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Phytotherapy use for disease control in aquaculture: a review of the last 5 years

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Abstract

Intensification of aquaculture practices brings about disease outbreaks, resulting in high mortality among farmed species and great economic losses. In order to prevent huge economic losses, various types of antibiotics have been used for the treatment of infections. Nevertheless, frequent use of synthetic antibiotics leads to an increase in antibiotics-resistant pathogenic bacteria and aggravating water pollution. Thereby, herbal medicine appeared to be an alternative for chemotherapy replacement due to its characteristics of being eco-friendly, having a high tolerance in animals, and being less toxic to the environment. Current research on the use of phytogenic compounds to combat some pathogens that cause diseases in aquaculture raises great worldwide interest due to their capability to act as immunostimulants, antibacterials, antioxidants, anti-parasites, and anti-viruses. This review paper aimed to review the past 5 years (2019–2023) on the usage of herbal medicine in disease mitigation, their mechanisms of action, and the effectiveness of various dosages and routes of administration during application in several aquatic species. The potential toxicological effects observed during the application of medicinal plants also were discussed.

Keywords Aquaculture · Extraction · Mechanism of action · Phytochemicals · Toxicological

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Introduction

Ever-increasing demand attributable to the growth of the population worldwide gives rise to the intensification of aquaculture. In 2020, global production of fish and aquaculture aquatic organisms reached 178 million metric tons, with 70% of that coming from Asia (FAO 2022). Corresponding to this kind of practice, outbreaks of diseases are the challenges hitting aquaculture, as they result in high mortality of farmed species and great economic losses. Until now, antimicrobials from the quinolones, tetracyclines, amphenicols, and sulfonamides classes are some of the most commonly prescribed drugs to stop disease outbreaks (Schar et al. 2020), particularly in a number of countries that are big producers for aquaculture products (i.e., China, India, Indonesia, and Vietnam), and all these four countries are projected to continue to have the highest consumption of antimicrobials in 2030 (Ferri et al. 2022).

The usage of these antimicrobials is governed by laws and policies from management unions, including the Food and Drug Administration (FDA), the Food and Agriculture Organization (FAO), the European Medicines Agency (EMA), and the European Union (EU), which established the maximum residue limits (MRLs) of chemicals in fish for consumption (Lulijwa et al. 2019). As of now, the approved antibiotics to be used in aquaculture are tetracycline, penicillin, quinolones, sulphonamides, and trimethoprim (Conti et al. 2015), whereas chloramphenicol, nitrofurans, vancomycin, dimetridazole, and cimaterol were prohibited due to their carcinogenic properties, bone marrow depressions, aplastic anemia, and acute nephrotoxicity (Bondad-Reantaso et al. 2012; Conti et al. 2015). Even though their use is under control, the excessive application and misuse in some of the developing countries have triggered some environmental issues, particularly water pollution and toxic sedimentation (Sodhi et al. 2021). Moreover, the majority of chemical antibiotics are polar molecules with low volatility, making them easily soluble in aquatic environments (Ramesh and Souissi 2018). More recently, most of the antibiotics used to treat diseases in aquaculture have been prohibited due to the possibility of the emergence of resistant and multi-resistance genes, which may infiltrate the seafood chain and pose a public health concern (Tadese et al. 2021).

Vaccination has worked well to reduce the use of antibiotics in some aquaculture sectors (e.g., Norwegian salmon) (Brudeseth et al. 2013) and against some common diseases in aquaculture (e.g., vibriosis, streptococcosis, and WSSV). However, it has some drawbacks, such as the need for a highly specific technique that requires a precise disease diagnosis and high production costs, which are not useful for tropical emergent diseases or small-scale production, and have long-lasting negative effects after injection. Additionally, they are also ineffective in multi-agent infections, where co-infections are frequent in aquaculture (Wei et al. 2022). As an alternative approach to antibiotics and vaccination, the use of medicinal plants in aquaculture has received much attention in the past decade, as they are safe, cheaper, less toxic, biodegradable, and more environmentally friendly (Kuebutornye and Abarike 2020; Zheng and Bossier 2023).

Since the 1990s, researchers have been interested in how herbal medicine is used in aquaculture due to their bioactive compounds (e.g., flavonoids, alkaloids, terpenoids, tannins, polysaccharides, and essential oils), which are not only used as chemotherapeutics but could also be added to animal feed as feed additives to boost their immune systems (Tadese et al. 2020; Zhu 2020; Amiruddin et al. 2021). Studies have shown that medicinal plants have a wide range of biological properties, such as stress-relieving, antimicrobial, antiviral, and antiparasitic effects. They also have low toxicity (Stratev et al. 2017; Zhu 2020) and are good for the environment and the economy (Cawthorn and Hoffman 2015). Their versatility in application (e.g., powdered, crude extract, isolated bioactive compounds) and in how they work with live bacteria or yeast and various animal product origins have become advantageous (Zhong et al. 2018; Tadese et al. 2020). Therefore, the present review seeks to highlight the use of medicinal plants in various forms (e.g., powdered, crude extracts, and isolated active compounds) in the fight against numerous diseases in aquaculture. Furthermore, the mechanisms of action of each biological activity of medicinal plants and the effectiveness of varying dosages and routes of administration during application in several aquatic species are also researched. Additionally, the toxicological effects of the medicinal plants from the previous research were also discussed. To achieve this goal, a literature search was performed on Web of Science and Google Scholar, which are time-limited to the years 2019–2023.

