). Despite various advanced therapies, fish diseases continue to have a great economic impact on commercial aquaculture. Strict adherence to biosecurity measures, scientific environment management along with eco-friendly treatment of disease are the top priorities for sustainable aquaculture. Infectious diseases result in high mortality and are a major concern in fish farming (Hasan *et al.,* 2013). Bacterial infections in fish are augmented by the wide fluctuation in physical and chemical parameters such as increased turbidity, temperature, salinity, pH, water conductivity and low dissolved oxygen of water (FAO, 2018). Early detection of bacterial pathogens from fish is important for effective disease control in intensive aquaculture conditions. Surveillance and monitoring of fish populations play a major role. Detection and characterization of the etiological agent during surveillance using biotechnological based tools in combination with other conventional methods are employed to assess the cause infection due to microbial strain diversity (OIE, 2018). Rapid diagnostic methods have been developed for the detection of pathogens in aquatic animals employing different immunological, molecular and biotechnological advancements. Immuno chromatography-based rapid kits, multiplex testing and micro-array technology are creating a new avenue in aquatic animal health management. Serology and immunodiagnostics methods such as agglutination (slide/latex), fluorescent antibody test (FAT/IFAT), immunohistochemistry (IHC), enzyme-linked immunosorbent assay (ELISA), and blot (dot-blot/dip-stick/western blot) are alternative approaches for the detection of fish and shellfish pathogens and epidemiological studies. Molecular techniques are also used for the identification and confirmation of pathogens, particularly those that are difficult to culture.

Advancements in biotechnology research have significantly reduced disease risk and new methodologies are contributing to effective fish disease management (Fig. 1). Though vaccination is efficacious in reducing the risk of bacterial and viral disease in fish, biological, scientific and technical restrictions prevent the fabrication and commercialization of vaccines for economically important fish diseases. Novel vaccine identification methods for different fish diseases are in progress using genomics, proteomics, knockout technologies and epitope mapping. Subunit or recombinant DNA vaccines have targeted specific pathogen components containing novel antigens produced using various expression systems (Cimica and Galarza, 2017). Besides vaccination, the role of improved nutrition, quality, and disease-resistance seed, good aquatic environment, judicial use of probiotics and immunostimulants with rapid detection of pathogens is critical in aquatic animal health management. The development of rapid and reliable testing methods has also made a substantial impact in reducing disease risk in recent years.

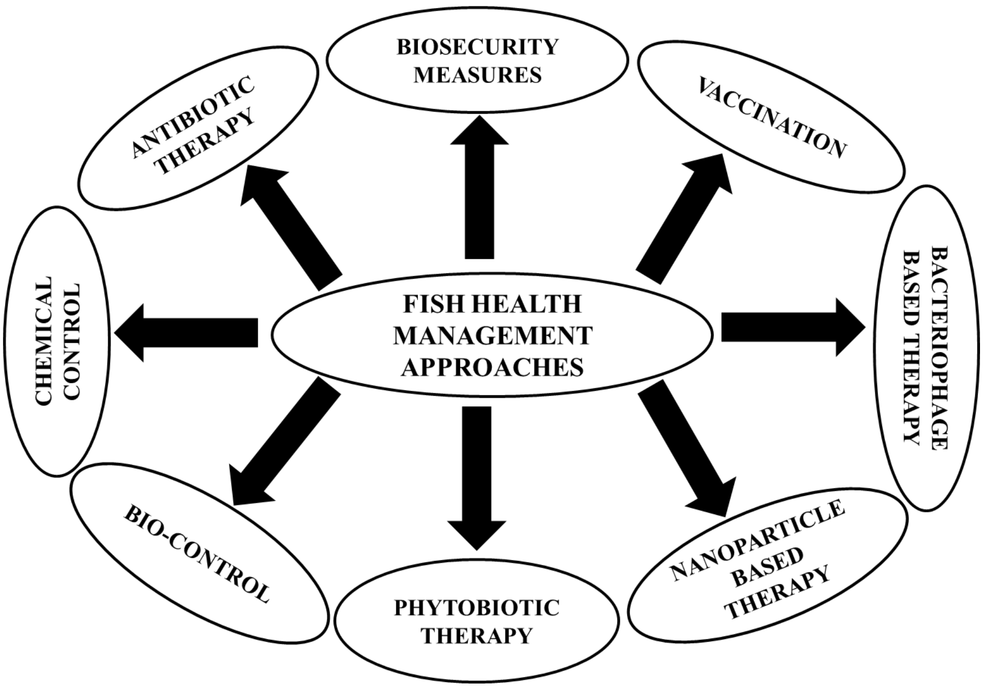

Figure 1: Recent approaches for fish health management.

