Medicinal plants: are they safe enough for fish health?

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Abstract

The antipathogenic, immunomodulatory, and other beneficial properties of medicinal plants have made them a potentially ideal alternative to chemotherapeutic treatments for fish health. Therefore, medicinal plants as alternative therapeutics in fish have been regarded by many studies as healthy and safe because plants are “natural” and thus considered harmless. However, some toxicology studies raise concerns for these medicinal plants’ safety and implications for application on fish. These studies reveal that the plant application may lead to some potential health risks on fish. The aim of this review is to briefly summarize and discuss the adverse effects of medicinal plants on fish health.

Keywords: Fish health management; Medicinal plants; Adverse effects; Monitoring safety

1. Introduction

The fish industry is one of the most important major suppliers of animal protein worldwide. The industry has grown steadily over the past 50 years, and today, fish has become the main source of protein for many countries (Naylor et al. 2000; Bostock et al. 2010; FAO 2020). To date, producers have increased the size and efficiency of intensive production systems for cost-effective fish production (Halwart et al. 2007; Naylor et al. 2021). They are designed to improve economies of scale and maximize productivity by optimizing fish conditions. However, these intensive systems have also increased the risk and impact of infection problems. Any pathogen that compromises the efficiency of the fish production system can pose a threat to food security worldwide (Pérez-Sánchez et al. 2018).

Chemical and antibiotic treatments are commonly used for controlling pathogens. However, these have several disadvantages, including their high cost and posing detrimental effects to the environment when used in high dosage (Chuah et al. 2016; Watts et al. 2017). Furthermore, antibiotics cause immunosuppression and accumulation of residues in fish organs (Bulfon et al. 2013). Vaccination, which is another treatment method, can sometimes be ineffective against pathogens since immune responses are not well developed when applied to fry (Falaise et al. 2016). In addition, there is currently a lack of commercial vaccines against pathogens, especially parasite species (Adams 2019). Therefore, it is crucial to expand alternative therapeutics knowledge to develop sustainable systems to control fish pathogens. The increasing economic and environmental significance of fish diseases has enhanced the importance of studies of the medicinal plant benefits on fish health, as they have the potential to contribute to disease prevention and control strategies. Medicinal plants have pharmacological and therapeutic characteristics such as antifungal, antibacterial, antiviral, and antiparasitic or sometimes they can be an immunostimulant, growth promoter, or antistress agent due to the natural compounds they contain (Awad and Awaad 2017; Doan et al. 2020; Elumalai et al. 2021).

In scientific studies, most medicinal plants are proposed as biological control agents when used as directed and under the supervision of knowledgeable individuals (Reverter et al. 2017). However, as the application of medicinal plant research grows and many new candidates are introduced as antipathogenic or immunostimulant agents, concerns surrounding their safety are not thoroughly considered. Most medicinal plants used in fish production are still in the early research phase for setting, species of plant, dose, and exposure time. Toxic and therapeutic effects may occur at the same doses in plant administration for fish in any treatment method (Zhang et al. 2014; Zoral et al. 2018). In addition, medicinal plants contain multiple unknown biologically active constituents which have not been adequately researched for pharmacological and toxicological properties in fish health (Zoral et al. 2018). The limited knowledge of the potential adverse effects of these plants makes it difficult to encourage their rational use during bath or oral treatment.

The purpose of the review is to provide guidance on reviewing the adverse effects of medicinal plants on fish health.

2. Characterization of adverse effects of medicinal plant

Even after a successful medicinal plant application for a target disease or to promote fish health has been identified, this treatment still faces serious challenges. Unacceptable plant toxicities may threaten fish health with the increased dose and/or the duration of treatment. For example, side effects can sometimes occur as anti-nutritional (Dongmeza et al. 2006), immunosuppressive (Baba et al. 2018), and hepatotoxic and nephrotoxic effects (Zoral et al. 2018). Therefore, some clinical tools are crucial for monitoring plant adverse effects on fish health. In the context of standard guideline toxicology studies, scientists primarily characterize adversity based on the median lethal concentration (LC_{50}), morphologic endpoints at the cell, tissue, or organ level using histopathology, in combination with hematology and blood chemistry data (Wester and Canton 1991; OECD 2019; Teh et al. 1997; Green and McCormick 1999; Zakęś et al. 2008).

Evaluation of hematology parameters is a sensitive index, and important in the correct interpretation of the physiological state of the fish, minimizing side effects and determining the safe effective dose during medicinal plant applications (Burhop et al. 2004; Ramaiah 2007; Vazquez and Guerrero 2007; Gbadamosi et al. 2008; Ozer et al., 2008; Lorenz et al. 2018; Fazio 2019). In many toxicology studies, diagnosis of anemia is based on detecting a reduction in red cell mass (evaluated by measurement of hematocrit (Hct), RBCs count and hemoglobin concentration (Hb) (Maita 2007). Available evidence showed that the consumption of medicinal plants caused significant alteration in hematological biomarkers resulting in anemia in fish (Irkin et al. 2014; Gabriel et al. 2015). The white blood cells (WBCs) participate in both innate and adaptive immune responses by expressing cell-specific immune-relevant genes (Esmaeili 2021). Although most studies have shown the positive effects of various plant extracts on WBCs numbers (Nhu et al. 2019; Yousefi et al. 2021), one study showed that the administration of high doses of *Aloe vera* (L.) Burm.f. extract in feed reduced WBCs numbers in fish (Harikrishnan et al. 2011).

Blood biochemistry parameters are important diagnostic tools for the detection of abnormalities and monitoring the function of internal organs in fish (Allison 2012). Aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) are enzymes that are indicators of response to liver damage, liver function, and environmental stress (Hoffman and Solter 2008; Haschek et al. 2010; Kong et al. 2012; Kim and Kang 2016; Bhat et al. 2017; Silva et al. 2018; Cai et al. 2020). Some studies reported that various medicinal plant diets increased AST, ALT, and ALP levels resulting in hepatotoxicity in fish (Rao et al. 2006; Mohebbi et al. 2011; Kavitha et al. 2012; Zhang et al. 2020; Hoseini et al. 2021; Wangkahart et al. 2022).

3. Medicinal plants and their adverse effects on fish health

Several current studies have addressed the safe use of plants and determined their toxicological effects during treatment application in fish (Table 1).

3.1. Garlic (*Allium sativum* L.)

Allium sativum L., commonly known as "garlic," is a member of the Amaryllidaceae family and is cultivated throughout the world. Garlic has been used for many years to prevent and control disease and has been proven to have immunostimulant, antibacterial, antifungal, antiviral, and antiparasitic effects against fish pathogens (Nya and Austin 2009; Thanikachalam et al. 2010; Militz et al. 2013; Fridman et al. 2014; Reverter et al. 2014). The investigation of the effect of the garlic diet against fish pathogens is well documented but mostly based on observation of the survival rate of fish during infection or target pathogen burden in fish. Overdose of garlic diet known to be hepatotoxic and nephrotoxic was presented in several researches. Garlic extract in feed (2 g/kg body weight of fish) caused pathological changes in the liver such as pyknotic cells with cytoplasmic vacuoles in spiny catfish (*Chrysichthys auratus*) (Al-Salahy and Mahmoud 2003). In addition, the kidney exhibited cell degeneration such as necrotic cells, and extensive damage of the renal tubules after 11 days (Al-Salahy and Mahmoud 2003).

Moreover, El-Barbary (2018) reported that 20 g/kg of garlic supplemented feed for 14 days showed cases of liver damage such as intravascular hemolysis in blood vessels and vacuolar degeneration of hepatocytes in Nile tilapia (*Oreochromis niloticus*). A previous study reported that AST and ALT in rainbow trout (*Oncorhynchus mykiss*) were significantly ($p < 0.05$) higher in 40 and 50 g/kg of garlic supplemented feed groups at the end of 8 weeks (Mohebbi et al. 2011). In another study, ALT level in sturgeon (*Huso huso*) was increased but not significantly during the 1 g/kg of garlic supplemented feed after 60 days.

According to Irkin et al. (2014), using 20 g/kg of garlic powder in feed caused a decreased RBCs, Hb, and Hct, resulting in anemia in sea bass (*Dicentrarchus labrax*) for 60 days feeding.