Biological activities of medicinal plants and their mechanism of actions

Medicinal herbs are plants that are used to treat and heal ailments (Van Hai 2015), which are made up of secondary metabolites (SMs) like polysaccharides, alkaloids, tannins, flavonoids, terpenoids, and saponins, as well as minerals and vitamins (Zhang et al. 2022). The extraction of SMs can be differentiated into conventional (e.g., soxhlet, maceration, hydrodistillation) and non-conventional techniques (e.g., ultrasound-assisted extraction (UAE), pulsed-electric field assisted extraction (PEF), enzyme-assisted extraction (EAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), and supercritical fluid extraction (SFE), where the choice depends on the medicinal plant type, the solvent used, the temperature, and the provision of solvent to the sample (Abubakar and Haque 2020). However, conventional extraction techniques generally utilize organic solvents and are time-consuming (Zhang et al. 2018), leading to the substitution of non-conventional or green technique methods (Rodríguez-Pérez et al. 2015; Zhang et al. 2018). Moreover, the extraction process is a key factor in determining the yield and effectiveness of the finished product; hence, the extraction conditions must be optimized in order to maximize desired bioactivities and extract yields (Jeong et al. 2014). For instance, crude extracts are usually extracted with different solvents (e.g., water, methanol, chloroform, and ethyl acetate), and methanol is indicated to have a high extraction yield with numerous bioactive compounds (Truong et al. 2019). All of the extracted bioactive compounds will have different biological effects, such as growth promoters, immunostimulants, antioxidants, antibacterial, antiparasitic, and antiviral effects (Reverter et al. 2014; Wei et al. 2022; Zhu 2020). Table 1 summarizes the recent techniques used for plant extraction.

As growth promoter

Several plant extracts have been proven to be feasible for use as growth promoters or appetite stimulators by elevating digestive enzymes and the growth rate of aquatic animals (Takaoka et al. 2011). Growth performance amelioration is also influenced by nutrient digestibility and absorption by enhancing the digestive enzymes, which further improves the capacity of gut digestion (Heidarieh et al. 2013). Khanal et al. (2021) reported a growth increase in juvenile common carp (*Cyprinus carpio*), when the diet was supplemented with 0.4 and 0.8% of *A. vera* extract. It is speculated that *A. vera* was capable of increasing the villus length and

Table 1 Summary of common extraction used in plant

Methods	Extraction solvent	Temperature needed	Time for extraction	Volume of solvent needed	References
Pulsed-electric field assisted extraction (PEF)	Ethanol–water mixture, dimethyl sulfoxide (DMSO)	30 – 40 °C	0–6 h	50 – 100 mL	Calleja-Gómez et al. (2022)
Enzyme-assisted extraction (EAE)	Water, ethanol–water mixture, n-hexane	40 – 60 °C	n.m	n.m	Alavarsa-Cascales et al. (2022)
Maceration	Water, ethanol, ethyl acetate, chloroform, butanol, etc	Room temperature	1–5 days	150 mL/25 g sample	Tambun et al. (2021)
Ultrasound-assisted extraction (UAE)	Ethanol, acidified water, alcohol, acetone, water	20–60 °C	< 30 min	50 – 100 mL	Kumar et al. (2021)
Microwave-assisted extraction (MAE)	Ethanol, acetone, acetonitrile, hexane, and 2-propanol	n.m	Few min	10 – 30 mL	Tambun et al. (2021)
Pressurized liquid extraction (PLE)	Hexane, methyl chloride, isopropanol, and ethanol	n.m	15–50 min	15 – 40 mL	Stéphane et al. (2021)

n.m not mentioned

enterocyte height, intensifying the surface area of the intestinal mucosa, and decreasing the villus width, which in turn provided maximum surface area for nutrient absorption (Valladão et al. 2017). In addition, Parsa et al. (2016) found that consuming *A. vera* gel led to a favorable shift in polysaccharide and *Lactobacillus* populations, both of which are good bacteria in the gastrointestinal tract and promote gut homeostasis. Hasani (2022) also revealed that chinacea, garlic, thyme, and peppermint are also the other common medicinal herbs that are commonly used as growth boosters in aquaculture. In addition, vanillin contained in nettle extract is able to increase feed palatability and the growth rate of rainbow trout (*Onchorhynchus mykiss*) fingerlings (Zare and Mirzakhani 2021).

Zemheri-Navruz et al. (2020) reported that the inclusion of olive leaf extract at 0.1 and 0.25% for 60 days in common carp (*Cyprinis carpio*) increased the digestive enzymes (i.e., a-amylase, protease, and lipase) in the fish intestine and the expression of the growth-related genes (i.e., GH and IGF-I) in the brain, liver, kidney, and muscle tissue of the fish. Ghafari-farsani et al. (2022a) reported that the inclusion of Persian shallot (*Allium hirtifolium*) extract at 1–2% in rainbow trout (*O. mykiss*) fingerling feed diet for 60 days significantly improved their growth rate with a minimum FCR. Additionally, their total protein values, albumin, and globulin were also increased, along with other non-specific immune responses including SOD, CAT, glutathione peroxidase (GPx), total immunoglobulin, and lysozyme activity. Terzi et al. (2021) also reported a decreased ($p<0.05$) FCR, improved innate immunity responses in the kidney and gut, and boosted disease resistance of rainbow trout against *Yersinia ruckeri* after feeding with 1% of *Prunus domestica* extract for 21 days, with no marked changes in major organs (i.e., gills, kidney, liver, and spleen) after the treatment. In a different investigation, rainbow trout fed a diet supplemented with barberry (*Berberis vulgaris*) root extract at 500 mg/kg for 8 weeks displayed better blood hematological parameters (Ramezanzadeh et al. 2020). Abd-elaziz et al. (2023) reported the significant increase in growth and immunostimulatory effects of *Pangasianodon hypophthalmus* fingerlings after being fed with 1–2% of phytobiotics, particularly the extracts of *Gingkgo biloba*, *Silybum marianum*, and *Myristica fragrans* for 56 days. This could be speculated by the presence of polyphenols and flavonoids in these plants to boost fish growth and immunity by improving digestive enzymes, appetite, and intestinal histomorphology (Ahmadifar et al. 2021).

Previously, Fang et al. (2020) reported that the presence of quercetin, terpenoid lactones, and polysaccharides in *G. biloba* extract stimulates immune responses in fish by increasing their serum myeloperoxidase, bactericidal, total immunoglobulins, complement, and lysozyme activities. Shi et al. (2020) reported that the inclusion of *Lactobacillus plantarum*-fermented *Astragalus membranaceus* root extracts improved common carp (*C. carpio*) growth and immunity through the increment of villus height and the improvement of several beneficial microbiomes in the intestine (i.e., *Cyanobacteria*, *Bacteroidetes, Proteobacteria*, *Fusobacteria*, *Acidobacteria*, *Actinobacteria*, *Firmicutes,* etc.). Some studies reported the ability of *Actinobacteria* to form a defensive barrier by colonizing the intestine and preventing pathogenic invasions, and they also secrete secondary metabolites that inhibit the growth of intestinal pathogenic bacteria (Xu et al. 2020).