Moreover, a number of biosecurity measures and better management practices (BMPs) have been adopted in aquaculture to exclude trans boundary introduction and spread of pathogens. Horizontal transmissions of pathogens are also prevented by strictly adhering to quarantine processes, introduction of specific pathogen-free (SPF) and specific pathogen-resistant (SPR) stock in culture practice. Probiotic microorganisms such as *Lactobacillus* spp., *Streptococcus* spp., *Bacillus* spp. etc. are also used in aquaculture to improve *digestive processes and immunity of aquatic animals, in addition to their anti-microbial* and anti-mutagenic activity against pathogens. Nowadays, Immunostimulants such as β-glucan, chitin, lipopolysaccharide, peptidoglycan, lactoferrin, levamisole are applied as dietary additives to boost the non-specific immune response of aquatic animals and increase resistance to specific pathogens as well.

Advancements in aquaculture therapeutics: Novel approaches to disease prevention and control

Disease outbreaks in aquaculture have increased the application of a variety of chemotherapeutants to control production loss. Chemotheraputants play a significant role in the maintenance of fish health and are also utilised in a number of other aquaculture activities, including water/soil treatment, disinfectants, piscicides, herbicides, organic fertilizers, inorganic fertilizers, feed additives and anesthetics. A detail overview on the usage of chemotherapeutants in the freshwater aquaculture sector is presented in Table 1 (Mohamed *et al.,* 2000).

Table 1: Chemotherapeutic Compounds used in Aquaculture.

Sl no.	Chemotherapeutic compound	Mode of application	Mechanism of action	Dosage
	Antibiotics			
A.	1. Oxytetracycline	Feed supplementation	Bacterial diseases	3-5 g/kg feed
	2. Chloramine-T	Immersion treatment	Bacterial gill disease, columnaris disease associated with *Flavobacterium columnare*	5 mg/L
	3. Erythromycin	Bath treatment	Bacterial diseases	4 mg/L
	4. Streptomycin	Long bath treatment	Bacterial diseases	4 mg/L
	5. Oxolinic acid	Feed supplementation	Bacterial diseases	3-5 g/kg feed
	6. Enrofloxacin	Bath treatment	Antibacterial agent	5-10 mg/L
	7. Neomycin	Bath treatment	Bacterial diseases	4 mg/L
	8. Florfenicol	Feed supplementation	Enteric septicemia of catfish	5 mg/L
	9. Nitrofurans	Feed supplementation	Bacterial diseases	3-5 g/kg feed
	10. Sulphadiazine	Water dispersible powder	Bacterial/fungal diseases	5 g/ 100 kg fish
	Disinfectants			
B.	1. Sodium chloride/ Malathion	Feed supplementation	*Epistylis* spp., *Zoothamnium* spp. *Dactylogyrus* spp. *Gyrodactylus* spp., *Argulus* spp., *Lernaea* spp., *Ergasilus* spp.	0.2 mg/L (Sodium chloride) 0.15-0.25 mg/L (Malathion)
	2. BKC	Bath treatment	Cleaning and disinfecting agent	2 mg/L
	3. Copper sulfate	Immersion treatment	Filamentous bacterial disease *Saprolegnia* spp., *Branchiomyces*	0.2-0.5 mg/L
	4. Sodium benzoate	Feed supplementation	Prevents growth of microbes and fungi	0.1-0.25% of feed
	5. Formalin	Applied into ponds	Protozoan parasite, Fungi of the Family Saprolegniaceae	15-25 mg/L
	6. Methylene blue	Bath treatment	Columnaris disease associated with *Flavobacterium*	1-2 mg/L

Table 1 continued:

Sl no.	Chemotherapeutic compound	Mode of application	Mechanism of action	Dosage
	7. Hydrogen peroxide	Bath treatment	control of mortality in freshwater-reared finfish eggs due to saprolegniasis, bacterial gill disease associated with *Flavobacterium branchiophilum*	50-75 mg/L
	8. Malachite green oxalate	Immersion treatment	Effective topical fungicide	0.15 mg/L
	9. Potassium permanganate	Long bath treatment	External bacterial and fungal infections	2 mg/L
	10. Acriflavin	Long bath treatment	Egg disinfection, ulcers, bacterial lesions and protozoan and monogenean infections	10 mg/L
C.	**Piscicide**			
	1. Saponin	Spreading in pond water	Kill predator fish, protozoan infections	20-30 mg/L, 5-25 mg/L
	2. Rotenone	Spreading in pond water	Kill predator fish	5-10 mg/L
	3. Organophosphate	Spreading in pond water	Infections by crustaceans, and monogeneans and ciliates	0.5-1 mg/L for 3 to 7 days
D.	**Culture system preparation**			
	1. Calcium hypochlorite	Spreading in pond bottom	Disinfect tanks and equipment, bactericidal effect	0.1-0.25 mg/L
	2. Lime	Spreading	Regulate phytoplankton abundance and pH	100-300 kg/ha
	3. Urea	Spreading	Fertilizer and a source of nitrogen	50-70 kg/ha
	4. Zeolite	Spreading	Improve pond bottom condition	300-500 kg/ha
	5. Calcium chloride	Spreading	Increase water hardness	500 kg/ha