In acute toxicity tests, safe doses of garlic for Nile tilapia have been assessed for *Trichodina* spp. treatment in two independent studies. LC_{50} of garlic extract for Nile tilapia fries was 6.186 e^{-8} mg/L for 96 h (El-Galil and Aboelhadid 2012) while the LC_{50} crude extract of garlic was 2259.44 mg/L for 2 h (Chitmanat et al. 2005). Although the age, size, and species of fish used in the two studies were the same, significant differences were found in the acute toxicity test. The tolerance level of fish for garlic extracts may vary depending on the dose of extract, exposure time of extract, the preparation method of extract, fish condition, water quality parameters such as ammonium, nitrite, dissolved oxygen, pH, and temperature, and other environmental stress factors.

3.2. Rosemary (*Rosmarinus officinalis* L.)

Rosmarinus officinalis L., commonly known as "rosemary," comes from the Lamiaceae family that is native to the Mediterranean region. Rosemary has been reported to have antibacterial, antiparasitic, immunostimulant, and other beneficial properties for fish health during bath and oral application (Abutbul et al. 2004; Zilberg et al. 2010; Zoral et al. 2017; Yousefi et al. 2019; Ebrahimi et al. 2020). However, some previous studies have reported that the use of rosemary has adverse effects on fish. For example, the use of 20 ml/100 g or more of rosemary supplemented feed for 20 days has shown to cause dose-dependent histopathological alterations such as pyknotic nucleus, cellular atrophy, irregular-shaped nucleus, and necrotic cell in the liver (Zoral et al. 2018). The presence of pyknotic nucleus and cytoplasmic vacuolation in the kidney was concluded that rosemary caused hepatotoxicity, and nephrotoxicity in common carp (*Cyprinus carpio*) (Zoral et al. 2018). A higher AST level was detected but not significantly during oral application of rosemary extract (Zoral et al. 2018). Interestingly, the study also found that 80 ml/100 g of rosemary supplemented feed has adverse effects on fish organs, but at the same time, this dose was effective against *Dactylogyrus minutus*, monogenean (Zoral et al. 2017 and Zoral et al. 2018). The low hepatosomatic index has been reported in great sturgeon (*Huso huso*) fed diets containing 2% rosemary extract after 8 weeks (Ebrahimi et al. 2020). Another study demonstrated that 168.5 ml/kg of rosemary supplemented feed in yellowtail kingfish (*Seriola lalandi*) gained less weight than the garlic diets (10 g/kg dried ground garlic and 3 g/kg Aquagarlic-P commercial) groups after 30 days (Ingelbrecht et al. 2020). This study might demonstrate liver function damage, leading to hepatotoxicity at high doses of rosemary extract or long-term feeding administration in fish.

Using solvent is the most common method of extraction of bioactive compounds from plant materials such as fruit, leaf, seed, and root (Huie 2002; Metrouh-Amir et al. 2015; Van Hai 2015). The lethal dose of rosemary leaf extract was investigated for common carp using two different solvents (Zoral et al. 2017). In the toxicity assays, the LC_{50} of rosemary ethanolic extract was 2.1 g/L while rosemary aqueous extract was 127.2 g/L at 30-min exposure in common carp. In addition, the active compound of 1,8-cineole in water and ethanol extract was determined to be 3.1 mg/100 g and 23 mg/100 g respectively. The important difference between the two results can be explained by the type of solvent used in the extract. Due to the presence of bioactive compounds of different chemical characteristics and polarities that may or may not be soluble in solvents, extract yields and the resulting bioactive compounds activities of plant materials vary with the type of solvent used (Jakopic and Veberic 2009; Gironi and Piemonte 2011; Hwang and Thi 2014). In addition, some studies have reported that the plant's production of bioactive molecules and their secondary metabolites vary depending on the seasonal change and geographical conditions (Prinsloo and Nogemane 2018). Hence, selecting the best solvent and collecting plants in specific seasons play a crucial role in the quality and quantity of the plant extraction.

3.3. Lang-du (*Euphorbia fischeriana* Steud.)

Euphorbia fischeriana Steud., commonly known as "lang-du" and a member of the Euphorbiaceae family, is commonly used as a medicinal plant supplement in northern China. The anthelmintic activity was observed against the eggs and adults of *D. vastator*, monogenean, using lang-du ethanolic acetate extract at 10 and 20 mg/L in vitro (Zhang et al. 2014). In in vivo adult parasite survival test, the effective dose of the ethyl acetate extract of lang-du was calculated at 14 mg/L after 48 h, which eliminated 80.1% of monogenean from goldfish (*Carassius auratus*) gills. However, the min anthelmintic dose (14 mg/L) of the ethyl acetate extract was almost the same lethal dose (LC_{50} = 13.65 mg/L) for fish. This study and the rosemary extract studies (Zoral et al. 2017 and Zoral et al. 2018) are strong evidence that the toxic and therapeutic doses of the target plant are sometimes very close. Therefore, the effects of plant extracts against pathogens and on fish health should be studied carefully.

3.4. Lemon balm (*Melissa officinalis* L.)

Melissa officinalis L. is a member of the family Lamiaceae and grows widely in central and southern Europe and in Asia Minor. The effects of dietary lemon balm have been reported as immunomodulator, growth promoter, antibacterial, and antioxidant agent in the scientific literature for fish health (Bilen et al. 2020; Mohammadi et al. 2020; Wang et al. 2022). However, the positive effects of lemon balm depend on the administration period and dose of extract in feed. The occurrence of nephrotoxicity was reported with histopathological alterations including degenerative and necrotic lesions in the kidney tubules of rainbow trout for 30 days of oral application using 400 and 1350 mg/kg of lemon balm supplemented feed (Jafarpour et al. 2018). In addition, the heart showed some local hemorrhage focal infiltration of lymphocytic inflammatory cells in the highest dose of the lemon balm extract. Researchers found that both doses of lemon balm cause genotoxicity, which indicates the potential DNA damage in erythrocytes (Jafarpour et al. 2018).

3.5. Neem (*Azadirachta indica* A. Juss.)

Azadirachta indica A. Juss., commonly known as “neem,” belongs to the Meliaceae family, is native to India, and was introduced to Africa, the Middle East, and the Caribbean. Neem has been reported to be antibacterial against *Vibrio harveyi*, antiparasitic against a flagellate protozoan of the Hexamitidae family, and immunostimulator using oral treatment in fish (Talpur and Ikhwanuddin 2013; Rather et al. 2016; Mondal et al. 2020). In addition, combined administration of neem, guava (*Psidium guajava* L.), and bitter leaf (*Vernonia amygdalina* Del.) increased disease resistance against co-infections of *Streptococcus agalactiae* and *Aeromonas jandaie* is possible in Nile tilapia after 8 weeks of oral application (Abarike et al. 2022). However, ALT, AST, and ALP significantly increased on the plant diet of 3 g/kg and above, and severe cell alterations such as pyknosis, necrotic cells, congestion of sinusoids, and cytoplasmic vacuolation occurred in the liver after 8 weeks of oral administration (Abarike et al. 2022). Nile tilapia was exposed to neem extract (1/10 LC_{50} = 112.5 mg/L) for 3 weeks (El-Badawi et al. 2015). After the bath treatment, vacuolar degeneration, hepatocytic necrosis, congestion of blood vessels, and pyknotic nucleus were observed in the liver. Typical severe histopathological alterations such as hyperplasia, lamellar fusion, epithelial uplifting, and necrosis were found in the gills. The posterior kidney was observed with severe tubular necrosis and cytoplasmic vacuolation at the end of bath application (El-Badawi et al. 2015). Aqueous neem extracts (LC_{50} = 6.03 mg/L at 96 h) caused erratic swimming and respiratory distress in redbelly tilapia (*Coptodon zillii*) (Omoregie and Okpanachi 1997).

3.6. Moringa (*Moringa oleifera* Lam.)

Moringa oleifera Lam. from the Moringaceae family is commonly known as "moringa" or "drumstick tree." It grows widely in many tropical and subtropical regions such as Africa, South and Central America, Mexico, Hawaii, and throughout Asia and Southeast Asia. The oral application of moringa extract for 50 days has been found to have antibacterial activity against *Aeromonas hydrophila*, immunostimulator, and growth promoter in gibel carp (*Carassius auratus gibelio* var. CAS III) (Zhang et al. 2020). In addition, ALT and AST levels were significantly lower in all doses of moringa diet (5–15 g/100 g of moringa supplemented feed) groups (Zhang et al. 2020). However, diet groups showed hepatosomatic index decreased. This finding is related to hepatotoxicity (Hamid et al. 2021) or increased metabolism of fish and protein synthesis resulting in the consumption of glycogen and lipid of hepatocytes for enhancement immunity (Chen et al. 2019). Interestingly, all treatment groups showed significantly higher ALP. This finding is evidence that the moringa diet in all doses can be a potential stressor or hepatotoxicity in fish.