As immunostimulant and antioxidant

Fish have a complex antioxidant system that includes superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx). These enzymes are the first line of defense against reactive oxygen species (ROS) stress by promoting their conversion into inert species (Wang et al. 2022). The accumulation of ROS beyond the fish's scavenging ability results in oxidative stress. In order to mitigate oxidative stress, an antioxidant enzyme system has been evolved to reduce the body's redox (Shi et al. 2020). Studies showed that phytotherapy as a dietary supplement is likely to induce innate immune factors as it contains a rich source of natural antioxidants that can scavenge excess ROS and avert oxidative damage (Dawood et al. 2020). Phytochemicals like phenolics and flavonoids consist of a diverse group of polyphenolic compounds whose structural backbones (i.e., flavan nuclei) are made up of two benzene rings connected by a heterocyclic ring of pyron or pyran (Rahimi et al. 2022). This structure permits multiple substitutions and formations subdivided into flavonols, flavanones, flavones, flavanonols, and flavanols (Speisky et al. 2022). The underlying mechanisms of bioactivity include the capacity of flavonoids to interact through their redox-active phenolic moieties to produce and suppress ROS through the quenching of free radical reaction cascades in lipid peroxidation by either transferring an electron or hydrogen atom of the hydroxyl group (Speisky et al. 2022). Figure 1 summarizes the antioxidant defense mechanisms that occur in fish.

An immunostimulant is a substance that increases the ability of defense mechanisms, thus making living organisms more resistant to infection and disease. In aquaculture, enhancing the immune systems of fish and shellfish is crucial for controlling and preventing the spread of diseases and also represents a new step in the development of pollution-free aquaculture. Lysozyme is a crucial antimicrobial enzyme that breaks down peptidoglycan in bacterial cell walls, causing cell burst, complement, and phagocytosis (Harikrishnan et al. 2011). In phytotherapy, the immunomodulatory effect can be achieved in four ways: by activating phagocytosis, stimulating fibroblasts, increasing respiratory activity, and making it harder for leukocytes to move (Vanichkul et al. 2010). A study conducted by Abarike et al. (2019) revealed a significant up-regulation of β-defensin, lysozyme, HSP70, SOD, and catalase genes in the intestine and kidney tissues of Nile tilapia when fed with a mixture of Chinese herbs: *Astragalus membranaceus*, *Crataegus hupehensis*, and *Angelica sinensis* in the ratio of 1:1:1 (10 g/kg) for 4 weeks (Abarike et al. 2019). Meanwhile, a diet supplemented with 2 g/kg of Assam tea extract (*Camellia sinensis*) enhanced growth performance, both humoral and mucosal immunity (e.g., lysozyme, PO, phagocytosis, and respiratory burst), and resistance against *Streptococcus agalactiae* infection in Nile tilapia fingerlings (Doan et al. 2019a). A similar experiment was conducted by Doan et al. (2019b), which revealed significant increase in growth performance, immune response, and disease resistance of Nile tilapia fingerlings against *S. agalatiae* after supplementation with 5 g/kg of Elephant's foot (*Elephantopus scaber*) for 8 weeks of feeding.

Other studies also reported that the oral feeding of oregano EO at a concentration of 4500 mg/kg diet altered the gut microbiota and improved the immunity and resistance of koi carp, *Cyprinus carpio* to *Aeromonas hydrophila* (Zhang et al. 2020). The fish fed with a commercial diet supplemented with guava leaf at 1.5–3% for 21 days significantly improved non-specific immunological responses (i.e., protease, antiprotease, and peroxidase) in hybrid tilapia (*O. niloticus* × *O. mossambicus*). A study by He et al. (2020) indicated that the essential oils (EO) of Celak (*Thymus quinquecostatus*) had potent antioxidant capacity by scavenging ROS and inhibiting lipid peroxidation. Also, Zhang et al. (2022) reported that the *Flos populi* extract was more effective than the control at increasing antioxidant enzymes in the liver and down-regulating Keap1. Wangkahart et al. (2022) found that when *O. niloticus* was fed 20 g/kg of *Aegle marmelos* fruit extract for 8 weeks, its antioxidant enzyme system (CAT, SOD, GPx, and GRD) went up, and its cholesterol level went down. Yilmaz (2019) and Yilmaz (2020) revealed improved tolerance to ammonia stress in *O. niloticus* and Nile tilapia after feeding with 20 mg/kg anthocyanin and 1.25%

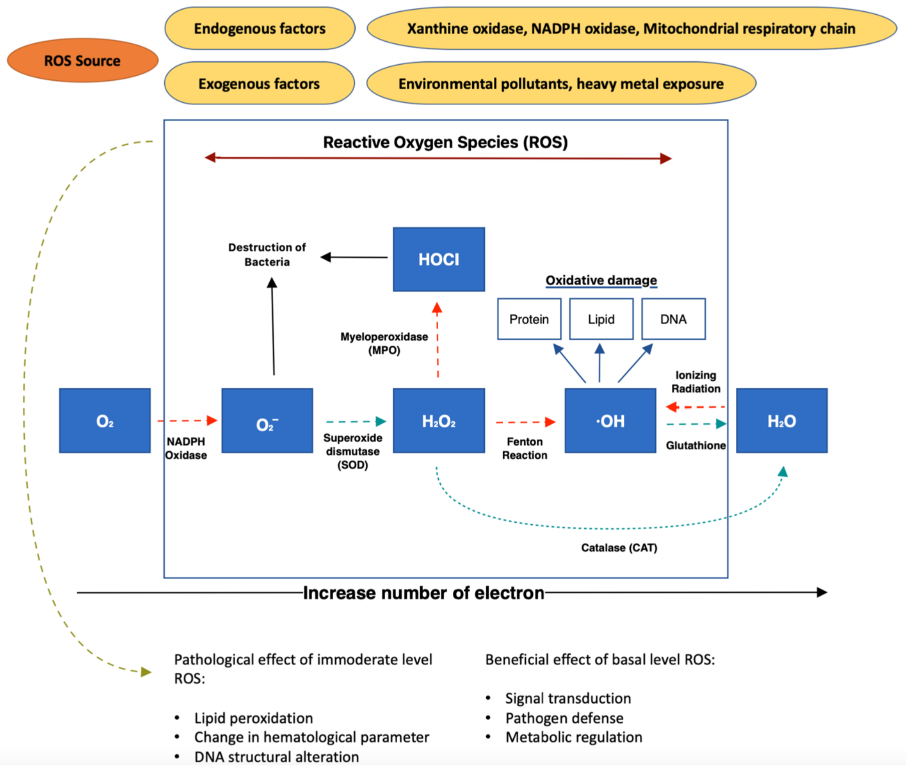

Fig. 1 Pathway of reactive oxygen species (ROS) formation and enzymatic defence mechanism against ROS along with source and effect of ROS towards animals