Chemotherapeutic agents are most often used to treat diseases like white spot, velvet disease, fin and tail rot, crustacean and monogenean infections, fungal infections, and dropsy in finfish. Antibiotics like chloramphenicol, erythromycin, oxytetracycline, and nitrofurans, along with chemicals such as formalin, malachite green, potassium permanganate, copper sulphate, and trichlorphon, are among the chemical substances commonly used in aquaculture. Inappropriate use of this therapeutic may result in host mortality, morphological abnormalities, the emergence of resistant bacterial strains, and risks to public health. The extensive use of antibiotics in aquaculture can contribute to the development of antimicrobial-resistant pathogenic bacteria both inside and outside the aquaculture facilities. Additionally, several antibiotics are moderately to heavily harmful to non-target bacteria and primary producers, affecting the aquatic habitats that receive aquaculture effluents (Andrieu *et al.,* 2015).

However, aquaculture industry has now realised that use of chemotherapeutics can result in environmental degradation, contributing to the development of drug-resistance pathogen. The quest for safer alternatives to disease prevention and antibiotics replacement has been a serious concern now-a-day. The use of medicinal plants and their by-products is growing in global aquaculture due to their biodegradable nature, wide availability, and simple cultivation method without being accumulated in animal tissues as residue. Terpenes and their derivatives occurring naturally in plants can serve as a promising alternative technique to reduce fish disease incidences helping fish to fight against pathogenic microorganisms. Research needed to be focused towards developing and bringing such environment friendly phytocompound alternative for the treatment of microbial diseases in aquaculture and associated production loss.

An insight into available derivatives of terpenes and terpenoids

Terpenes are frequently more biodegradable than synthesized compounds; hence, their use has positive economic and environmental effects (Thapa *et al.,* 2015). Terpenes and their derivatives are secondary plant metabolites with antibacterial properties against susceptible and resistant pathogens that are often present in EOs (Ruzicka, 1953). They are large hydrocarbon groups that are extremely nonpolar and are not soluble in water. Their structures follow the biogenetic isoprene rule (C5 rule) (Kim *et al.,* 2014). Terpenes are synthesized by two distinct metabolic pathways, such as the mevalonate pathway (MVA) and methylerythritol phosphate (MEP). Except for certain bacteria and terrestrial plants, these two routes are mutually incompatible in several species (Swamy *et al.,* 2016). Most archaea and eukaryotes use MVA pathway, whereas bacteria typically have MEP pathway. Terpenoids have terpene analogues with comparable physical characteristics; however, they are often more polar and less volatile. The glycosides are highly polar terpene derivatives and water-soluble solids that are connected to sugars. Some of the best analysed terpenoids are geraniol, linalyl acetrate, carvacrol, thymol, menthol and linalool (Fig. 2). The functional group of terpenoids determines their antibacterial activity.

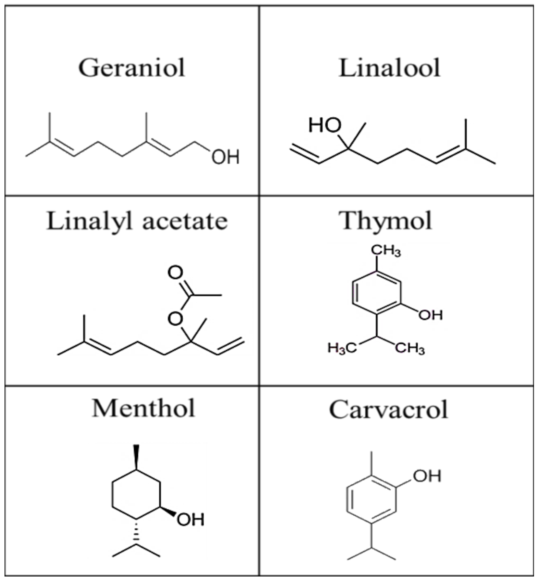

Figure 2: The chemical structure of terpenoids having antibacterial activity.

Terpenes can be categorised based on the number of isoprene units present in the structure; a prefix in the name indicates how many isoprene pairs were required to build the molecule. The monoterpenes and sesquiterpenes are the terpenes that are most commonly observed and differ in the quantity of isoprene units; nevertheless, lengthy chains like diterpenes and triterpenes also occur (Nazzaro *et al.,* 2013) (Fig. 3). Terpenes typically have 2, 3, 4, or 6 isoprene units; tetraterpenes, which have 8 isoprene units, belong to a different class of substances known as carotenoids.