Another study also found that organ dysfunction biomarkers such as AST, ALT, and ALP were gradually increased in the safe dose (12.40 mg/L) of moringa extract depending on exposure time in common carp for 35 days (Kavitha et al. 2012). This result is referred to as cellular damage in the liver. In addition, RBCs, Hct, Hb, and mean corpuscular hemoglobin concentration (MCHC) were significantly decreased in common carp, resulting in anemia in a safe dose of moringa extract (a non-lethal dose of 1/10th of 96 h LC_{50} value of 12.40 mg/L) (Kavitha et al. 2012).

Moringa contains saponin and tannin, which cause anti-nutritional factor (Madalla et al. 2013; Samtiya et al. 2020). All moringa diet (15–60 mg/100 g of moringa supplemented feed) showed poor diet palatability, and a significant reduction in feed intake in Nile tilapia (Madalla et al. 2013). In addition, all diet groups showed a significant decline in growth rate compared with the control group at the end of 8 weeks (Madalla et al. 2013). Similarly, Nile tilapia showed significantly low body mass gain, resulting to reduction in specific growth rate in all moringa methanolic extract diet (106–177 g/kg of moringa supplemented feed) groups compared to control after 10 weeks (Dongmeza et al. 2006). Interestingly, fish fed diets which contained lower amounts of tannins, saponins, and total phenolics in extract resulted in better growth parameters than other experimental diet groups. In light of this, the effects of different antinutrients should be viewed not only individually, but also in terms of the effects of different antimetabolites acting together, in relation to their proportions and quantities.

3.7. Olive (*Olea europaea* L.)

Olea europaea L. belongs to the Oleaceae family and is an important plant distributed in the Mediterranean region. A total of 1 and 2 g/kg of olive supplemented feed have been reported to alleviate pesticide, Danitol®, toxicity through the regulation of the haemolysis, SOD, CAT, ALT, AST, and ALP enzymes in common carp (Ghelichpour et al. 2020). In addition, Sokooti et al. (2021) reported that dietary olive extract in all doses (200 and 400 mg/kg of olive supplemented feed) had enhanced WBCs and lysozyme in common carp for 75 days of feeding. In another study, 1 g/kg of dietary supplementation olive extract diet has also been reported to upregulate the non-specific immune response genes including IL-8, IL-1β, and TNF-α in rainbow trout, leading to a high survival rate against *Yersinia ruckeri* (Baba et al. 2018). However, high doses of olive extract in feed including 2.5, 5, and 10 g/kg group showed immunosuppression effect on IL-8, IL-1β, and TNF-α, resulting in a low survival rate in *Y. ruckeri* challenge compared to the control after 60 days. A total of 5 and 10 g/kg of olive supplemented feed showed negative relative and specific growth rate in common carp after 60 days compared to all lower doses (1 and 2.5 g/kg of olive supplemented feed) and control (Zemheri-Navruz et al. 2020). In addition, digestive enzymes including α-amylase, protease, and lipase were found significantly lower in higher doses (5 and 10 g/kg). These adverse effects are a clear example that sometimes high doses did not yield the enhanced effects observed at lower concentrations (Sakai 1999). Therefore, there is no positive correlation between increased extract dose and guaranteed beneficial effect on fish health.

3.8. Russian olive (*Elaeagnus angustifolia* L.)

Elaeagnus angustifolia L. belongs to the Elaeagnaceae family. Vernacular names are Russian olive or oleaster. The plant is cultivated in western and central Asia, Europe, and some parts of North America (Hamidpour et al. 2017; Saboonchian et al. 2014). Russian olive extract in feed enhanced immune-related parameters such as total serum protein, globulins, lysozyme activity, lymphocytes production, and WBCs in common carp (Hoseini et al. 2021). However, fish fed with 30 g/kg of Russian olive supplemented feed showed significantly higher AST, and ALT induced hepatotoxicity after 8 weeks (Hoseini et al. 2021).

3.9. Date palm (*Phoenix dactylifera* L.)

Phoenix dactylifera L., originating from the regions of Southwest Asia and North Africa, is a plant from the Arecaceae family (Baliga et al. 2011). It has been reported to be a potent growth promoting agent in common carp (Hoseinifar et al. 2015; Ahmed et al. 2017; Mohammadi et al. 2018). In addition, the immunostimulatory effect of the date palm diet was reported on the skin, resulting in increase in skin mucosal immunity (Hoseinifar et al. 2015), and an increase in lysozyme activity (Mohammadi et al. 2018). The toxicity of the date palm diet was investigated in different doses (0.5, 1, 2, and 4 g/kg of palm date extract) using a blood chemistry assay on common carp. AST, ALT, and ALP on common carp significantly increased in a dose-dependent manner, resulting in hepatotoxicity after 60 days (Mohammadi et al. 2018).

3.10. Astragalus (*Astragalus membranaceus* or *Astragali Radix* Fisch. ex Bunge)

Astragalus membranaceus Fisch. ex Bunge from the Fabaceae family is one of the important Chinese traditional medicinal plants which is widely abundant in the northern and eastern parts of China, as well as Mongolia and Korea. It has been reported to play an important role in biological responses, including growth promoter, immunostimulatory activity, and antibacterial against *A. hydrophila* in Nile tilapia and common carp (Yin et al. 2006, 2009; Ardó et al. 2008; Zahran et al. 2014). However, there has been one report available regarding the astragalus diet that caused hepatotoxicity in juvenile pikeperch (*Sander lucioperca*) (Zakęś et al. 2008). Diets containing 1 g/kg astragalus powder and a mixture of two plant powders (0.5 g/kg astragalus + 0.5 g/kg lonicera (*Lonicera japonica* Thunb.) damaged the liver and intestinal cells after 8 weeks. Both diet groups showed cell alterations such as fatty vacuolation, decreased nuclear size of hepatocytes, and necrotic cells in the liver. The enterocyte and its nuclear size in the intestine were significantly decreased in all treatment groups with astragalus (Zakęś et al. 2008).

3.11. *Aloe vera* (L.) Burm.f.

Aloe vera (L.) Burm.f. is a member of the Asphodelaceae family and is widely distributed in tropical and subtropical regions. Previous studies reported that enrichment of diet with *A. vera* has been shown to be growth promoter, anti-depressant, and immunostimulant as well as an antibacterial agent against *Streptococcus iniae* and *S. agalactiae* in fish (Alishahi et al. 2010; Heidarieh et al. 2013; Gabriel et al. 2015). On the other hand, anemia has been reported due to the use of high doses of *A. vera* extract in Nile tilapia after 60 days (Gabriel et al. 2015). Lower hematological indices such as RBCs, Hb, and Hct were reported in the highest doses of supplemented *A. vera* (40 g/kg in feed), whereas no significant difference was observed at low doses of *A. vera* diet (5, 10, and 20 g/kg *A. vera* in feed) compared to control. In addition, the WBC count was found significantly lower in fish fed 40 g/kg *A. vera* supplemented diet group. Overdose of *A. vera* extract for a long time causes an immunosuppression effect in fish (Harikrishnan et al. 2011).

3.12. Bael (*Aegle marmelos* (L.) Corrêa)

Aegle marmelos (L.) Corrêa belongs to the Rutaceae family. Nowadays, this plant has grown in the South East Asia region and some parts of USA states such as Florida and Hawaii (Rana et al. 1997). The medicinal properties of *A. marmelos* have been reported to enhance digestive enzymes, growth performance, antioxidant capacity, and non-specific immune response against *Streptococcus agalactiae* in Nile tilapia and *A. hydrophila* in common carp (Pratheepa et al. 2010; Wangkahart et al. 2022). In addition, ALT and AST were found significantly lower in all treatment groups (5, 10, 15, and 20 g/kg of bael supplemented feed) after 8 weeks (Wangkahart et al. 2022). Interestingly, ALP activity increased significantly in a dose-dependent manner. High ALP levels indicate that stress or hepatotoxicity has occurred in the host during oral therapy.