of *Ceratonia siliqua* syrup for 96 h of ammonia stress, respectively. A high level of carvacrol and thymol in *O. vulgare* EO is able to scavenge free radicals, chelate transition metal ions, and decompose peroxides in fish after being supplemented for 8 weeks in the diet (Abdel-Latif et al. 2020).

As antibacterial and antifungal

Antimicrobial compounds (e.g., alkaloids, flavonoids, terpenoids, and phenolic compounds) interact with microbial cell enzymes and membrane proteins by hydrophobic and hydrogen bonding, thus altering cell membrane protein permeability and disrupting the structure of mitochondria (Razak et al. 2019). This initiates proton flux interference with extrinsic cells that promote or inhibit cell death as well as obstruct enzymes for amino acid biosynthesis (Novita et al. 2020). Zhang et al. (2022) and Pei et al. (2023) indicated that cinnamaldehyde could thrust into the bacterial and fungal cell membrane and destroy the cytoplasmic membrane integrity by elongating bacterial cell morphology, causing cell lysis. Valenzuela-Gutiérrez et al. (2021) showed that allicin in garlic exhibits inhibitory capacity against bacterial growth by inhibiting biofilm formation and virulence. Hardi et al. (2019) indicated that combined *Boesenbergia pandurata*, *Solanum ferox*, and *Zingiber Zerumbet* plant extracts are capable of treating and impede *A. hydrophila* and *Pseudomonas fluorescens* infection in tilapia by increasing the white and red blood cells, phagocytic index, and lysozyme activity. Avicenna marina (*Gracilaria* spp.) and *Sargassum* spp. have been used and proven to effectively stimulate resistance against *Vibrio* pathogens (Ghosh et al. 2021). A dietary combination of thymol (0.5 g/kg diet) and chitosan nanoparticles (5 g/kg diet) in the feed diet of *O. niloticus* improved the antibacterial activity by causing bacterial cell wall permeability and the release of bacterial toxins (Abd El-Naby et al. 2020).

A different study performed by Ceballos-Francisco et al. (2020) revealed the significant increase of protease and lysozyme enzymes in hybrid tilapia (*O. niloticus* × *O. mossambicus*) skin mucus after supplementation with guava, *Psidium guajava* L., at 1.5 and 3% for 21 days, where these enzymes are responsible for activation of the innate and adaptive immune systems in fish. Omitoyin et al. (2019) reported the improvement of gut morphology (i.e., villi length and width, and absorption area) of *O. niloticus* fingerlings after feeding with 0.75% *P. guajava* for 84 days. These occasions may have equally contributed to the fish's growth, as the increased area of absorption directly influenced the amount of nutrient absorbed (Markovic et al. 2009). Moreover, the improvement of gut morphology will also benefit healthy mucosal epithelium, which can help prevent infection from opportunistic bacteria (Ringø et al. 2007). Zhai and Li (2019) reported the effectiveness of oral supplementation of Chinese herbs (San-Huang-San) in combination with enrofloxacin for 5 days to treat AHPND infection in *L. vannamei*. Diets containing 0.2% *Psidium guajava* and 1% of *Mimosa pudica* boosted the immune system and survival of the striped catfish (*Pangasianodon hypophthalmus*) against *Edwardsiella ictaluri* infection (Nhu et al. 2019).

Additionally, the inclusion of *Z. officinale* at 6 or 10 g/kg results in increased non-specific immune responses in *O. mykiss* to *Yersinia ruckeri* (Soltanian et al. 2019). Hernández-Cabanyero et al. (2023) revealed the success of phytobiotic additives with 30% of EO microencapsulated mixture (thyme and cinnamon) to significantly reduce the *Litopenaeus vannamei* infection against V_{pAHPND} after 4 weeks of administration, where the results showed decreased in shrimp mortality with the lowest percentage of V_{pAHPND} carriers, and fewer pathological effects. Their studies were also in line with Quiroz-Guzmán et al. (2022) and made speculations that it could be due to the antibacterial potential of the phytobiotic compounds, which impaired the bacterial colonization in the gut microbiota and promoted the beneficial bacterial community in the shrimp intestine. A similar study was conducted by Quiroz-Guzmán et al. (2022), however with a different administration where they used the injection method, to result in significant antibacterial properties against *V. parahaemolyticus* (i.e., an increase of 85% in survival) when supplementing *P. vannamei* with a mixture of 2% of a mix of *Curcuma longa* and *Lepidium meyenii* for 6 weeks. Quiroz-Guzmán et al. (2022) suggested that the antibacterial properties of phytobiotics were enhanced by promoting a better bacterial community (e.g., *Proteobacteria, Acinetobacter, Actinobacteria, and firmicutes).* According to Li et al. (2018), the *Proteobacteria* phylum is present highest in the gut and intestine of *P. vannamei* and is the most stable bacteria group in healthy shrimp. Thymol and carvacrol compounds are believed to promote antibacterial activity by inhibiting the ergosterol biosynthesis and membrane degradation of pathogens (Behbahani et al. 2018).