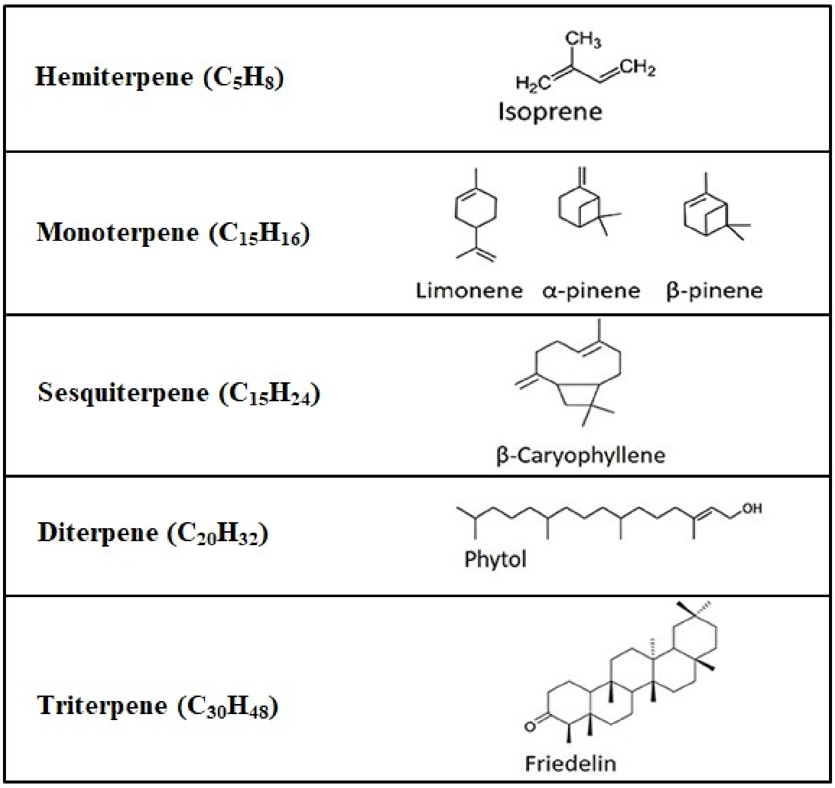

Figure 3: Structures of terpenes and derivatives.

Monoterpenes ($C_{10}H_{16}$), which exist in plants, contain two isoprene units. Monoterpenes and monoterpenoids include geraniol, carvacrol, thymol, menthol, terpineol (found in lilacs), limonene (found in citrus fruits), myrcene (found in hops), linalool (found in lavender), hinokitiol (found in cypress trees) or pinene (found in pine trees) (Lorenzi *et al.,* 2009). Against Gram-positive and Gram-negative bacteria, carvacrol, thymol, menthol, and geraniol were effective terpenes. Geraniol effectively reduces the vulnerability of Gram-negative, multi-drug resistant (MDR) *Enterobacter aerogenes, Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* by acting as a strong efflux pump inhibitor (Dorman and Deans, 2000). Carvacrol, eugenol, and thymol have a significant level of antibacterial activity (Knowles, 2005). Moreover, *Salmonella typhimurium* and *Staphylococcus aureus* cannot form biofilms while exposed to carvacrol (Koo *et al.,* 2002).

Sesquiterpenes ($C_{15}H_{24}$) consist of three isoprene units, including humulene, farnesenes, farnesol, and geosmin. Farnesol has a moderating impact on the development of biofilms from *Streptococcus mutans* and *Streptococcus sobrinus* (Gomes *et al.,* 2009). It also revealed antimicrobial effect against *S. aureus* and *S. epidermidis*, preventing the formation of biofilms (Rukayadi and Hwang, 2006). The capacity of *Staphylococcus mutans* to attach to cells was reported to be reduced by 60% by a phenol sesquiterpene called xanthorrhizol (Gonçalves *et al.,* 2011). Sesquiterpenes added to antibiotic discs led to a noticeably larger inhibitory zone (Reyes-Zurita *et al.,* 2009).

Diterpenes ($C_{20}H_{32}$) are composed of four isoprene units and form the basis for biologically important compounds such as retinol, retinal, and phytol. Cafestol, kahweol, cembrene and taxadiene (precursor of taxol) are the common diterpenes and diterpenoids. Salvipisone and aethiopinone, the two diterpenoids isolated from the roots of *Salvia sclarea* have antibacterial and antibiofilm properties towards *S. aureus*, *Enterococcus faecalis* and *S. epidermidis*. Diterpenes with antibiotics are widely used in combination therapy.