3.13. Kulekhara (*Hygrophila auriculata* (Schumach.))

Hygrophila auriculata (Schumach.) belongs to the family of Acanthaceae. This genus is distributed in tropical region of Asia and Africa (Sethiya et al. 2018). It is reported for its growth promoter property, an immunostimulant agent, and a supporter of hematology parameters in *Cirrhinus mrigala* (Indian major carp) (Kumar et al. 2022). However, the ALP significantly increased in all treatment groups (5, 10, and 15 g/kg of kulekhara supplemented diet).

3.14. Chaff-flower (*Achyranthes aspera*)

A medicinal plant, *Achyranthes aspera* L., is a member of the Amaranthaceae and is commonly distributed throughout the tropical regions of India (Chakrabarti and Rao 2012). The growth promoter and immunostimulatory properties of the plant are well studied in walking catfish (*Clarias batrachus*) and various carps like catla (*Catla catla*) and rohu (*Labeo rohita*) (Sharma et al. 2019, 2021). A total of 1, 10, and 50 g/kg of chaff flower diet groups significantly increased ALP level in rohu after 4 weeks (Rao et al. 2006).

3.15. Turmeric (*Curcuma longa* L.)

The perennial herb *Curcuma longa* L. belongs to the family Zingiberaceae. It is a native species in the tropical and subtropical regions of Asia, America, and Africa (Yu et al. 2002). It was reported a growth promoter and an immunostimulant in fish (Fagnon et al. 2020). Although this herb had positive effects on health parameters, fish fed 0.1–5 g/kg turmeric added feed showed significantly higher ALP levels after 60 days (Sahu et al. 2008).

3.16. Safflower (*Carthamus tinctorius* L.)

Carthamus tinctorius L. belongs to the family Asteraceae and is a natural plant of Central Asia. Safflower is another candidate medicinal plant used as an antibacterial, immunomodulator, and growth promoter for fish health (Dadras et al. 2016; Zargari et al. 2018). Toxicity assay of the safflower was investigated on zebrafish (*Danio rerio*) (Xia et al. 2017). According to LC_{50} assay, 345.6 mg/L was toxic for zebrafish embryos at 96-h bath treatment. Furthermore, teratogenetic effects such as pericardial oedema, tail malformation, notochord malformation, abnormal head-trunk angle, scoliosis, yolk edema, and growth retardation were observed after 48-h bath treatment. In addition, concentrations of plant extract that are safe for embryos significantly altered and inhibited larvae swimming ability, indicating neurotoxicity (Xia et al. 2017). It revealed significant inhibition of heart rate at 48 and 72 h post fertilization at 250 mg/L bath administration which was evidence of developmental toxicity to the cardiac (Xia et al. 2017). The researchers also found that the levels of defense enzymes (SOD, CAT, and GPX) were significantly manipulated in zebrafish larvae, while the MDA content increased, the caspase-3 activity decreased, and the mRNA levels of the related genes might have triggered toxicity. Therefore, due to developmental abnormalities, safflower exposure is responsible for oxidative stress and increased apoptosis in zebrafish (Xia et al. 2017).

3.16. Other medicinal plants

Some studies have reported that zebrafish embryos show adverse effects when exposed to various plant extracts that have previously shown antipathogenic properties in fish (Reverter et al. 2014). For example, *Momordica cochinchinensis* Spreng. (39.4 ng extract/embryo) caused cardiotoxicity, oxidative stress, and apoptosis (Du et al. 2021). An increase in dose from 5 to 100 g/kg of *Tinospora cordifolia* (Willd.) Miers ex Hook.f. & Thomson extract led to increased mortality of zebrafish embryos after 48 h (Romagosa et al., 2016). Another toxicity experiment found that an aqueous extract of *Orthosiphon stamineus* Benth. (LC_{50} = 1.68 mg/ml) significantly caused cardiotoxicity in zebrafish embryos (Ismail et al. 2017). In addition, *Andrographis paniculata* (Burm.f.) Wall. (LC_{50} = 0.52 mg/ml), *Cinnamon zeylanicum* Blume (LC_{50} = 0.98 mg/ml), *Curcuma xanthorrhiza* Roxb. (LC_{50} = 0.74 mg/ml), and *Eugenia polyantha* Barb. Rodr. (LC_{50} = 0.92 mg/ml) caused significant adverse effects, resulting in decreased survival rate, malformation of organs, abnormal heartbeats, and delay in hatching rates in zebrafish embryos after 48 h (Ismail et al. 2017).

4. Negative effects on non-target organisms

Although medicinal plant substances are biodegradable, their use in water can have an adverse effect on non-target organisms that are more sensitive than fish, such as plankton. For example, *Melaleuca alternifolia* Cheel extract presented acute toxicity against nontarget *Daphnia magna*, with a 24-h LC_{50} of 80.64 mg/kg (Conti et al. 2014). In another study, *Ichthyophthirius multifiliis* infection in small scaled pacu (*Piaractus mesopotamicus*) was treated using 2-h daily baths of *M. alternifolia* extract for 5 days, using a concentration of 50 mg/kg (Valladão et al. 2015).

5. Concluding Remarks and Future Research

Medicinal plant treatment sometimes carries a risk of adverse effects, as in the examples above. Therefore, a brief discussion of some of the adverse effects of plants on fish health studies will ensure their safe use in fish. In the future, medicinal plants will continue to have great potential as alternative therapeutic agents for fish health management. To date, a limited number of medicinal plants have been studied to have beneficial effects as well as adverse effects on fish health. According to adverse effects studies, the tolerance level of fish for toxicity depends upon fish species, condition, age, plant species, type of plant extract, concentration of plant extract, and bath or oral administration time.

There is still an urgent need for more detailed toxicology studies focusing on the adverse effects of some medicinal plants not mentioned in this review. A lack of knowledge of toxicology leads to misinterpretation and misunderstanding. Hence, such toxicology studies will help establish a suitable and safe dosage of antipathogenic, immunostimulant, or other beneficial properties of plants for fish health management.

5. References

Abutbul, S., Golan-Goldhirsh, A., Barazani, O., Zilberg, D., 2004. Use of *Rosmarinus officinalis* as a treatment against *Streptococcus iniae* in tilapia (*Oreochromis* sp.). Aquaculture 238, pp. 97–105. doi.org/10.1016/j.aquaculture.2004.05.016

Adams A., 2019. Progress, challenges and opportunities in fish vaccine development. Fish Shellfish Immunol., 90, pp. 210-214. doi.org/10.1016/j.fsi.2019.04.066

Alishahi M., Ranjbar M.M., Ghorbanpour M., Peyghan R., Mesbah M., Razi J.M., 2010. Effects of dietary *Aloe vera* on some specific and none specific immunity in the common carp (*Cyprinus carpio*). Int. J. Vet. Res., 4, pp. 189-195. doi.org/10.22059/IJVM.2010.21352

Allison R.W., 2012. Laboratory Evaluation of the Liver. Thrall M.A., Weiser G., Allison R., Campbell T.W. (Eds.), Veterinary hematology and clinical chemistry, John Wiley & Sons, Ames (IA) (2012), pp. 298-312.

Al-Salahy, Mahmoud B, 2003. Metabolic and histological studies on effect of garlic administration on the carnivorous fish chrysichthysauratus. Egyptian journal of biology, 5, pp. 94-107.

Ardó L., Yin G., Xu P., Váradi L., Szigeti G., Jeney Z., Jeney G., 2008. Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of Nile tilapia (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila*. Aquaculture, 275(1-4), pp. 26-33. doi.org/10.1016/j.aquaculture.2007.12.022

Awad E., Awaad A., 2017. Role of medicinal plants on growth performance and immune status in fish. Fish Shellfish Immunol., 67, pp. 40-54. doi.org/10.1016/j.fsi.2017.05.034

Baba E., Acar Ü., Yılmaz S., Zemheri F., Ergün S., 2018. Dietary olive leaf (*Olea europea* L.) extract alters some immune gene expression levels and disease resistance to *Yersinia ruckeri* infection in rainbow trout *Oncorhynchus mykiss*. Fish Shellfish Immunol., 79 (2018), pp. 28-33. doi.org/10.1016/j.fsi.2018.04.063

Bhat I.A., Rathor P.K., Mir I.N., Gireesh-Babu P., Goswami M., Sundaray J.K., Sharma R., 2017. Toxicological evaluation and effective dose selection of eurycomanone, a quassinoid of *Eurycoma longifolia* plant in fishes. Aquaculture, 481, pp. 94-102. doi.org/10.1016/j.aquaculture.2017.08.030