As antiparasitic

Fish are susceptible to a variety of parasitic diseases that come from protozoan, worm, and parasitic crustaceans (Zhang et al. 2022). Ivermectin, praziquantel, formalin, formaldehyde, and trichlofon are the chemotherapeutic agents to control parasitic disease (Thing et al. 2016); however, they may directly leave toxic residues in fish body and environment, which indirectly damage consumer health (Pandey 2013). Generally, there are few biochemical compounds that possess antiparasitic activity, such as polyphenols, terpenoids, saponins, and alkaloids (Zhang et al. 2022). According to Zhou et al. (2021), the treatment of *Dioscorea colletti* extract, which contains diosin as a major compound, completely eliminated *Gyrodactylus kobayashii* in goldfish (*Carrasius auratus*) when used at 10 mg/L after 48 h of exposure. Most of the microvilli on the tegument surface dropped, and serious tegumental damage was seen, which impaired the survival of parasite (Zhou et al. 2021). Whereas, the elimination of marine parasitic leech *Zeylanicobdella arugamensis* hybrid groupers (*Epinephelus fuscoguttatus*×*E. lanceolatus*) was achieved after treatment with *Dillenia suffruticosa* extract at 100 mg/mL for 8 min (Shah et al. 2021).

The toxic effects of EO on parasites are usually caused by changes in membrane permeability, swelling, vacuolization, cytoplasm leakage, and death (Zhang et al. 2013). Alavinia et al. (2018) also showed the in vitro and in vivo effects of tannic acid on *Ichthyophthirius multifiliis* in zebrafish, where they found that tannic acid may damage the parasites' plasma membrane and kill them. The mode of action for these antiparasitic drugs may involve raising intracellular osmotic pressure and accumulating free radicals, which induce membrane destruction (Fu et al. 2019). Some herbal extracts also possess antiparasitic activity by inhibiting reproductive maturation and hindering embryonic development (Banerjee et al. 2014). Ginger and other active compounds such as dioscin devastate the microvilli from the tegument surface (e.g., anterior attachment organ and posterior opistohaptor), which decreases parasitic movement and larvae survival rates and further threatens the survival rate (Trasviña-Moreno et al. 2019; Zhou et al. 2021). Fu et al. (2019) reported that the exposure of 1–4 mg/L of the 10-gingerol isolated from *Z. officinale* effectively protects against the infestation of *Ichthyophthirius multifiliis* in grass carp. Meanwhile, Gonzales et al. (2020) reported the potential of 500 mg/L of *C. citratus* EO being effective against monogeneans in 5 min (i.e., *Anacanthorus spathulatus*, *Mymarothecium boegeri*, and *Notozothecium janauachensis*) isolated from *C. macropomum*, whereas the lower concentrations of the EO (400 and 300 mg/L) were effective against the parasites in 10–30 min of exposure. Hamdan et al. (2022) also revealed that exposure to *Melaleuca cajuputi* leaf extract at 0.85 g/L for 1 h caused severe damage to adult female and male *Probopyrus buitendijki*.

As antiviral

In the aquaculture industry, viral infections are frequent diseases that are highly contagious, spread quickly, impact a range of hosts, and have high fatality rates. Currently, the majority of viral diseases in aquaculture are not treatable by drugs and are typically preventive in nature (e.g., disinfection and control of the aquaculture environment); however, the results are not encouraging. According to several researchers, medicinal plants may engage in a number of mechanisms before exhibiting their antiviral potential. These mechanisms include direct inactivation of viral particles, interruption of viral attachment and penetration phases, inhibition of virus replication, participation in transcriptional regulation, disruption of virus protein synthesis or expression, inhibition of viral cell-to-cell transmission, and immunomodulatory roles (Takebe et al. 2013). The existence of the hydroxy group -OH in polyhydroxy isocopalane may impede DNA virus replication and amino acid synthesis on the active site of the host cell.

There are many antiviral phytomolecules that can be applied in the prevention of viral disease, such as polyphenols, flavonoids, and terpenes (Behl et al. 2021). Based on the study of Liu et al. (2019), *Curcuma kwangsiensis* is able to combat grouper iridoviral infection; the same goes for the water extract of *Thlaspi arvense* (Xiao et al. 2019). In another study, it was disclosed that quercetin from *Illicium verum* may obstruct Singapore grouper iridovirus (SGIV) from attaching to the host cell's receptor and destroy the virus structure (Liu et al. 2020). The in vivo antiviral effects of licorice, *Glycyrrhiza uralensis* water extract, which contains glycyrrhizic acid as a major compound, exhibited anti-SGIV activity by destroying the viral structure and interfering with the viral invasion (Li et al. 2022).

A separate study reported the antiviral activity of *G. uralensis* root extract against viral hemorrhagic septicemia (VHSV). Bioactive compounds from licorice, including glycyrrhizin (GL) and glycyrrhetinic acid (GLA), prevented viral attachment and replication and therefore inhibit the early fusion steps of VHSV in olive flounder, *Paralichthys olivaceus* (Lim et al. 2020). Genipin and geniposidic acid have been discovered in *Gardenia jasminoides* extract as being able to inhibit WSSV replication in crayfish, *Procambarus clarkii* (Huang et al. 2019; 2020). Luteolin from *Lonicera japonica* extract, particularly ranging from 6.25 to 25 mg/kg, was reported to block WSSV genome replication (e.g., *ie1* and *Vp28*) and enhance apoptosis to suppress WSSV replication during the early infection stage in *Procambarus clarkia* in vivo (Jiang et al. 2022). Liu et al. (2022) investigated the effect of the bioactive compound matrine from *Sophora flavescent* against white spot syndrome virus infection (WSSV) in crayfish. Inclusion of hesperetin additive in feed at 50 mg/kg is able to effectively inhibit WSSV viral replication in crayfish by down-regulating the expression of Toll-like receptor and crustin 1, up-regulating the expression levels of NF-kappaB and C-type lectin, and increasing THC, PO, and SOD expression (Qian and Zhu 2019).

Proteins containing a C-type lectin domain have several functions for shrimp immunity, including cell–cell adhesion, immunity to pathogens, and apoptosis (Cambi et al. 2009). A triterpene saponin derived from *Bupleurum falcatum* at a dose of 6 mg/kg is able to significantly inhibit spring viraemia of carp virus (SVCV) nucleoprotein and glycoprotein gene expression in the kidney and spleen of zebrafish (Shen et al. 2019). The inclusion of EO from *Mentha piperita* at 0.25% in the tilapia diet improved fish immunity and survival against *S. agalactiae* by increasing the total number of leukocytes, thrombocytes, and plasma proteins (de Souza Silva et al. 2019). The methanolic extract of *Andrographis paniculata* supplemented in the diet of *Labeo rohita* at 50 μL increased the non-specific immune responses (i.e., hemoglobin and total erythrocyte-leucocyte counts), phagocytic index, and survival rates against *A. hydrophila* while maintaining the health of major organs, including the gills and liver (Palanikani et al. 2020). Other recent medicinal herbs utilized in aquaculture are summarizes in Table 2.

