*Eco. Env. & Cons. 28 (3) : 2022; pp. (1431-1445)*
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*ISSN 0971–765X*

**DOI No.: http://doi.org/10.53550/EEC.2022.v28i03.047**

# A review on Immunostimulatory and antioxidant potential of herbs, *Curcuma longa* L., *Camellia sinensis* L. *Zingiber officinale* and *Allium sativum* Linn. in fish health: a sustainable approach for a healthy aquaculture

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(Received 14 August, 2021; Accepted 7 December, 2021)

**ABSTRACT**

The usage of antibiotics and chemotherapeutics agents in aquaculture and their undesired consequences leads to the search for other alternatives. Plants are rich in active compounds includes alkaloids, flavonoids, terpenoids, phenols and polypeptides which proved to maintain a good health status in fish by acting as growth promoters, stress resistance and preventions of infections. Phytomedicine provides a cheaper source for the treatment and greater accuracy than chemotherapeutic agents without causing toxicity. There are diverse medicinal plants in India which are being used extensively in aquaculture for several purposes. Present review focuses only on the immunostimulatory and antioxidant property of some common herbs; turmeric, green tea, ginger and garlic owing to their effectiveness and availability. The studied herbs possess potent immunostimulant and antioxidant compounds which conferring early activation to the non-specific defence mechanisms of fish and boosted the immune responses like phagocytic and respiratory burst activity, lysozyme activity, complement proteins, immunoglobulins (Igs), protease, anti-protease and alkaline protease (ALP) and leucocytes promotion etc. It also inhibits the generation of oxygen anions and scavenges free radical by increasing the antioxidant enzymes activity like catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx) and superoxide dismutase (SOD) during emergence of physiological disturbances. These herbs will promote the eco-friend health management of highly commercial fish species without compromising the aquatic environment and ensure sustainable aquaculture with higher yield and nutrient content.

***Key words :*** *Antioxidant, Fish health, Growth promoter, Herbs, Immunostimulants, Phytochemicals, Survival*

## Introduction

Herbal medicine, the backbone of traditional medicine in many countries, primarily in the developing countries are being used by a large portion of the world population for primary health care because of better cultural acceptability, better compatibility and lesser side effects. Plants are rich in a wide variety of phytochemical compounds which have the beneficial capabilities to improve the health status of fish.

Phytochemicals in herbs enhanced the innate immune system, have antimicrobial properties and are redox-active molecules with antioxidant qualities which can bring better physiological condition in fish health (Citarasu, 2010). It presents a viable alternative to antibiotics and other banned drugs being safer for organism and humans, as well as to the environment.

Aquaculture is a growing industry in Asian countries. The United Nations, Food and Agriculture Organization (2018) estimated feeding nine billion people in the world by 2050. Fish has become an ideal candidate for the necessary accomplishment of animal protein because of highly digestible food material with high nutritive value comprising essential amino acids like lysine, methionine, arginine and essential fatty acids, vitamin A, D and minerals. As fish protein requirement has been increasing in the global market and the aquaculture industry has become the only problem solver. Nowadays, fish culture is growing into new directions adopting new methodologies and techniques to fulfil the appetite for animal protein. The most effective and widely used fish diet in aquaculture industry includes fish meal and fish oil. But its rising market value urged a need for replacement by more sustainable alternatives. Such curiosity has enforced aquaculturists to search for substitution in fish diets without affecting the nutritive quality of fish protein. So for the continual aquaculture development, there lies an essential need for cost-effective and eco-friendly substitutes on which fish farmers could rely on. Phytochemicals are plant-derived compounds found to be effective in disease resistance, stimulate immunity and prevent stress (Chakraborty and Hancz, 2011). Phytochemical usage as herbal medicine is most popular in Asian countries. Thus, herbs richness in phytochemicals provides a convenient approach as an alternative to high priced commercially available diets for the high production in fish culture with profitability in the aquaculture industry. Despite being rich in bioactive compounds in plants, the presence of anti-nutritional factors (ANF) may limit their inclusion in the fish feed which could be overcome by adopting different processing techniques before incorporating the plant ingredients in feed for better utilization of nutrients by fish available in plants.