Triterpenes ($C_{30}H_{48}$) and triterpenoids containing six isoprene units are found in a wide variety of plants that are utilised in traditional medicine (Ludwiczuk *et al.,* 2017). The primary component of shark liver oil is the linear triterpene squalene, which undergoes biosynthetic process to produce lanosterol or cycloartenol, the structural components of the steroids (Alamgir, 2018). Oleanic acid (OA), bonianic acid A, bonianic acid B, ergosterol peroxide, ursolic acid (UA), maslinic acid, amyrin, betulinic acid and betulinaldehyde are the mostly described triterpenes and triterpenoids. Oleanic acid (OA) has a high antipathogenic activity against pathogens such as *Mycobacterium tuberculosis* (Jiménez-Arellanes *et al.,* 2013). Bonianic acid A and B isolated from *Radermachera boniana* have shown activity against *M. tuberculosis* (Cunha *et al.,* 2010). Ergosterol peroxide and ursolic acid also exhibited synergistic action against *M. tuberculosis*. Oleanic acid and ursolic acid extracted from *Miconia ligustroides* have shown antibacterial properties against *Bacillus cereus, Vibrio cholerae, S. choleraesuis, K. pneumoniae* and *S. pneumoniae*. Maslinic acid, a triterpenoid molecule found in fruit and leaves of *Olea europaea*, when supplemented to the regular diet of trout can function as a growth factor and have a significant anabolic impact on protein metabolism of fish (Fernández-Navarro *et al.,* 2008). Rainbow trout, *Oncorhynchus mykiss* fed with maslinic acid supplementation exhibited a greater protein-accumulation rate and weight of white muscle. The total DNA, RNA, and protein was also reported to be greater than the control.

Terpenes and derivatives from diverse origins

Terpene, a natural substance found in plants and animals, possess various therapeutic benefits. It mediates antagonistic and efficient interactions inside the body. Microbes, animals, and plants can be protected from abiotic and biotic stressors, diseases and predators using terpenes. It comes in a variety of forms and types. Many species use terpenes for similar purposes. EOs are made of different concentrations of 20-60 compounds (Chouhan *et al.,* 2017). Terpenes are the main constituents of EOs. They are derived through the isoprenoid pathway. They are produced from the specialized plant tissues. They are also secreted from the tissues of the specialized plants (Iriti *et al.,* 2006). Podduturi *et al.,* (2017) mentioned another source of terpenes in filamentous *Actinobacteria* (popularly named as actinomycetes). Common in freshwater environments, filamentous actinobacteria are known for producing a wide variety of structurally distinct terpenes. The author also reported the production of volatile terpenes by various geosmin-producing *Actinobacteria* (*Saccharopolyspora spinosa*, *Streptomyces filamentosus*, and *S. rimosus*). Terpenes can have several different chemical functional groups. It has an alcohol group, viz., linalool, geraniol, carveol, citronellol, terpineol, menthol, borneol, and bisabolol, etc. It consists aldehyde group, viz., citral and citronellal. It also has phenol group, viz., thymol and carvacrol. Tarpenes also have a ketone group, viz., carvone and camphor. It has an ether group, viz., eucalyptol, and it also consists of a hydrocarbon group, viz., cymene, pinene, limonene, and phellandrene. The evaluation of the biological activity of EOs have been carried out over the years in order to identify new compounds with antibacterial activity for industrial applications (Sonker *et al.,* 2015). Different plants, like tea (*Melaleuca alternifolia),* thyme, *Salvia lavandulifolia* (Spanish sage), citrus fruits, viz., lemon, orange, mandarin, etc. contain terpenes. Tarpenes have a variety of therapeutic benefits. Tea plant oil contains terpenes which are a volatile EO and it has antibacterial properties (Perry, 2000). It is applied as an active component in the treatment of cutaneous infections (Carson, 2006). The thyme plant produces terpene alcohols and phenols, which have antibacterial and antifungal characteristics (Bound, 2015). Several studies have also suggested the presence of terpene in microbes (Li *et al.,* 2018). Microbes such as *Haematococcus pluvialis*, *Xanthophyllomyces dendrorhous*, *Thraustochytrid* sp. are also a rich source of terpene (Dominguez *et al*., 2005; Atienza *et al.,* 2012). In addition, through carbon chain extension, marine microalgae can synthesize a variety of bioactive secondary metabolites. Marine fish display better skin colours and skin smoothness when fed on microalgal extract in comparison to those without microalgal extract. The skin colour improvement could be due to the presence of terpene in microalgal extract (Xie and Elsheikh, 2022). Different terpenoids show distinct effects on skin pigmentation, which could be due to the minor differences that exist in their chemical structure that effect the absorption efficiency of individual terpenes (Tejera *et al.,* 2007). Different terpenoid compounds can be transferred, accumulated and metabolized by higher-level predators from their microbial hosts (Dominguez *et al.,* 2005; Van Nieuwerburgh *et al.,* 2005). The effectiveness of terpene transmission depends on nutrient dynamics primarily by the phytoplankton population in a defined ecological niche and transmission into higher trophic levels is facilitated by various channel efficiencies of secondary predators (Nie *et al.,* 2011). The extraction process is also an important step for the use of phytocompounds. Many extraction methods, including traditional ones like distillation, maceration, soxhlet extraction, percolation, reflux extraction, and reflux extraction, have all been employed to obtain extracts from plants (Kumar *et al.,* 2023).