Application technology of medicinal plants in aquaculture

Generally, the efficacy of medicinal plants is tightly related to the abundance of bioactive compounds, which are influenced by several geographical areas, the part of the plant used, the age of the harvesting plant, etc. Besides, the different materials of the same plant (e.g., dried powdered plant or extract) will also contribute to different metabolites and potentials. According to Reverter et al. (2021), powdered plants were the most commonly used materials, followed by ethanolic extracts, EO, and aqueous extracts, where the trend mainly depended on the cost and safety of the extract produced. The reason why extraction using alcoholic solvents is preferred to aqueous might be due to the solvent polarity, which can

Table 2 Recent medicinal plants used in aquaculture

Species; age	Plant used	Part of plant used; dosage	Administration; duration	Outcomes	References
Pangasianodon hypophthalmus; juveniles	*Silybum marianum*	n.m; 0.1–0.3% in diet	Feeding; 60 days	Improve fish growth, enhance digestive enzymes, and boost the fish's immunity, with no adverse effects on the morphometry of the hepatopancreatic and intestine organs	Abdel-Latif et al. (2023)
C. carpio; juveniles	Pennyroyal EO single compound	n.m.; 250 mg/kg in diet	Feeding; 8 weeks	Improve the growth performance, digestive enzymes, and physiological immunological responses of fish	Yousefi et al. (2023a)
C. carpio; juveniles	*Tanacetum balsamita* EO	n.m.; 100–200 mg/kg in diet	Feeding; 8 weeks	Improve fish digestive enzymes, growth, immunity, and antioxidants, and reduce stress in ammonia-exposed fish	Yousefi et al. (2023b)
O. niloticus; fingerlings	*Salvia hispanica*	Seeds; 10 g/kg in diet	Feeding; 8 weeks	Improve growth performance, antioxidants, and immune parameters	Abd El-Naby et al. (2023)
Mystus cavasius; n.m	*Spirulina platensis*	n.m.; 7.5–10% in diet	Feeding; 10 weeks	Improve the growth and immunological responses of fish and reduce mortality when challenged *with A. hydrophila*	Al Mamun et al. (2023)
C. carpio hematopterus; n.m	*Urtica dioica*	Leaves; 1.0 g/kg in diet	Feeding; 90 days	Improve the hematological and biochemical parameters of fish, with no mortality until the end of the experiment	Jangpangi et al. (2023)

Table 2 (continued)

Species; age	Plant used	Part of plant used; dosage	Administration; duration	Outcomes	References
Clarias gariepinus; n.m	*Melaleuca cajuputi*	Leaves; 12.7 and 25.4 mg/L	Bathing; 30 days	Accelerated epidermal cell migration of the wound, epidermal covering, and cell proliferation in 1 h of plant extract exposure, with no negative effects on fish health	Hassan et al. (2023)
O. niloticus; n.m	*Bougainvillea glabra*	Leaves; 4.5% in diet	Feeding; 30 days	Improve growth performance and disease resistance against *Enterococcus faecalis*	Uma et al. (2022)
Rutilus caspicus; n.m	*Satureja hortensis* EO	n.m.; 200 mg/kg in diet	Feeding; 60 days	Improve the growth performance, antioxidant activities, and immune responses of fish	Ghafarifarsani et al. (2022b)
Oreochromis niloticus; juveniles	Red thyme × pepper rosemary in the ration of 1:3	n.m; 1.2 mg/g in diet	Feeding; 20 days	Improve growth performance, oxidative stress, immune and hematological responses, and resistance against *A. hydrophila*	de Rezende et al. (2021)
O. niloticus; n.m	*Tribulus terrestris*	n.m.; 500 and 700 mg/kg in diet	Feeding; 45 days	Improve fish growth performance and reproductive efficiency	Hassona et al. (2020)
Colossoma macropomum; n.m	*Mentha piperita*	Leaves; 0.54 g/kg in diet	Feeding; 30 days	As an antiparasite against *Neoechinorhynchus buttnerae* and improve fish growth rates	de Souza Costa et al. (2020)

Table 2 (continued)

Species; age	Plant used	Part of plant used; dosage	Administration; duration	Outcomes	References
C. carpio; n.m	*Origanum vulgare*	n.m; 15 g/kg diet	Feeding; 8 weeks	Boost the antioxidative status and immunity against *A. hydrophila*	Abdel-Latif et al. (2020)
Carrasius auratus gibelio; juveniles	Fermented *Moringa leaves*	Leaves; 40% in diet	Feeding; 50 days	Enhance growth, antioxidant and immune responses, and resistance against *A. hydrophila*	Zhang et al. (2020)
O. niloticus; n.m	*Cantella asiatica*	Leaves and stems; 5 and 10 g/kg in diet	Feeding; 61 days	Improve serum lysozyme and peroxidase activities, phagocytosis, and respiratory burst; however, there are no effects on fish growth or survival rate	Srichaiyo et al. (2020a)
O. niloticus; fingerlings	*Fishwort, Houttuynia cordata*	Leaves and stem; 10 g/kg in diet	Feeding; 72 days	Improve serum and fish mucosal immunity, however, with no changes in growth or survival rates	Srichaiyo et al. (2020b)
O. niloticus; n.m	*Houttuynia cordata*	Leaves and stems; 10 g/kg in diet	Feeding; 72 days	Increase blood serum and immunity, however, with no effects on growth or survival rate	Srichaiyo et al. (2020b)
C. carpio; n.m	*Artemisia annua*	Leaves; 0.5–1.0 g/kg in diet	Feeding; 8 weeks	Improve growth, antioxidant capacity, and immunity	Sarhadi et al. (2020)
O. niloticus; n.m	Isolated compound; Menthol EO	0.25% of menthol EO in diet	Feeding; 30 days	Improve the immunity, antioxidative, and immune responses of fish intoxicated with chlorpyrifos	Dawood et al. (2020)

Table 2 (continued)