Herbal treatment boosts the immunity and antioxidant response in fish during exposure to environmental stress. Herbs can act as immunostimulants by directly activating the innate or non-specific defence mechanisms of fish and elevating the specific immune response. The non-specific defence mechanisms of fishes include neutrophil activation, production of peroxidase and oxidative radicals, together with initiation of other inflammatory factors (Ainsworth *et al*., 1991). The herbs contain many immunologically active components such as polysaccharides, organic acids, alkaloids, glycosides and volatile oils, which can enhance immune functions such as bacteriolytic activity and leukocyte promoted function in fishes (Chansue *et al*., 2000). Therefore, the medicinal plants have been used as medicine to treat different fish diseases and to control of other aquatic organisms like shrimp, especially in the countries like China, Mexico, India, Thailand and Japan (Yin *et al*., 2008). In fish culture, stress associated with chemical, biological and physical disturbances in the aquatic environment may result in mortality and lead to substantial economic losses. Plants contain a wide variety of biochemical compounds with antioxidant properties. These could help organisms to cope up with oxidative stress caused by environmental stressors, hence, keep the fish physiological fitness in a better state. Oxidative stress can damage various important biological molecules by releasing Reactive oxygen species (ROS). The most significant targets of cellular injury are proteins, DNA and lipids of cell membranes. Lipids attacked by free radicals subsequently convert to lipid peroxidation. This metabolite is toxic and capable to damage most cells and tissues (Mohiseni *et al*., 2016; Singh *et al*., 2013b). Antioxidants are one of the main parts of the herbal structure with a high content of bioactive compounds which shown to have the ability to inhibit ROS generation and to scavenge free radicals (Citarasu, 2000). Plants are a potential source for providing natural antioxidants. Therefore, in recent years there is increasing the interest in the use of the medicinal plant in Aquaculture. Present review article discusses some of the usefulness of four important herbs rich in immunostimulant and antioxidant compounds owing to its effectiveness and availability with some exemplifications of their successful use in aquaculture.

**Phytochemicals of the studied herbs**

Phytochemicals are biologically active chemical compounds in plants, also known as secondary plant metabolites having biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. Based on structure, phytochemicals are predominantly classified into polyphenols, terpenoids, flavonoids, alkaloids, pigments, steroids and essential oils (Chakraborty and Hancz, 2011) However, the chemical composition of medicinal plants varies under different geographic location, harvest time and climatic conditions. Moreover, the handling of cultivation and other factors are also responsible for the variation of biochemical compounds in plants. The major bioactive compound present in the studied herbs are enlisted in Table 1.

**Immunostimulatory effects of herbs on fish health**

There are two branches of the immune system in fish depending on the functionality such as natural or non-specific immune system and acquired or specific immune system and the former is considered to be the most important ones for immunisation in fish and regarded as the first line of defence against pathogen. Innate immunity constitutes humoral, cellular components and epithelial barriers. Lysozyme (LYZ) or complement proteins are included as humoral elements and the major cellular elements included monocytes, macrophages and granulocytes in fish innate immunity. Fish mucus, gills and flakes are considered as the first line of defence as fish mucus contain lectins, immunoglobulins, lysozyme and complement proteins (Elumalai *et al.,* 2019). Lysozyme and complement proteins play a significant role during pathogen attack. The skin of teleost fish also acts as the most important immune barriers resembled skin-associated lymphoid tissue (SALT), act as an important constituent of the mucosal immune system (Xu *et al.,* 2013). Immunoglobulin M and enzymes such as protease, anti-protease, alkaline protease (ALP), peroxidase are considered to be important components of the fish immune system. Cytokines are the important regulators of immune responses which include interleukins (IL) like 1β, IL-8, Tumour necrosis factor-alpha (TNFα) as pro-inflammatory molecules induces inflammation to pathogen invasion and tissue injury stimulates cellular components for the production of other cytokines. On the contrary, IL-10 acts as anti-inflammatory cytokine and mitigates immune responses (Hoseinifar *et al*., 2019). Non-specific immunity parameters also include respiratory burst, myeloperoxidase, haemagglutination, haemolytic and bacterial agglutination activities which are the cumulative actions of all the anti-bacterial agents present in fish body (Singh *et al.,* 2013a). Measurement of all these parameters helps in accessing the immunological condition of fish. Application of chemical immunostimulants to aquatic health management has immense potential, but due to its high cost, unwanted side effects such as immunosuppression using too high doses of immunostimulants or non-desirable effects caused by prolonged use of such compounds has increased the interest of using cost-effective and lesser side effects compounds as available in medicinal herbs. Following is a brief discussion of few effective and