Terpenes and terpenoids in good aquaculture practices

The addition of herbal plants and their EOs in fish feed ingredients gives beneficial results connected to active metabolites and functional elements (Elumalai *et al.,* 2020). The importance of EOs and immunostimulants as natural antioxidants has increased. The structural component of EOs is gastric acid resistant, assuring its efficiency and impact (Aydın and Barbas, 2020; Zeng *et al.,* 2015). Moreover, these volatile oils improve palatability and influence genes involved in controlling appetite in the hypothalamic−pituitary organs of fish. EOs have an effective antimicrobial impact on harmful bacteria, reducing their activity and destroying their cell walls (Dawood *et al.,* 2022). As terpenes and terpenoids are secondary metabolites of plants, frequently present in EOs, they have been shown to have antibacterial action against a broad range of bacteria, whether Gram-positive or Gram-negative. Combination therapies Terpene-based combination therapies are often used in medical technology, particularly as antifungal medications (Lewis and Kontoyiannis, 2001). Some research has been conducted to assess the effect of EOs and their principal constituents on parasitic flatworms of the class Monogenea, which are one of the major hazards to aquaculture (Ogawa, 2015). In addition, the acyclic monoterpenoids geraniol and β-citronellol have anthelmintic activities within this category of organic substances (Barros *et al.,* 2009). Studies on the anthelmintic activity of geraniol and β-citronellol against monogeneans show that both terpenoids are capable of killing these parasites in short-duration baths. However, terpenes have yet to be investigated in terms of their use as antimicrobials in the clinical phase. Terpenes are a diverse group of chemical compounds that are lipophilic, providing a wide range of structural variations that impact their mode of action. β-caryophyllene has low antibacterial efficacy against a panel of microorganisms (Fidyt *et al.,* 2016).

There is a broad correlation between the antibacterial characteristics of terpenes and their ability to modulate bacterial growth. Terpenes, due to their hydrophobic properties, can penetrate through the cell membrane of bacteria and cellular organelles, disarranging the structure of the phospholipidic bilayer and increasing permeability. This results in the leakage of particular molecules and ions in the bacteria (Hassoun and Emir Çoban, 2017). Terpenes also prevent the formation of structural macromolecules and the enzymatic reactions responsible for energy metabolism by destabilising the membrane structure of cellular organelles like the endoplasmic reticulum and mitochondria. The importance of EO and immunostimulants as natural antioxidants has increased. The structural component of EO is gastric acid resistant, assuring its efficiency and impact (Zeng *et al.,* 2015; Aydın and Barbas, 2020). Moreover, these volatile oils improve palatability and influence genes involved in controlling appetite in the hypothalamic−pituitary organs of fish. EO has an effective antimicrobial impact on harmful bacteria, reducing their activity and also destroying their cell walls (Dawood *et al.,* 2022). It has a stronger antibacterial effect on Gram-positive bacteria than on Gram-negative bacteria. This may be because the cell wall of Gram-negative bacteria has an extra component with hydrophilic characteristics that may prevent the terpenes' hydrophobic structure from passing through (Tariq *et al.,* 2019). Terpene-based combination therapies are often used in medical technology, particularly as antifungal medications (Lewis and Kontoyiannis, 2001). Some research has been conducted to assess the effect of EOs and their principal constituents on parasitic flatworms of the class Monogenea, which are one of the major hazards to aquaculture (Ogawa, 2015). In addition, the acyclic monoterpenoids geraniol and β-citronellol have anthelmintic activities within this category of organic substances (Barros *et al.,* 2009). Studies on the anthelmintic activity of geraniol and β-citronellol against monogeneans show that both terpenoids are capable of killing these parasites in short duration baths. However, terpenes have not yet been extensively investigated for their antimicrobial applications at the clinical level. Several studies have reported enhanced phagocytic activity, lysozyme activity, respiratory burst activity, and overall immunomodulatory effects following the application of plant extracts containing terpenes and their derivatives (Fig. 3).