Species; age	Plant used	Part of plant used; dosage	Administration; duration	Outcomes	References
O. niloticus; juveniles	*O. vulgare, Melissa officinalis*	Leaves; 0.5% in diet	Feeding; 60 days	Improve growth, immune responses, and resistance against *A. hydrophila*	Mohammadi et al. (2020)
O. niloticus; n.m	*M. piperita*	Leaves; 0.25% in diet	Feeding; 50 days	Increase fish survival against *S. agalactiae*	de Souza Silva et al. (2019)
L. vannamei; post-larvae	Piperine isolated compound from *Piper nigrum* and *Piper longum*	n.m; 2.2 g/kg in diet	Feeding; 53 days	Improve shrimp growth, immunity, digestibility, and disease resistance against *V. parahaemolyticus*	Shin et al. (2023)
Macrobrachium rosenbergii; n.m	*M. cajuputi*	Leaves; 15 g/kg in diet	Feeding; 45 days	Improve growth, immunological responses, and resistance against *A. hydrophila*	Sahimi et al. (2022)
L. vannamei; post-larvae	*Solanum ferox* × *Zingiber zerumbet*	n.m; 100 mL/L of *S. ferox* and *Z. zerumbet*	n.m; n.m	Improve shrimp growth, survival rate, and hemato-immunological performances	Rahardjo et al. (2022)
L. vannamei; n.m	Isoquinoline alkaloid, sanguinarine (Iqs) isolated compound from *Macleaya cordata*	n.m.; 0.5 and 1.0% mg/kg in feed	Feeding; 30 days	All Iqs-fed groups showed better growth performance, immune responses, and survival rates against *V. parahaemolyticus*, with no histopathological lesions in the hepatopancreas or intestine	Bussabong et al. (2021)

Table 2 (continued)

Species; age	Plant used	Part of plant used; dosage	Administration; duration	Outcomes	References
Scylla paramamosain; intermolt males	*Galla chinensis*	Stem wood; 20 mg/L	Bathing; 7 days	Reduce low cumulative mortality to 25% in *V. parahaemolyticus*-infected crabs only after 2 days of therapeutic exposure, and return to a normal condition like control in the following days	Wu et al. (2021)
O. niloticus; juveniles	*Glycyrrhiza glabra L*	Root; 10 g/kg in diet	Feeding; 60 days	Result with significant increases ($p < 0.05$) in growth parameters, immunological responses, and fish survival against *A. hydrophila*	Abdel-Tawwab and EL-Araby (2021)
Penaeus vannamei; larvae	*O. vulgare*	5.0 mg/kg in diet	Feeding; 102 days	Act as antivirulence against vibriosis, reduce shrimp mortality in grow-out ponds to 40%, and increase shrimp production	Domínguez-Borbor et al. (2020)

n.m not mentioned

extract out more phytochemical compounds to optimize the biological potential; however, solutions with concentrated bioactive molecules may somehow display toxic effects on the treated fish and crustaceans. Moreover, Lech and Reigh (2012) have explained that the use of dried powdered plants is cheap compared to extracting them, but they end up having low biological activity due to the presence of indigestible and anti-nutritional compounds.

For the plant extracts, their biological activity is highly dependent on the extraction methods (e.g., solvent polarity, equipment, temperatures, etc.) used, which will influence the presence of bioactive compounds in the extract itself. The varied availability of phytochemicals may have an impact on the outcome of therapy. In addition to powdered and plant extracts, EO is frequently used as a feed supplement in aquaculture products due to its low toxicity and has also been regarded as safe (GRAS) by the US Food and Drug Administration (FDA 2016). Domínguez-Borbor et al. (2020) suggested that the feed supplementation of *O. vulgare* EO for *P. vannamei* from mysis growth stage below 2.5 μg/mL, whereas up to a maximum of 10 μg/mL in post larvae. Meanwhile, the inclusion of *O. vulgare* EO in the diet for 8 weeks was reported to significantly raise the SOD and CAT, whereas it reduced the MDA levels to boost the antioxidative status in common carp fingerlings (Abdel-Latif et al. 2020).

There are some studies that report the potential of medicinal plants with a mixture of other supplements (e.g., commercial antibiotics, probiotics). For example, a study conducted by Sadeghi et al. (2020) reported that the inclusion of lemon peel (1.5–3%) with *Bacillus licheniformis* at 10^8 CFU/mL in fish diets for 8 weeks improved their growth, immunity, and resistance against *A. hydrophila.* However, the combination of plant extracts with other supplements somehow showed exceptional results due to the chemical components of plants having a multi-targeted effect or ameliorating active substance absorption (Yousef and Haliem 2022). Other than the presence of bioactive compounds, dosages also play an important role in the efficiency and safety of the solution used, as too low dosages may not give the desired effect on fish, whereas too high dosages may impair fish growth, immunity, and survival (Orso et al. 2022). Generally, higher dosages may be used on powdered plant (e.g., 0.1 to 420 mg/100 g of fish/day), followed by ethanolic (e.g., 0.2 to 160 mg/100 g of fish/day) and aqueous extract (e.g., 0.03–200 mg/100 g of fish/day), whereas the lowest doses are used with EO (e.g., 0.005–30 mg/100 g of fish/day) (Reverter et al. 2021). For example, the inclusion of *Sargassum horneri* in the fish diet at 240 mg/100 g fish/day resulted in a significant growth rate for black sea bream; however, the inclusion of the same plant but in a higher amount decreased the fish growth ($p < 0.05$) (Shi et al. 2019). Reverter et al. (2021) concluded that treatment ranging from 2 to 4 weeks of most plant extracts were commonly effective than 8 weeks and more. Therefore, the choosing of the optimum dosages and duration to give the best effects on treated fish (produce no toxic effects) becomes the main objective of most studies.