**Table 1.** Bioactive compounds found in *Curcuma longa* L., *Camellia sinensis* L. *Zingiber officinale* and *Allium sativum* Linn.

| Sl. No | Family | Herbs | Major compounds |
|---|---|---|---|
| 1 | Zingiberaceae | *Curcuma longa* L. (Turmeric) | Phenolic compound (curcuminoids),alkaloids, flavonoids, saponins, turmeron, gingibaron , atlanton, sesquiterpenoids and monoterpenoids, carotenoids, Vitamin C and E. |
| 2 | Theaceae | *Camellia sinensis* L. (Green tea) | Flavonoids (catechin), epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate and gallocatechin, caffeine, theanine phenolic compound (gallic acid), alkaloid, steroids, terpenoids and saponin. |
| 3 | Zingiberaceae | *Zingiber officinale* **(**Ginger) | Polyphenol (gingerol), shogaol, flavonoids, alkaloids, glycosides, saponins, steroids, terpenoids, tannins, paradols and zingerone, zingiberene, β-bisabolene, α-farnesene, β-sesquiphellandrene and α-curcumene, mono- and sesquiterpenes. |
| 4 | Amaryllidaceae | *Allium sativum* Linn. (Garlic) | Organosulfur compounds (alliin, ajoenes, allicin and S-allyl-cysteine), saponins, alkaloids, gluco sides, flavonoids, steroids, shogaols, gingerols, vitamins, carotenoids and polyphenols. |

locally available herbs to encourage the application of natural products to improve the immune system of fisheries, achieve greater weight gains and feed conversions and thereby increase the production and consequently economic gains for producers. The idea was to develop an alternative to the excessive use of antibiotics by using natural products which are friendly to the environment to strengthen the immune system of fisheries.

**Turmeric (*Curcuma longa* L.)**

Turmeric, a medicinal plant, has been used for thousands of years in Indian Ayurvedic medicine. Turmeric is considered as the most immunostimulant natural herb, belongs to the family Zingiberaceae. Curcuminoids are the main bioactive molecules of turmeric which is hydrophobic and polyphenolic with pharmacological properties like anti-inflammatory, antitumor, antiangiogenic, antimutagenic, hepatoprotective, gastroprotective, anticancer, immunomodulatory, antimicrobial and wound healing (Prasad and Aggarwal, 2011). Intraperitoneal administration of curcumin in *Labeo rohita (L. rohita)* increased its non-specific immunity and further enhancement in bacterial agglutination activities with low doses curcumin which might due to antibacterial effect of curcumin against the fish pathogen, *Aeromonas hydrophila (A. hydrohila)* (Bahera *et al*., 2011). Abdel-Tawab and Abbass (2016) also reported a positive effect of turmeric powder supplementation at a level of 2.0 g/kg diet on innate immunity and disease resistance in common carp against aeromoniosis. This work is supported with the work of Abdelrazek *et al.* (2017) which showed improvement in TLC, DLC, IL- 2,4, anti-bacterial enzyme activity (nitric oxide and LYZ) resulted in higher growth performances of Nile tilapia (*Oreochromis niloticus)* fed with 2g/kg turmeric diet. Addition of Turmeric powder (TP) alone and in combination with black pepper powder (BPP) in the diet of Cadmium(Cd) intoxicated *Clarias gariepinus* (*C. gariepinus*) restored the LYZ activity and immunoglobulin level which undergoes reduction during exposure to Cd without supplementation of TP and BPP (El-Houseinya *et al*., 2019). Dietary supplementation of curcumin at 15g/kg to *Cyprinus carpio (C. carpio)* for 8 weeks stimulates skin immune mucosal parameters and mucosal enzymatic activities includes higher LYZ activity, increased IgM level, elevated ALP activity and protein level, higher peroxidase activity in skin mucous of fish (Giri *et al.,* 2019). Expression of immune-related genes such as anti-inflammatory cytokines IL-10 were found to be up-regulated and down-regulation of expression of pro-inflammatory cytokines IL-1β, TNFα, signalling molecules NF-εB, p65 and Toll-like receptor-22 (TLR22) were observed in the head kidney, hepatopancreas and intestine of common carp fed with 5g/kg supplemented curcumin (Giri *et al.,* 2019). Similar results were also observed with turmeric incorporated (10g/kg) diet fed common carp, *C. carpio* exposed to copper (Fazelan *et al*., 2020). Earlier studies on herbal administration in fish also showed inverse expression of pro- and anti-inflammatory cytokines.