Bilen S., Altief T.A.S., Özdemir K.Y., Salem M.O.A., Terzi E., Güney K., 2020. Effect of lemon balm Effect of lemon balm (*Melissa officinalis*) extract on growth performance, digestive and antioxidant enzyme activities, and immune responses in rainbow trout (*Oncorhynchus mykiss*). Fish Physiology and Biochemistry, 46, pp. 471-481. doi.org/10.15832/ankutbd.870923

Bostock J., McAndrew B., Richards R., Jauncey K., Telfer T., Lorenzen K., Little D., Ross L., Handisyde N., Gatward I., Corner R., 2010. Aquaculture: global status and trends. Phil. Trans. R. Soc. B., 365, pp. 2897-2912. doi.org/10.1098/rstb.2010.0170

Bulfon C., Volpatti D., Galeotti M., 2013. Current research on the use of plant-derived products in farmed fish. Aquac. Res., 46, pp 513-551. doi.org/10.1111/are.12238

Burhop K., Gordon D., Estep T., 2004. Review of hemoglobin-induced myocardial lesions. Artif. Cells Blood Substit. Immobil. Biotechnol., 32, pp. 353-374. doi.org/10.1081/bio-200027429

Cai X., Zhang J., Lin L., Li Y., Liu X., Wang Z., 2020. Study of a noninvasive detection method for the high-temperature stress response of the large yellow croaker (*Larimichthys crocea*). Aquaculture Reports, 18, 100514. doi.org/10.1016/j.aqrep.2020.100514

Chakrabarti R., Rao Y.V., 2012. *Achyranthes aspera* enhances immunity and antigen clearance in common carp, *Cyprinus carpio* L. Journal of Fish Diseases, 35, pp 389–392. doi.org/10.1111/j.1365-2761.2012.01356.x

Chen X.Q., Zhao W., Xie S.W., Xie J.J., Zhang Z.H., Tian L.X., Liu Y.J., Niu J., 2019. Effects of dietary hydrolyzed yeast (*Rhodotorula mucilaginosa*) on growth performance, immune response, antioxidant capacity and histomorphology of juvenile Nile tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol., 90, pp. 30-39. doi.org/10.1016/j.fsi.2019.03.068

Chitmanat C., Tongdonmuan K., Nunsong W., 2005. The use of crude extracts from traditional medicinal plants to eliminate *Trichodina* sp. in tilapia (*Oreochromis niloticus*) fingerlings. Songklanakarin. J Sci Technol 27 (Suppl. 1), pp. 359–364.

Chuah L.O., Effarizah M.E., Goni A.M., Rusul G., 2016. Antibiotic Application and Emergence of Multiple Antibiotic Resistance (MAR) in Global Catfish Aquaculture. Curr. Environ. Health Rep. 3, pp. 118–127. doi.org/10.1007/s40572-016-0091-2

Conti B., Flamini G., Cioni P.L., Ceccarini L., Macchia M., Benelli G., 2014. Mosquitocidal essential oils: are they safe against non-target aquatic organisms? Parasitology Research, 113, pp. 251–259. doi.org/10.1007/s00436-013-3651-5

Dadras H., Hayatbakhsh M.R., Shelton W.L., Golpour A., 2016. Effects of dietary administration of Rose hip and Safflower on growth performance, haematological, biochemical parameters and innate immune response of Beluga, *Huso huso* (Linnaeus, 1758). Fish Shellfish Immunol., 59, pp. 109-114. doi.org/10.1016/j.fsi.2016.10.033

Dongmeza E, Siddhuraju P, Francis G, Becker K., 2006. Effects of dehydrated methanol extracts of moringa (*Moringa oleifera* Lam.) leaves and three of its fractions on growth performance and feed nutrient assimilation in Nile tilapia (*Oreochromis niloticus* L.). Aquaculture, 261, pp. 407–22. doi.org/10.1016/j.aquaculture.2006.08.006

Doan H.V., Soltani E., Ingelbrecht J., Soltani M., 2020. Medicinal Herbs and Plants: Potential Treatment of Monogenean Infections in Fish. Rev Fish Sci Aquacult., 28(2), pp. 260-282. doi.org/10.1080/23308249.2020.1712325

Du Z.C., Xia Z.S., Huang Y.F., Peng Y., Cao B.B., Li C.Q., Liang Y.F., Zhao F.H., Zhang M.Z., Chen Z.M., Hou X.T., Hao E.W., Deng J.G., 2021. Cardiotoxicity induced by *Cochinchina momordica* seed extract in zebrafish. J. Appl. Toxicol., 41(8):1222-1231. doi: 10.1002/jat.4108. Epub 2021 Jan 14. PMID: 33445225. doi.org/10.1002/jat.4108

Ebrahimi E., Haghjou M., Nematollahi A., Goudarzian F., 2020. Effects of rosemary essential oil on growth performance and hematological parameters of young great sturgeon (*Huso huso*). Aquaculture, 521, 734909. doi.org/10.1016/j.aquaculture.2019.734909

El-Badawi A., Gaafar A.Y., Abbas H.H., Authman M.M.N., 2015. Toxic Effects of Neem Seeds Oil on Nile Tilapia (*Oreochromis niloticus*) and Application of Different Trials of Control. Research Journal of Pharmaceutical, Biological and Chemical Science, 6(1), pp. 645-658.

El-Barbary M.I., 2018. Impact of Garlic and Curcumin on the Hepatic Histology and Cytochrome P450 Gene Expression of Aflatoxicosis *Oreochromis niloticus* Using RT-PCR. Turkish Journal of Fisheries and Aquatic Sciences 18, pp. 405-415. doi.org/10.4194/1303-2712-v18_3_06

El-Galil M.A.A., Aboelhadid S.M., 2012. Trials for the control of trichodinosis and gyrodactylosis in hatchery reared *Oreochromis niloticus* fries by using garlic. Vet Parasitol 185, 57–63. doi.org/10.1016/j.vetpar.2011.10.035

Elumalai P., Kurian A., Lakshmi S., Faggio C., Esteban M.A., Ringø E., 2021. Herbal Immunomodulators in Aquaculture. Rev Fish Sci Aquacult., 29(1), pp. 33-57. doi.org/10.1080/23308249.2020.1779651

Esmaeili M., 2021. Blood Performance: A New Formula for Fish Growth and Health. Biology 2021, 10, 1236. doi.org/10.3390/biology10121236

Fagnon M.S., Thorin C., Calvez S., 2020. Meta-analysis of dietary supplementation effect of turmeric and curcumin on growth performance in fish. Reviews in Aquculture, 12(4), pp. 2268-2283. doi.org/10.1111/raq.12433

FAO, 2020. The State of World Fisheries and Aquaculture 2020; FAO: Rome, Italy, 2020.

Falaise C., François C., Travers M.A., Morga B., Haure J., Tremblay R., Turcotte F., Pasetto P., Gastineau R., Hardivillier Y., Leignel V., Mouget J.L., 2016. Antimicrobial Compounds from Eukaryotic Microalgae against Human Pathogens and Diseases in Aquaculture. Mar. Drugs, 14, 159. doi.org/10.3390/md14090159

Fazio F., 2019. Fish hematology analysis as an important tool of aquaculture: A review. Aquaculture, 500, pp. 237-242. doi.org/10.1016/j.aquaculture.2018.10.030

Fridman S., Sinai T., Zilberg D., 2014. Efficacy of garlic based treatments against monogenean parasites infecting the guppy (*Poecilia reticulata* (Peters)). Vet. Parasitol., 203 (1–2) pp. 51-58. doi.org/10.1016/j.vetpar.2014.02.002

Gabriel N.N., Qiang J., He J., Ma X.Y., Kpundeh M.D., Xu P., 2015. Dietary *Aloe vera* supplementation on growth performance, some haemato-biochemical parameters and disease resistance against *Streptococcus iniae* in tilapia (GIFT). Fish & Shellfish Immunol., 44, pp. 504–514. doi.org/10.1016/j.fsi.2015.03.002

Gbadamosi O. K., Adeparusi E.A, Olanrewaju J., 2008. Basic haematogical parameters in African catfish, *Clarias gariepinus* (Burchell, 1822) fed ascorbic acid supplemented diets. Res. J. Anim. Sci., 2, pp. 17-21.