There are a variety of ways to administer medicinal plants to aquatic life, including injection, bathing or immersion, and oral. Essential oils are normally dispensed via bathing or as dietary supplements for aquatic organisms (Bandeira Junior et al. 2022), where they can instantaneously affect the bacterial cell by regulating the gut bacteria flora and physiological function of the host (Ghafarifarsani et al. 2022a). Meanwhile, herbal extracts in powdered form are typically given orally by mixing with feed pellets (Dadgar et al. 2019). Oral administration is the most practical and favored method, particularly in extensive aquaculture, because it is stress-free and suitable for fish of all sizes. According to Kuebutornye and Abarike (2020), oral administration of medicinal plants through feed formulation is commonly used in tilapia aquaculture. Injection is scarcely utilized for treating infection because it is rapid and efficient; however, it is costly, strenuous, and will impose stress on the animal. Palanikani et al. (2020) reported that no significant difference was observed in terms of immunity and survival of *L. rohita* against *A. hydrophila* when supplemented with *A. paniculata* extract through several administration methods (i.e., injection, oral feeding, and diffusion). In addition, bath treatment is extensively used in ectoparasite treatment alone (Forwood et al. 2013).

Toxicological studies of medicinal plants in aquaculture

Even though herbal plants have demonstrated numerous advantages in acting as growth promoters, immunostimulants, antibacterial or antifungal agents, anti-parasite agents, and antivirals, in addition to being perceived as low-risk, it is important to carefully consider any potential health risks and hazards of herbal remedies. These medicinal plants, at certain concentrations, might also exert adverse effects on aquatic animals. Therefore, some clinical tools (e.g., LC_{50}, blood biochemistry, hematology parameters, and histopathology) are crucial to monitoring plant adverse effects on aquatic life. Most studies determine the in vivo *acut*e toxicity of medicinal plants using Artemia *nauplii* as a model animal representing shrimp and other crustaceans; however, the results are not accurate due to the lower sensitivity of *Artemia* (Zheng and Bossier 2023).

Numerous studies have indicated that plant extracts can have a toxic effect on aquatic organisms if an overdose has been taken. Akinsanya et al. (2016) manifested that fish displayed abnormal behaviors such as erratic swimming, hyperactivity, rapid opercula movement, and excessive mucus secretion prior to death during the exposure of plant extracts. Ekanem et al. (2007) observed some pathological changes in exposure fish within the 96-h exposure period of *Adenia cissampeloides* and *Blighia sapida,* such as moribund swimming, depigmentation, and increased opercular motion, which eventually led to spine fracture and death. Whereas Kavitha et al. (2012) demonstrated the elevation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), and a reduction in plasma protein and glucose levels when fish were exposed to *Moringa oleifera* seed extract, which indicates the damage to the cellular and hepatic systems of the treated animals. Abarike et al. (2022) reported the liver injuries (i.e., pyknosis nuclei, hydropic changes, erythrocyte congestion, and vacuolation) of Nile tilapia after being fed with > 5 GBNL g/kg (a mixture of guava, bitter, and Neem leaf extracts) after 8 weeks of administration. Shi et al. (2022) reported the mild injury of the kidney in *C. carpio* when fed with a minimum and high dosage of *A. membranaceus* and *L. plantarum*-fermented *A. membranaceus* for an 8-week period, and they suggested only supplying 0.1% (w/w) of them to reduce the side effects. Abdel-Latif et al. (2020) reported the normal histomorphology of the spleen, hematopoietic tissues, and the anterior kidneys in all treated fish with a 5–20 g/kg diet of *O. vulgare* EO, which contains high constituents of carvacrol and thymol. Moreover, the exposure of *C. citratus* through bath treatment at 60 mg/L, which contains higher geranial and neral EO, was the optimum concentration for *C. macropomum*, and if exceeded, it caused hyperplasia, lamellar fusion, detachment, and aneurysms in the *C. macropomum* gills (Gonzales et al. 2020). The inclusion of 0.25% of menthol EO in fish diet reduced the toxicity effects of chlorpyrifos with little congestion and no telangiectasia of the secondary filaments compared to control (Dawood et al. 2020). The inclusion of *O. vulagre* EO at 0.5–1% in fish diet improved histopathological damages and apoptosis in gills, kidneys, and hepatic tissues of *C. carpio* exposed to cypermethrin synthetic insecticides (Khafaga et al. 2020). Bussabong et al. (2021) revealed that the inclusion of sanguinarine isolated compound from *Macleaya cordata* extract at 0.5 and 1.0% in feed resulted in no histopathological lesions in both the intestine and hepatopancreas of *L. vannamei*. In a separate study, Rahardjo et al. (2022) revealed an increase in *L. vannamei* growth production, survival, and hemato-biochemical parameters when included with an 80 ml/L mixture of *Solanum ferox* and *Zingiber zerumbet* in the diet; however, this was accompanied by tissue changes in the gills, including vacuolation and hyperplasia, which may be caused by stress factors from plant extract exposure (Rahardjo et al. 2022). The supplementation of 10 g/kg of *Salvia hispanica*-enriched diets improved the intestine histomorphometry parts and also the length, width, absorption area, and goblet cells in the intestine tissue (Abd El-Naby et al. 2023). All these features will have positive effects on the gut health and nutrient absorption, thus improving the fish's performance (Nicholson et al. 2012). A similar result was revealed by Amin et al. (2019) when the Nile tilapia were fed 10 g/kg of *Ziziphus mauritiana* for 12 weeks, where positive results were observed in the intestine histomorphometry (i.e., increased villi heights and widths, absorption area, and thickness of the mucosal layer).

Conclusion and future directions

Although medicinal plant treatment is recognized as an alternative against disease with little adverse effect and minimal sequelae to animals and the environment, the toxicological study of herbal remedies in aquaculture needs further investigation. Furthermore, there were fewer studies on synergistic and antagonistic outcomes for combinations of herbal medicines to treat certain diseases in aquaculture, which requires the establishment of a safe concentration or dosage for administration. Moreover, regulatory policies on herbal medicine products need to be standardized globally and comply with the drug regulatory framework because adulteration of herbal products, improper storage, and improper preparation may affect the quality and purity of herbal medicines. In a nutshell, it can be concluded that the integration of immunoprophylaxis, legally permitted antibiotic and prebiotic utilization, biosecurity measures, and medicinal plant feed supplementation may greatly improve fish health and their survival rate.

Author contribution All authors have contributed to the final manuscript. JJYN: data curation, formal analysis, writing—original draft, writing—review and editing. NAHY: data curation, formal analysis, writing—original draft, writing—review and editing. NAE, NASN, and NAH: writing—review and editing, validation, and investigation. FA and ANI: writing—review and editing, validation, and investigation. MH: conceptualization, writing—original draft, validation, writing—review and editing.

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