**Green tea (*Camellia sinensis* L.)**

Green tea is the most widely consumed beverage after water, due to its healthy, sensory, stimulant, relaxing and cultural properties (Ahmed and Stepp, 2012). The beneficial effects of green tea are owing to their polyphenolic compounds. Among the polyphenols, flavonoids especially catechins, are the leading functional components, which include mainly epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC), epigallocatechin-3-gallate (EGCG) and gallocatechin (GC). EGCG is the most abundant catechin in green tea accounts for at least 59% of the total catechin have anti-infection properties (Steinmann *et al*., 2013) and are reported to have anti-inflammatory, antioxidative and anti-bacterial property. Structurally catechins in green tea have two benzene rings and a dihydropyran heterocycle with a hydroxyl group on C-3, which found to be potent antioxidants and also responsible in improving non-specific immunity in fish (Harikishnan *et al*., 2011). Some studies also have shown that green tea could be a useful supplement to fish diets improving disease resistance, survival rate, growth rate, antioxidant and immune system functions.

EFCG found to be more effective at enhancing serum LYZ activity, improving phagocytic activity and the alternative complement activity at least at the inclusion level of 32 mg $kg^{-1}$ diet in rainbow trout (*Oncorhynchus mykiss)* (Thawonsuwan *et al*., 2010). Green tea supplement in fish proved to be a promising immunostimulant, which could improve fish physiological performances and health. The survival of fish challenged with *A. hydrophila* also increased with increasing green tea level in the fish diet (Yoshida *et al*., 1993). Dietary supplementation of green tea leaves containing flavonoids catechins enhanced its immunity to fight against bacterial infection, aeromoniosis in Nile tilapia, *O. niloticus* (Abdel-Tawwab *et al.*, 2010). Dietary green tea supplementation positively enhanced the non-specific humoral and cellular immune responses and disease resistance of kelp grouper (*Epinephelus bruneus*) to *Vibrio carchariae* (Harikishnan *et al.*, 2011). Previous report showed that decaffeinated green tea also can enhance the immunity of rainbow trout (Sheikhzadeh *et al.*, 2011) and that catechins increased nonspecific immunity in grass carp (Sun *et al.,* 2012). An *in-vitro* study also revealed that anti-Grass carp reovirus activity of intraperitoneal injection of EGCG in grass carp, *Ctenopharyngodon idellus* can increase LYZ activity at the administration of 100mg/kg concentration (Wang *et al.,* 2018). Whereas, there are reports of poor growth performances of fishes fed with green tea products which may be due to low consumption of such diets because of high fibre content. Therefore more research work is needed related to its effective dose and mode of administration of green tea supplemented diet in fish.

### Ginger (*Zingiber officinale*)

Ginger contains about 400 different compounds which facilitate growth, anti-stress, enhances resistance to infectious diseases by increasing non-specific and specific immune responses in fish. Ginger may play diverse biological roles in anti-oxidative, anti-inflammatory, hypolipidemic, anti-carcinogenic, anti-nausea, anti-thrombotic, cardiovascular and antibacterial processes. Ginger contains macro and micro minerals such as Na, P, Mg, Ca, P, Fe, Zn, Cu and Mn and anti-nutritional factors (ANF) like phytate, oxalate, tannin and saponin (Iheanacho *et al,* 2018). The rhizome of ginger has been reported to possess a broad-spectrum of prophylactic and therapeutic activity constituent isolated from ginger, zingerone can be used as an immunostimulant and disease resistance against infectious viral and bacterial diseases (Chang *et al*., 2011).