Ghelichpour M., Mirghaed A.T. Dawood M.A.O., Hoseinifar S.H., Van Doan H., 2020. Alteration of haematological and antioxidant parameters in common carp (*Cyprinus carpio*) fed olive (*Olea europea*) leaf extract after exposure to danitol®. Aquac. Res., 52(3), pp. 1088-1095. doi.org/10.1111/are.14964

Gironi F. Piemonte V., 2011. Temperature and solvent effects on polyphenol extraction process from chestnut tree wood. Chem. Eng. Res. Des., 89, pp. 857–862. doi.org/10.1016/j.cherd.2010.11.003

Green B.S., McCormick M.I., 1999. Influence of larval feeding history on the body condition of *Amphiprion melanops*. Journal of Fish Biology 55, pp. 1273-1289. doi.org/10.1111/j.1095-8649.1999.tb02075.x

Halwart M., Soto D., Arthur J.R. (eds), 2007. Cage aquaculture: regional reviews and global overview. Rome, Italy: FAO fisheries technical paper no. 498. Rome, Italy: Food and Agriculture Organization of the United Nations.

Hamid N., Juaid M., Wang Y., Pu S., Hia P., Pei D., 2021. Chronic exposure to PPCPs mixture at environmentally relevant concentrations (ERCs) altered carbohydrate and lipid metabolism through gut and liver toxicity in zebrafish. Environ. Pollut., 273, 116494. doi.org/10.1016/j.envpol.2021.116494

Hamidpour R., Hamidpour S., Hamidpour M., Shahlari M., Sohraby M., Shahlari N., Hamidpour R., 2017. Russian olive (*Elaeagnus angustifolia* L.): From a variety of traditional medicinal applications to its novel roles as active antioxidant, anti-inflammatory, anti-mutagenic and analgesic agent. J. Tradit. Complement. Med. 7, pp. 24–29. doi:org/10.1016/j.jtcme.2015.09.004

Harikrishnan R., Balasundaram C., Heo M.S., 2011. Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. Aquaculture, 317, pp. 1-15. doi.org/10.1016/j.aquaculture.2011.03.039

Haschek W.M., Walling M.A., Rousseaux C., 2010. Fundamental of Toxicologic Pathology, Academic Press, San Diego, California.

Heidarieh M., Mirvaghefi R.A., Sepahil A., Sheikhzadel N., Shahbazfar A.M., Akbari M., 2013. Effects of dietary *Aloe vera* on growth performance, skin and gastrointestinal morphology in rainbow trout (*Oncorhynchus mykiss*). Turk. J. Fish. Aquat. Sci., 3, pp. 367-373. doi.org/10.4194/1303-2712-v13_2_20

Hoffman W.E., Solter S.F., 2008. Diagnosis enzymology of domestic animals. In: Kaneko JJ et al (eds) Clinical biochemistry of domestic animals, 6th ed. Academic, San Diego, pp 351–378.

Hoseini S.M., Mirghead A.T., Iri Y., Hoseinifar S.H., Doan H.V., Reverter M., 2021. Effects of dietary Russian olive, *Elaeagnus angustifolia*, leaf extract on growth, hematological, immunological, and antioxidant parameters in common carp, *Cyprinus carpio*. Aquaculture, 536 (15), 736461. doi.org/10.1016/j.aquaculture.2021.736461

Huie C.W., 2002. A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants. Anal Bioanal Chem, 373, pp 23–30. doi.org/10.1007/s00216-002-1265-3

Hwang, E.S.; Thi, N.D., 2014. Effects of Extraction and Processing Methods on Antioxidant Compound Contents and Radical Scavenging Activities of Laver (*Porphyra tenera*). Prev. Nutr. Food Sci., 19, pp 40–48. doi.org/10.3746/pnf.2014.19.1.040

Ingelbrecht J., Miller T.L., Lymbery A.J., Maita M., Torikai S., Partridge G., 2020. Anthelmintic herbal extracts as potential prophylactics or treatments for monogenean infections in cultured yellowtail kingfish (*Seriola lalandi*). Aquaculture, 520, 734776. doi.org/10.1016/j.aquaculture.2019.734776

Irkin L.C., Yigit M., Yilmaz S., Maita M., 2014. Toxicological Evaluation of Dietary Garlic (*Allium sativum*) Powder in European Sea Bass Dicentrarchus labrax Juveniles. Food and Nutrition Sciences pp. 989-996. doi.org/10.4236/fns.2014.511109

Ismail H.F., Hashim Z., Soon W.T., Rahman N.S.A., Zainudin A.N., Majid F.A.A., 2017. Comparative study of herbal plants on the phenolic and flavonoid content, antioxidant activities and toxicity on cells and zebrafish embryo. J. Tradit. Complement. Med., 7, pp. 452-465. doi.org/10.1016/j.jtcme.2016.12.006

Jafarpour M., Amniattalab A., Fard A.A., 2018. Genotoxicity and Histopathology Effects of *Melissa officinalis* Aqueous Extract on the Blood and Vital Tissues of *Oncorhynchus mykiss* Fish. Iranian Journal of Toxicology, 12(6), pp. 13-18. doi.org/10.32598/IJT.12.6.564.1

Jakopic J., Veberic R. 2009. Extraction of phenolic compounds from green walnut fruits in different solvents. Acta Agric. Slov., 93, pp. 11–15. doi.org/10.2478/v10014-009-0002-4

Kavitha, C., Ramesh, M., Kumaran, S.S., Lakshmi, S.A., 2012. Toxicity of *Moringa oleifera* seed extract on some hematological and biochemical profiles in a freshwater fish, *Cyprinus carpio*. Exp. Toxicol. Pathol. 64, pp. 681–687. doi.org/10.1016/j.etp.2011.01.001

Kong X., Wang S., Jiang H., Nie G., Li X., 2012. Responses of acid/alkaline phosphatase, lysozyme, and catalase activities and lipid peroxidation to mercury exposure during the embryonic development of goldfish *Carassius auratus*. Aquatic Toxicology, 120-121, pp. 119-125. doi.org/10.1016/j.aquatox.2012.05.005

Kumar J., Priyadharshini M., Madhavi M., Begum S.S., Ali A.J., Musthafa M.S., Faggio C., 2022. Impact of *Hygrophila auriculata* supplementary diets on the growth, survival, biochemical and haematological parameters in fingerlings of freshwater fish *Cirrhinus mrigala* (Hamilton, 1822). Comp. Biochem. Physiol. Part A Mol. Integr. Physiol., 263, 111097. doi.org/10.1016/j.cbpa.2021.111097

Lorenz E.K., Barone R.C.S., Franca W.G., Sabioni R.E., Koch J.F.A., Cyrino J.E.P., 2018. Performance, hematology and immunology of *Salminus brasiliensis* fed diets containing swine liver hydrolysate. Aquaculture, 483, pp. 46-52. doi.org/10.1016/j.aquaculture.2017.09.040

Madalla N., Agbo N.W., Jauncey K., 2013. Evaluation of aqueous extracted moringa leaf meal as a protein source for Nile tilapia juveniles. Tajas, 12(1), pp. 53–64.

Maita M., 2007. Fish health assessment. In: Nakagawa H, Sato M, Gatlin DM (eds) Dietary supplements for the health and quality of cultured fish. CAB International, Washington, pp 11–35.

Metrouh-Amir H., Duarte C.M.M. Maiza F., 2015. Solvent effect on total phenolic contents, antioxidant, and antibacterial activities of *Matricaria pubescens*. Industrial Crops and Products, 67, pp. 249-256. doi.org/10.1016/j.indcrop.2015.01.049

Militz T.A., Southgate P.C., Carton A.G., Hutson K.S., 2013. Dietary supplementation of garlic (*Allium sativum*) to prevent monogenean infection in aquaculture. Aquaculture, vol. 408-409, pp. 95-99. doi.org/10.1016/j.aquaculture.2013.05.027

Mohammadi G., Rafiee G., El Basuini M.F., Doan H.V., Ahmed H.A., Dawood M.A.O., Abdel-Latif H.M.R., 2020. Oregano (*Origanum vulgare*), St John's-wort (Hypericum perforatum), and lemon balm (*Melissa officinalis*) extracts improved the growth rate, antioxidative, and immunological responses in Nile tilapia (*Oreochromis niloticus*) infected with *Aeromonas hydrophila*. Aquaculture Reports, 18, 100445. doi.org/10.1016/j.aqrep.2020.100445

Mohebbi A., Nematollahi A., Dorcheh E.E., Asad F.G., 2012. Influence of dietary garlic (*Allium sativum*) on the antioxidative status of rainbow trout (*Oncorhynchus mykiss*). Aquac. Res., 43 (2012), pp. 1184-1193. doi.org/10.1111/j.1365-2109.2011.02922.x