In Indian major carp (*Cirrhinas mrigala*), intramuscular dosing (100 µL) of a combination of three medicative plant components, azadirachtin (a tetra-nortriterpenoid), camphor (a terpenoid) and curcumin (a polyphenol) markedly increased serum lysozyme activity and generation of reactive oxygen and reactive nitrogen by peripheral blood leukocytes (Harikrishnan *et al.,* 2009). Immunocompetent cells have been shown to be activated and to proliferate *in vitro* by the diterpene lactone andrographolide that has been found as a significant phytoconstituent of *Andrographis paniculata*. It also exhibits immunomodulating characteristics by inducing the production of important cytokines and an immune activation marker (Panossian *et al.,* 2002). The addition of plant extract of *A. paniculata* to the Crucian carp (*Carassius auratus*) diet results enhanced phagocytosis in the white blood cells (Chen *et al.,* 2003). Tilapia subjected to intraperitoneal injection of hot water extract (4 or 8 µg.g-1) of the Chinese herb *Toona sinensis* which contain triterpenes showed increase in respiratory burst, lysozyme activity and phagocytic cell activity (Wu *et al.,* 2010). Triterpene from *Azadirachta indica* called azadirachtin increased leukocyte count, respiratory burst activity and the primary and secondary antibody response to SRBC (sheep erythrocytes) in tilapia (Logambal and Michael, 2001). Addition of acetone extract from herbal plants such as *Cynodon dactylon*, *Aegle marmelos*, *Withania somnifera* and *Zingiber officinale* in the diet of tilapia, *O. mossambicus* also improve phagocytic profile, lysozyme activity and leucocrit values (Immanuel *et al.,* 2009). Several alkaloids, triterpenoids, β-sitosterol, steroidal lactones and volatile oils are present in these plants as strong bioactive compounds, and studies have demonstrated that they have anti-inflammatory, anti-stress, anti-oxidant, and immunomodulatory activities. Numerous alkaloids have physiological effects and strong pharmacological activity, making them useful as medications (Garba *et al.,* 2023). Furthermore, *Achyranthes aspera* root extract has different triterpenoid saponins and improve disease resistance to *A. hydrophila* in *Labeo rohita* (Rao *et al.,* 2006). Channel catfish given Orego-Stim (OS), a commercial product comprising oregano EO produced from *Origanum heracleoticum* that containing a mixture of carvacrol and thymol, showed lower mortality after *A. hydrophila* infection (Zheng *et al.,* 2009). *Cratoxylum formosum*, a Thai herbal plant, has been demonstrated to boost innate immune response of tilapia and increase the disease resistance of fish to *Streptococcus agalactiae* (Rattanachaikunsopon and Phumkhachorn, 2010). Many antimicrobial phytochemicals, including camphor, polyphenols, and terpenes (1,8-cineol, o-pinene, limonene, terpineol-4-ol, and α-terpineol) are found in *Rosmarinus officinalis. Streptococcus iniae* infected tilapia showed reduction in mortality rate after fed supplimented diet using ethyl acetate extract of *R. officinalis* (Abutbul *et al.,* 2004). The transcription of virus may be inhibited or blocked by bioactive herbal ingredients that may also lower viral replication in the host cells. They stimulate the immunity of the host and may improve the capacity to deal with the physiological impacts of acute stress associated with aquaculture activities such as transportation, handling, inadequate stocking density, netting, and fluctuating water quality. Likewise, phytochemicals may alter the enzymatic systems essential in fish detoxifying metabolism. To completely recognise the therapeutic efficacy of these substances, it is vital to have thorough knowledge of the appropriate dosage, period, method of administration, and molecular basis for the functional activity of various plant bioactive components. The effects of terpenes and their derivatives obtained from different plants that are used as feed additives or injected intramuscularly to different species are compiled in Table 2.

Table 2: Terpenes and their derivatives as feed additives in aquaculture.

Sl No	Fish Species	Terpenes and derivatives used	Sources and dosage	Effect on fish	Reference
1	*Oreochromis niloticus*	β-glucans	Mushroom stalk waste extract 0.5 – 10 g/kg	Growth promoting activity and improvement of SOD, CAT in stress	Ahmed *et al.,* 2017
2	*Oreochromis niloticus*	Phenolic compounds	Corn silk extract at 3.5 g/kg feed	Improve lysozyme activity, Reduce liver peroxidation during oxidative stress	Žilić *et al.,* 2016
3	*Carassius auratus*	Phenolic compounds	*Andrographis paniculata* extract	Enhance phagocytosis in the white blood cells	Chen *et al.,* 2003
4	*Ictalurus punctatus*	Essential oil	*Origanum heracleoticum* and *Origanum vulgare L.* 0.05% extract	Fish showed lower mortality after *Aeromonas hydrophila* infection and higher growth	Zheng *et al.,* 2009

Table 2 continued:

Sl No	Fish Species	Terpenes and derivatives used	Sources and dosage	Effect on fish	Reference
5	*Oreochromis niloticus*	Triterpenes	Hot water extract of *Toona sinensis* at 4 or 8 µg/g	Increase in respiratory burst, lysozyme activity and phagocytic cell activity	Wu *et al.,* 2010
6	*Oreochromis niloticus*	Triterpenes	Azadirachtin, extract of *Azadirachta indica*	Increased leukocyte count, respiratory burst activity and the primary and secondary antibody response	Logambal and Michael, 2001
7	*Labeo rohita*	Triterpenoid	*Achyranthes aspera* root extract at 0.1 – 0.5%	Improve disease resistance to *Aeromonas hydrophila*	Rao *et al.,* 2006
8	*Oreochromis mossambicus*	Phenolic compounds	*Cratoxylum formosum* extract at 1.5 ml/g	Boost innate immune response and increase the disease resistance	Rattanachaikunsopon and Phumkhachorn, 2010
9	*Oreochromis niloticus*	Phenolic compounds	Ethyl acetate extract of *Rosmarinus officinalis* at 1.2 ml/g	*Streptococcus iniae* infected fish showed reduction in mortality rate	Abutbul *et al.,* 2004
10	*Danio rerio*	β-glucans	endosperm of oat grains	Immunomodulatory function	Udayangani *et al.,* 2017
11	*Oreochromis mossambicus*	Limonene	*Citrus sinensis* (Orange peel) extract at 5 g/kg	Higher survival rate against *Streptococcus iniae* and act as an immunostimulant and promoting growth	Acar *et al.,* 2015
12	*Labeo victorianus*	Phenolic compounds	Bitter lemon fruit peel extract at 10 – 20 g/kg	higher erythrocyte count, leucocyte count, haematocrit, and neutrophil levels	Ngugi *et al.,* 2017
13	*Mugil cephalus*	Phenolic compounds	Sorghum distillery residue at 200 g/kg feed	boosts antioxidant activities and inhibits the oxidation of low-density lipoproteins in the plasma	Lee *et al.,* 2009
14	*Dicentrarchus labrax L.*	Phenolic compounds	Grape seeds extract at 100 mg/kg	Expression of a robust and protective adaptive immune response	Arciuli *et al.,* 2017
15	*Oreochromis mossambicus*	Essential Oil	*Citrus limon* (lemon peel) extract at 10 g/kg feed	Improved immuno-haematological parameters	Baba *et al.,* 2016