Non-specific immunity was increased in rainbow trout eating a diet containing 1% of a dried aqueous ginger extract with high phagocytosis and extracellular burst activity of blood leukocytes (Dugenci *et al*., 2003; Haghighi *et al*., 2013). Khafagy *et al.* (2014) reported that ginger provides significant additions to enforce the immune system on catfish. Increased in TLC, lymphocyte and neutrophil showing higher immunostimulatory efficiency of ginger than turmeric in *Cirrhinhus mrigal (C. mrigal)* infected by *Pseudomonas aeroginosa (P. aeroginosa)* (Sivaguunathan *et al*., 2011). Elevation in some haematological indices also considered as stimulation in the immune system and indicated efficient functions of the organs like thymus, spleen and bone marrow responsible for the formation of blood cells. Iheanacho *et al.* (2018) reported highest values of red blood cells, haemoglobin, platelet and hematocrit in *C. gariepinus* juvenile fed with 1.0% ginger diet compared to 0%, 0.5%, 1.5%, 2.0% ginger incorporated diet. Dietary supplementation of ginger increased total Ig levels in *L. rohita* (Sukumaran *et al*., 2016) and zebrafish, *Denio rerio* (Ahmadifar *et al*., 2019) but showed no effects on *O. niloticus* (Brum *et al*., 2017). Ahmadifar *et al*. (2019) reported that 3% of ginger supplementation increased immunological and biological response in Zebrafish. Ginger administration positively restored plasma LYZ activity, bactericidal activity of common carp under high stocking density thereby prevented immunosuppression caused by such stressful condition (Fazelan *et al*., 2019). Studies also demonstrated that the dietary effects of ginger had a different effect on the expression of cytokines. It was observed that 10-30g ginger per kg diet showed no effect on the expression of TNFα, IL-1β and IL-8 (Ahmadifar *et al.,* 2019). On the other hand, 6-10g/kg ginger administration down regulated the expressions of pro-inflammatory cytokines TNFα and IL-1β whereas up-regulated the expression of anti-inflammatory cytokine molecule IL-10 and transforming growth factor-beta gene (Sukumaran *et al.,* 2016). Therefore there is a need for further studies in the expressions of cytokines molecule of ginger treated fish.

### Garlic (*Allium sativum* Linn.)

Garlic contains about 33 sulfur compounds like alliin, allicin, ajoene, allyl propyl and etc. Allium species have immune-enhancing activities like promotion of lymphocyte synthesis, cytokine release, phagocytosis and natural killer cell activity (Kyo *et al.*, 1998). Garlic has an immune raising activity that involves the promotion of lymphocyte formation, cytokines release, phagocytosis and natural killer cells activity (Kyo *et al.*, 1998). Presence of allicin component, the most abundant and biologically active compound of all thiosulfinates in garlic mostly responsible for its immuno-stimulatory role as it is highly permeable through biological membrane influencing its absorption, concentration and bioavailability in target tissues (Miron *et al*., 2000).

Supplementation of *A. sativum* in the diet at a concentration of 1, 5 and 10g per kg stimulates the production of LYZ, serum bactericidal, total white blood cell counts, erythrocyte count and neutrophils, lymphocytes, NBT and monocytes count indicating immunostimulatory effect during 30 days feeding trial in *L. rohita,* improved the surivival rate and more resistant to infection by *A. hydrophila* in *L. rohita* compared to other groups (Sahu *et al*. 2007). Rainbow trout (*Oncorhynchus mykiss*) fed with garlic diets containing 0.5 and 1.0 g garlic per 100 g of feed showed significant enhancement of phagocytic activity, respiratory burst, LYZ, antiprotease and bactericidal activities and reduction in mortality after *A. hydrophila* infection (Nya *et al*., 2009). The incorporation of garlic peel powder into the diet (0.5%) resulted in enhanced serum proteins, RBC and survival rates in African catfish, *C. gariepinus* challenged with *A. hydrophila* (Thanikachalam *et al*., 2010). In female Guppy (*Poecilia reticulata*), 0.15 ml/kg garlic extract supplemented diets increased LYZ activity in the skin mucus and total Ig level, ACH50 values significantly increased from 0 to 0.15 ml/kg but declination started when garlic concentration have increased from 0.15 to 0.20ml/kg (Motlagh *et al*., 2020). Dietary garlic supplementation mitigated zearalenone (mycotoxin) toxic effects by enhancing haematological parameters include RBCs, Hb and Ht, increased in serum total protein, albumin and globulin contents, as well as higher LYZ activity reflecting enhancement in innate immunity on European sea bass (*Dicentrarchus labrax*) challenged to *Vibrio alginolyticus* infection (Abdel-Tawwab *et al*., 2020). It was observed that 1% and 1.5% garlic supplementations in *C. carpio* improved the lysozyme level by mitigating the ammonia induced lysozyme activity, also ameliorated the decreasing of ACH50 and plasma bactericidal activity after exposing to ambient ammonia (Yousefi *et al*., 2020).