Mondal P., Garai P., Chatterjee A., Saha N.C., 2020. Toxicological and therapeutic effects of neem (*Azadirachta indica*) leaf powder in hole-in-the-head (HITH) disease of fish *Anabas testudineus*. Aquaculture Research, 52(2), 715-723. doi.org/10.1111/are.14928

Naylor R.L., Goldburg R.J., Primavera J.H., Kautsky N., Beveridge M.C.M., Clay J., Folke C., Lubchenco J., Mooney H., Troell M., 2000. Effect of aquaculture on world fish supplies. Nature, 405 (6790), pp. 1017-1024. doi.org/10.1038/35016500

Naylor R.L., Hardy R.W., Buschmann A.H., Bush S.R., Cao L., Klinger D.H., Little D.C., Lubchenco J., Shumway S.E., Troell M., 2021. A 20-year retrospective review of global aquaculture. Nature, 591, pp. 51–563. doi.org/10.1038/s41586-021-03308-6

Nhu T.Q., Bich Hang B.T., Bach L.T., Buu Hue B.T., Quetin-Leclercq J., Scippo M.L., Phuong N.T., Kestemont P., 2019. Plant extract-based diets differently modulate immune responses and resistance to bacterial infection in striped catfish (*Pangasianodon hypophthalmus*). Fish Shellfish Immunol., 92, pp. 913-924. doi.org/10.1016/j.fsi.2019.07.025

Nya E.J., Austin B., 2009. Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis 32, pp. 963–970. doi.org/10.1111/j.1365-2761.2009.01100.x

Omoregie, E. and Okpanachi, M.A. (1997): Acute toxicity of water extracts of bark of the neem plant, *Azadirachta indica* (Lodd) to the cichlid, *Tilapia zilli* (Gervais). Acta Hydrobiol., 39(1/2), pp. 47–51.

Ozer J., Ratner M., Shaw M., Bailey W., Schomaker S., 2008. The current state of serum biomarkers of hepatotoxicity. Toxicology 245:194–205. doi.org/10.1016/j.tox.2007.11.021

Pérez-Sánchez T., Mora-Sánchez B., Balcázar J.L., 2018. Biological approaches for disease control in aquaculture: Advantages, limitations and challenges. Trends Microbiol., 26 (11), pp. 896-903. doi.org/10.1016/j.tim.2018.05.002

Pratheepa V., Ramesh S., Sukumaran N., 2010. Immunomodulatory effect of *Aegle marmelos* leaf extract on freshwater fish *Cyprinus carpio* infected by bacterial pathogen *Aeromonas hydrophila*. Pharmaceutical Biology, 48(11), 1224-1239. doi.org/10.3109/13880201003713598

Prinsloo G., Nogemane N., 2018. The effects of season and water availability on chemical composition, secondary metabolites and biological activity in plants. Phytochemistry Reviews, 17, pp. 889–902. doi.org/10.1007/s11101-018-9567-z

Ramaiah S.K, 2007. A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. Food Chem. Toxicol., 45, pp. 1551-1557. doi.org/10.1016/j.fct.2007.06.007

Rana B.K., Singh U.P., Taneja V., 1997. Antifungal activity and kinetics of inhibition by essential oil isolated from leaves of Aegle marmelos. J. Ethnopharmacol., 57, pp. 29-34. doi.org//10.1016/s0378-8741(97)00044-5

Rao Y.V., Das B.K., Jyotyrmayee P., Chakrabarti R., 2006. Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. Fish Shellfish Immunol., 20(3), pp. 263-273. doi.org/10.1016/j.fsi.2005.04.006

Rather M.A., Bhat I.A., Sharma N., Gora A., Ganie P.A., Sharma R., 2016. Synthesis and characterization of *Azadirachta indica* constructed silver nanoparticles and their immunomodulatory activity in fish. Aquaculture Research, 48(7), 3742-3754. doi.org/10.1111/are.13199

Reverter M., Bontemps N., Lecchini D., Banaigs B., Sasal P., 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. Aquaculture 433, pp. 50–61. doi.org/10.1016/j.aquaculture.2014.05.048

Reverter M., Bontemps N., Sasal P., Saulnier D., 2017. "Use of Medicinal Plants in Aquaculture," in Diagnosis and Control of Diseases of Fish and Shellfish, B. Austin and A. Newaj-Fyzul, Eds., pp. 223–261, JohnWiley & Sons Ltd., 1st edition. doi.org/10.1002/9781119152125.ch

Romagosa C., David E., and Dulay R.M.R., 2016. Embryo-toxic and teratogenic effects of *Tinospora cordifolia* leaves and bark extracts in zebrafish (*Danio rerio*) embryos. Asian Journal on Plant Sciences and Research, vol. 6, no. 2, pp. 37–41.

Saboonchian F., Jamei R., Sarghein S.H., 2014. Phenolic and flavonoid content of *Elaeagnus angustifolia* L. (leaf and flower). Avicenna J Phytomed, 4, p. 231. doi.org/10.22038/AJP.2014.1975

Sahu S., Das B.K., Mishra B.K., Pradhan J., Samal S.K., Sarangi N., 2008. Effect of dietary Curcuma longa on enzymatic and immunological profiles of rohu, *Labeo rohita* (Ham.), infected with *Aeromonas hydrophila*. Aquaculture Research, 39, pp. 1720-1730. doi.org/10.1111/j.1365-2109.2008.02048.x

Sakai M., 1999. Current research status of fish immunostimulant. Aquaculture 172 pp. 63–92. doi.org/10.1016/S0044-8486(98)00436-0

Samtiya M., Aluko R.E., Dhewa T., 2020. Plant food anti-nutritional factors and their reduction strategies: an overview. Food Prod Process and Nutr., 2, 6. doi.org/10.1186/s43014-020-0020-5

Sethiya N.K., Ahmed N.M., Shekh R.M., Kumar V., Singh P.K., Kumar V., 2018. Ethnomedicinal, phytochemical and pharmacological updates on *Hygrophila auriculata* (Schum.) Hiene: an overview. Journal of Integrative Medicine 16 (5), pp. 299–311. doi.org/10.1016/j.joim.2018.07.002

Sharma J., Kumar N., Singh S.P., Singh A., Harikrishna V., Chakrabarti R., 2019. Evaluation of immunostimulatory properties of prickly chaff flower *Achyranthes aspera* in rohu *Labeo rohita* fry in pond conditions. Aquaculture, 505, pp. 183-189. doi.org/10.1016/j.aquaculture.2019.02.065

Sharma J., Singh A., Begum A., Sonia, Krishna V. H., Chakrabarti R., 2021. The impact of *Achyranthes aspera* seeds and leaves supplemented feeds on the survival, growth, immune system and specific genes involved in immunostimulation in *Clarias batrachus* fry challenged with *Aeromonas hydrophila* in pond conditions. Fish Shellfish Immunol., 118, pp. 11–18. doi.org/10.1016/j.fsi.2021.08.026

Shen Y., Wang D., Zhao J., Chen X., 2018. Fish red blood cells express immune genes and responses. Aquacult. Fish., 3 (2018), pp. 14-21. doi.org/10.1016/j.aaf.2018.01.001

Silva M.J.D.S., Costa F.F.B.D., Leme F.P., Takata R., Costa D.C., Mattioli C.C., Luz R.K., Miranda-Filho K.C., 2018. Biological responses of Neotropical freshwater fish *Lophiosilurus alexandri* exposed to ammonia and nitrite. Sci Total Environ., 616-617, pp. 1566-1575. doi.org/10.1016/j.scitotenv.2017.10.157

Sokooti R., Dezfoulnejad M.C., Baboli M.J., 2021. Effects of olive leaf extract (*Olea europaea Leecino*) on growth, haematological parameters, immune system and carcass composition in common carp (*Cyprinus carpio*). Aquac. Res., 52(6), pp. 2415-2423. doi.org/10.1111/are.15091

Talpur, A.D., Ikhwanuddin, M., 2013. *Azadirachta indica* (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, *Lates calcarifer* challenged with *Vibrio harveyi*. Fish Shellfish Immunol. 34, 254–264. doi.org/10.1016/j.fsi.2012.11.003

Teh S.J., Adams S.M., Hinton D.E., 1997. Histopathological biomarkers in feral freshwater fish populations exposed to different types of contaminant stress. Aquatic Toxicology, 37, pp. 51-70. doi.org/10.1016/S0166-445X(96)00808-9