Aquaculture is a rapidly expanding industry, and environmentally safe, natural alternatives to antibiotics are increasingly in demand. Plant secondary metabolites possess enormous economic importance in the food, pharmaceutical, chemical, and agricultural sectors due to their diverse biological activities and functional properties. Research on natural product synthesis began several decades ago and led to the development of nature-identical compounds (NICs), which are chemically synthesized substances that are structurally identical to the bioactive compounds found in plant essential oils (EOs) and oleoresins (ORs) (Beltrán and Esteban, 2022).

Among plant secondary metabolites, terpenes and their derivatives represent the most abundant and structurally diverse class of natural compounds. Terpenoids exhibit a broad range of biological activities, reflecting their structural diversity, which has made them valuable in both traditional and modern applications. In aquaculture systems, stress caused by chemical, biological, and physical factors can lead to increased mortality and significant economic losses. Stress generally results in physiological alterations that disrupt homeostasis and compromise fish health.

Most phytochemicals are redox-active compounds, meaning they can scavenge free radicals and inhibit the formation of reactive oxygen species. Terpenoids, polyphenols, and organosulfur compounds are three major groups of phytochemicals known for their antioxidant properties. Numerous scientific studies have highlighted the ability of phytochemicals to protect fish against the detrimental effects of chronic oxidative stress. These natural antioxidants constitute a diverse group of plant-derived substances with significant protective potential.

Phytocompounds may serve as promising alternatives to conventional antibacterial agents in aquaculture. Recent research and emerging technologies have demonstrated that many phytochemicals exhibit a broad spectrum of activity, with moderate to strong antibacterial effects against both marine and freshwater pathogens. Nevertheless, further research is required to optimize the use of medicinal plants and fully harness the safety and efficacy of phytocompounds, which may ultimately prove superior to synthetic or semi-synthetic alternatives.

Future prospects

Phytochemicals offer significant health and performance benefits, observed in terrestrial organisms and humans, with ample information available on their use in aquaculture. Advancements in aquaculture automation and technology are crucial for enhancing the antibacterial activity of terpenes and derivatives. The use of herbal compounds, particularly essential oils (EOs) rich in terpenes, is rapidly expanding in aquaculture to ensure industry sustainability. EOs exhibit positive effects on growth, immunity, and combatting bacterial and parasitic infections in aquatic animals. Collaboration with the commercial pharmaceutical industry could further enhance the beneficial components of EOs for fish production. There is still a limited understanding of the molecular mechanisms through which essential oils (EOs) promote fish immunity and growth, highlighting the need for cell culture studies and pathway characterization to gain further insights. Determining optimal EO dosages against pathogens affecting economically important fish globally is crucial, considering the synergistic interactions among their bioactive components. However, the use of terpenes can lead to an off-odour in fish, but this can be resolved through depuration, which involves placing the fish in clean water (Wu *et al.,* 2022). Further research is needed to assess the immunomodulatory potential of plant extract oils on fish and shellfish mucosal surfaces, including the stomach, gills, and tissues, under varying conditions.

Polyculture, recirculatory aquaculture, and integrated multi-trophic aquaculture (IMTA) are gaining popularity for their economic and environmental benefits in intensive fish production. Studies highlight the potential of terpenes and their derivatives in enhancing disease resistance, promoting growth, and improving health in farm-reared fish. The increasing interest in environmentally friendly nutritional supplements as alternatives to antibiotics in aquaculture is notable. However, the species-specific benefits of herbal feed supplements must be cautiously considered, with separate evaluations required for each farmed fish species and production stage, from fry to fingerling and grow-out phases. Regulatory frameworks, especially in many countries, impose strict controls on natural therapeutics, feed additives, and supplements, potentially limiting their widespread use in animals and fish.

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Conflicts of interest

The authors declare that this review was conducted without any conflict of interest with any party.

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