### Antioxidant effect of herbs on fish health

Vertebrates possess enzymatic and non-enzymatic antioxidant systems as a defence against oxidative stress. In aquaculture, physiological stress response may be caused by various husbandry practices such as elevated rearing densities, thermal stress, handling, low dissolved oxygen and transportation stressful conditions (Tort, 2011). SOD and CAT are the key enzymes that convert the highly toxic ROS into less harmful oxygen species for the host (Reyes-Becerril *et al.,* 2017). Stress may also be induced by antibiotics and chemotherapeutics which is now used widely in aquaculture, has been associated with enhanced ROS generation. It can seriously affect immune function and attack all biological molecules, especially PUFA (poly-unsaturated fatty acids) and lead to the formation of lipid peroxidation products, collectively, the thiobarbituric acid reactive substances (TBARS) content and conjugated dienes (CD), which are toxic to the body. Malondialdehyde (MDA) is the main product of lipid peroxidation with strong biotoxicity and brings cellular damage affecting the normal function of the organ (Sharma *et al.,* 2015). Most common ROS include superoxide anion, hydrogen peroxide ($H_2O_2$), peroxyl radicals ($ROO^-$) and reactive hydroxyl radicals ($OH^-$). Nuclear factor erythroid 2-related factor 2(Nrf2), the key regulator of antioxidant enzymes protects the cell from ROS and other electrophiles components by generating detoxifying antioxidant enzymes (Kobayashi and Yamamoto, 2016). Chemical compounds and reaction capable of generating potential toxic oxygen species/free radicals are referred to as 'pro-oxidants' and antioxidants are the compounds which scavenge them suppressing their formation or opposing their actions. In a normal cell, there is an appropriate balance between pro-oxidant and antioxidant. However, this balance can be shifted towards the pro-oxidant when production of oxygen species is increased or when levels of antioxidants are diminished. This state is called 'oxidative stress' and can result in serious cell damage if the stress is massive or prolonged. Antioxidants include enzymes catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx) and superoxide dismutase (SOD), Vitamin A, E, carotenoids, peptides, amino acids and phenolic compounds (tocopherols, ubiquinones), which is found in the cell plasma, mitochondria of cell membranes.

Researchers suggest that two-thirds of the world's plant species have medicinal value; in particular, many medicinal plants have great antioxidant potential. Herbal antioxidants have been successfully employed as rejuvenators for several centuries in the Indian systems of alternative medicine. Antioxidants properties of the studied herbs on fish health were briefly discussed in the following.

### Turmeric (*Curcuma longa* L.)

Turmeric is a great antioxidant and the compounds responsible for its antioxidant property are the curcumin and curcuminoids. Studied have shown the antioxidant capacity of superoxide anion and hydroxyl radical scavenging ability, glutathione reductase, CAT, total SOD, GPx and glutathione-S-transferase activities and glutathione content in the intestine were significantly increased with increasing curcumin supplementation in Crucian carp, *Carassius auratus* (Jiang *et al*., 2016). 0.5% dose of curcumin may directly scavenge free radicals by reducing in lipid peroxidation products, collectively, the thiobarbituric acid reactive substances (TBARS) content, SOD activity increased in all the curcumin-treated groups of *Anabas testudineus,* Bloch assuring their protective role *in vivo* too (Manju *et al*., 2011). It uses different pathways to bring about its effect. More than eighty molecular targets have been reported for curcumin so far. Studies have shown that curcuminoids are very effective in scavenging free radicals and neutralize them. Thus, if the natural body mechanism not able to fight against these free radicals, turmeric can assist the body to do so very effectively. The free radical scavenging activity of curcumin comes either from the phenolic (OH) group or CH2 group of the β-diketone moiety (Sokmen and Khan, 2016). Compounds of curcumin which contain a phenolic group at position-4 of the aromatic ring were found to be highly potent antioxidants with higher antioxidant values than the synthetic antioxidant butylated hydroxytoluene (Sokmen and Khan, 2016). Curcumin may induce the expression of Nrf2 mRNA. *In vitro* and *in vivo* study in the teleost model, *Anabas testudineus* using curcumin analogue salicyclcurcumin along with aquaculture feed also inhibited the formation of lipid peroxidation products and stimulate SOD activity (Manju *et al*., 2008). Curcumin supplementation at 10 or 15 g/kg in *C. carpio* increased SOD and CAT enzyme activities in fish and decreased in MDA level indicating the enhancement of endogenous antioxidant defence mechanism in curcumin fed common carp (Giri *et al.,* 2019). Dietary supplementation of 10-15g/kg of curcumin upregulated Nrf2 mRNA expression in head kidney, hepatopancreas and intestine, whereas highest Nrf2 mRNA expression was registered in the head kidney at the administration of curcumin at 10g/kg (Giri *et al*., 2019). Curcumin administration at 200 mg/kg of the diet increased the hepatic Nrf2 levels in rainbow trout under the stressful condition of high stocking density (Akdemir *et al*., 2017). Cortisol and glucose level are the indicators of stress level in fish. 10 and 20 g/kg turmeric diet mitigated the stress level of cortisol and glucose in common carp exposed to copper toxicity (Rajabiesterabad *et al*., 2020).