Thanikachalam K, Kasi M, Rathinam X., 2010. Effect of garlic peel on growth, hematological parameters and disease resistance against *Aeromonas hydrophila* in African catfish *Clarias* *gariepinus* (Bloch) fingerlings. Asian Pacific J Trop Med 3:614–618. doi.org/10.1016/S1995-7645(10)60149-6

Valladão G.M., Gallani S.U., Pilarski F., 2015. Phytotherapy as an alternative for treating fish disease. J. Vet. Pharmacol. Ther., 38(5), pp. 417-428. doi.org/10.1111/jvp.12202

Van Hai N., 2015. The use of medicinal plants as immunostimulants in aquaculture. Rev. Aquac., 446, pp. 88-96. doi.org/10.1016/j.aquaculture.2015.03.014

Vazquez G.R., Guerrero G.A., 2007. Characterization of blood cells and hematological parameters in *Cichlasoma dimerus* (Teleostei, Perciformes). Tissue Cell., 39, 151-160. doi.org/10.1016/j.tice.2007.02.004

Wangkahart E., Wachiraamonloed S., Lee P.T., Subramani P.A., Qi Z., Wang B., 2022. Impacts of *Aegle marmelos* fruit extract as a medicinal herb on growth performance, antioxidant and immune responses, digestive enzymes, and disease resistance against *Streptococcus agalactiae* in Nile tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol., 120, 402-410. doi.org/10.1016/j.fsi.2021.11.015

Wang Q., Mei J., Cao J., Xie J., 2022. Effects of *Melissa officinalis* L. Essential Oil in Comparison with Anaesthetics on Gill Tissue Damage, Liver Metabolism and Immune Parameters in Sea Bass (*Lateolabrax maculatus*) during Simulated Live Transport. Biology, 11(1), 11. doi.org/10.3390/biology11010011

Watts J.E.M., Schreier H.J., Lanska L., Hale M.S., 2017. The Rising Tide of Antimicrobial Resistance in Aquaculture: Sources, Sinks and Solutions. Mar. Drugs 15, 158. doi.org/10.3390/md15060158

Wester, P.W., Canton J.H., 1991. The usefulness of histopathology in aquatic toxicity studies. Comparative Biochemistry and Physiology (C), 100, pp. 115-117. doi.org/10.1016/0742-8413(91)90135-G

Xia Q., Ma Z., Mei X., 2017. Assay for the developmental toxicity of safflower (*Carthamus tinctorius* L.) to zebrafish embryos/larvae. Journal of Traditional Chinese Medical Sciences, vol. 4, no. 1, pp. 71–81. doi.org/10.1016/j.jtcms.2017.05.004

Xie E., Li F., Wang C., Shi W., Huang C., Fa K., Zhao X., Zhang D., 2020. Roles of sulfur compounds in growth and alkaline phosphatase activities of *Microcystis aeruginosa* under phosphorus deficiency stress. Environmental Science and Pollution Research volume 27, pages21533–21541. doi.org/10.1007/s11356-020-08480-2

Yin G., Jeney G., Racz T., Xu P., Jun X., Jeney Z., 2006. Effect of two Chinese herbs (*Astragalus radix* and *Scutellaria radix*) on non-specific immune response of tilapia, *Oreochromis niloticus*. Aquaculture, 253(1-4), pp. 39-47. doi.org/10.1016/j.aquaculture.2005.06.038

Yin G., Ardó L., Thompson K.D., Adams A., Jeney Z., Jeney G., 2009. Chinese herbs (*Astragalus radix* and *Ganoderma lucidum*) enhance immune response of carp, *Cyprinus carpio*, and protection against *Aeromonas hydrophila*. Fish Shellfish Immunol., 26(1), 140-145. doi.org/10.1016/j.fsi.2008.08.015

Yousefi M., Hoseini S.M., Vatnikov Y.A., Kulikov E.V., Drukovsky S.G., 2019. Rosemary leaf powder improved growth performance, immune and antioxidant parameters, and crowding stress responses in common carp (*Cyprinus carpio*) fingerlings. Aquaculture, 505, 473-480. doi.org/10.1016/j.aquaculture.2019.02.070

Yousefi M., Ghafarifarsani H., Hoseinifar S.H., Rashidian G., Doan H.V., 2021. Effects of dietary marjoram, *Origanum majorana* extract on growth performance, hematological, antioxidant, humoral and mucosal immune responses, and resistance of common carp, Cyprinus carpio against *Aeromonas hydrophila*. Fish Shellfish Immunol. 108, pp. 127-133. doi.org/10.1016/j.fsi.2020.11.019

Yu Z.F., Kong L.D., Chen Y., 2002. Antidepressant activity of aqueous extracts of *Curcuma longa* in mice. Journal of Ethnopharmacology 83, pp. 161–165. doi.org/10.1016/S0378-8741(02)00211-8

Zahran E., Risha E., AbdelHamid F., Mahgoub H.A., Ibrahim T., 2014. Effects of dietary *Astragalus* polysaccharides (APS) on growth performance, immunological parameters, digestive enzymes, and intestinal morphology of Nile tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol. 38(1), pp. 149-157. doi.org/10.1016/j.fsi.2014.03.002

Zargari A., Mazandarani M., Hoseini S.M., 2018. Effects of safflower (*Carthamus tinctorius*) extract on serum antibacterial activity of rainbow trout (*Oncorhynchus mykiss*) against *Aeromonas hydrophila*, *Streptococcus iniae* and *Yersinia ruckeri*. International Journal of Aquatic Biology, 6, 1. doi.org/10.22034/ijab.v6i1.392

Zakęś Z., Kowalska A., Demska- Zakęś K., Jeney G., Jeney Z., 2008. Effect of two medicinal herbs (*Astragalus radix* and *Lonicera japonica*) on the growth performance and body composition of juvenile pikeperch [*Sander lucioperca* (L.)]. Aquaculture Research, 39(11), pp. 1149-1160. doi.org/10.1111/j.1365-2109.2008.01977.x

Zemheri-Navruz F., Acar Ü., Yılmaz S., 2020. Dietary supplementation of olive leaf extract enhances growth performance, digestive enzyme activity and growth related genes expression in common carp *Cyprinus carpio*. Gen. Comp. Endocrinol., 296, pp. 113541. doi.org/10.1016/j.ygcen.2020.113541

Zhang X.P., Li W.X., Ai T.S., Zou H., Wu S.G., Wang G.T.,2014. The efficacy of four common anthelmintic drugs and traditional Chinese medicinal plant extracts to control *Dactylogyrus* *vastator* (Monogenea). Aquaculture 420, pp. 302–307. doi.org/10.1016/j.aquaculture.2013.09.022

Zhang X., Sun Z., Cai J., Wang J., Wang G., Zhu Z., Cao F., 2020. Effects of dietary fish meal replacement by fermented moringa (*Moringa oleifera* Lam.) leaves on growth performance, nonspecific immunity and disease resistance against *Aeromonas hydrophila* in juvenile gibel carp (*Carassius auratus gibelio* var. CAS III). Fish Shellfish Immunol., 102, 430-439. doi.org/10.1016/j.fsi.2020.04.051

Zilberg, D., Tal, A., Froyman, N., Abutbul, S., Dudai, N., Golan-Goldhirsh, A., 2010. Dried leaves of *Rosmarinus officinalis* as a treatment for streptococcosis in tilapia. J. Fish Dis. 33, 361–369. doi.org/10.1111/j.1365-2761.2009.01129.x

Zoral M.A., Futami K., Endo M., Maita M., Katagiri T., 2017. Anthelmintic activity of *Rosmarinus officinalis* against *Dactylogyrus minutus* (Monogenea) infections in *Cyprinus carpio*. Vet. Parasitol., 247 (2017), pp. 1-6. doi.org/10.1016/j.vetpar.2017.09.013

Zoral M.A., Ishikawa Y., Ohshima T., Futami K., Endo M., Maita M., Katagiri T., 2018. Toxicological effects and pharmacokinetics of rosemary (*Rosmarinus officinalis*) extract in common carp (*Cyprinus carpio*). Aquaculture, 495 pp. 955-960. doi.org/10.1016/j.aquaculture.2018.06.048

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Ethics approval and consent to participate

Not applicable.

Human and animal ethics

Not applicable.

Competing interests

The author declares no competing interests.