**Green tea (*Camellia sinensis* L.)**

The components of green tea such as catechins, flavonols, flavanones, phenolic acids and glycosides have powerful natural antioxidant property. The green tea phenolic compounds of highest concentration are gallic acid (GA), gallocatechin (GC), catechin (C), EC, EGC, ECG, EGCG, *p*-coumaroylquinic acid (CA) and gallocatechin-3-gallate (GCG) with EGCG being the most abundant by weight. Green tea also contains condensed and hydrolyzable tannins. Green tea has the highest concentration of polyphenols compared to other teas, including EGCG, which may be why green tea can induce apoptotic cell death in cancer better than other teas (Lin *et al*., 2003). Intake of green tea increased the activity of SOD in rainbow trout eggs and also decreased the level of malondialdehyde, a marker of oxidative stress (Asadpour *et al*., 2012). Thawonsuwan *et al.* (2010) also showed that EGCG increased the availability of antioxidant vitamin E and lowered the levels of lipid hydroperoxide in the liver of rainbow trout. Dietary tea inclusion in feeding could be an important source of Manganese (Mn) with metabolic repercussions on antioxidant mechanisms in fish (Pérez-Jiménez *et al.,* 2013). Tea waste or tea residue also contains polyphenols, which was reported to have strong antioxidant activity. EGCG treatment reduced MDA content and increased the antioxidant enzyme, GSH helps to reduce lipid oxidation and convert active oxygen molecules into the non-toxic compound in grass carp challenged to grass carp reovirus (Wang *et al*., 2018).

**Ginger (*Zingiber officinale*)**

Ginger is a strong antioxidant substance with the major constituents of zingerone, gingerdiol, zingiberene, gingerols and shogoals which may either mitigate or prevent the generation of free radicals. The antioxidant property of ginger is extremely significant as it could be used as a preventive agent against several diseases in aquaculture. Eleazu and Eleazu (2012) studied antioxidant potentials of six varieties of ginger and all the varieties were observed to possess strong antioxidant activities and had high quantities of phenols, which may be responsible for their antioxidant activities. The presence of tocopherols like a-tocopherol and d-tocopherol in addition to phenol and flavonoid in ginger responsible for its antioxidant property (Sukumaran *et al*., 2016).

Supplementation of ginger (1.0% ) in the diet found increased SOD and CAT levels in all tissues and decreased the malondialdehyde (MDA) levels in liver and gut tissues in *O. niloticus* (Sahan *et al*., 2016). It may be due to various bioactive substances (shogoal, gingerols, zingerone, etc.) found in ginger. Association of ginger and vitamin C in the diet seemed to be the more effective stimulator of ROS and promoters of antioxidant defences against *Vibrio harveyi* infection in *Epinephelus fuscoguttatus,* brown-marbled grouper (Apines-Amar *et al*., 2012). Ginger at 3% level caused catalase gene expression in zebrafish (Ahmadifa *et al*., 2019). Similarly, ginger administration significantly up-regulated the expression of SOD-1 and GPx enzyme as well as increased mRNA expression of Nrf2 in *L. rohita* fingerlings (Sukumaran *et al*., 2016).

**Garlic (*Allium sativum* Linn.)**

Extracts of fresh garlic contain antioxidant phytochemicals including water-soluble compounds, lipid-soluble organo-sulfur components and flavonoids, which bring about antioxidant action by enhancing the oxidant enzymes superoxide SOD, CAT and GPx (Borek, 2001). Numerous studies determined garlic and its bioactive sulphur compounds to be potent antioxidants by displaying radical–scavenging activity and modulating cellular antioxidant enzyme activity Among the variety of organosulfur compounds, *S*-allyl cysteine (SAC) and *S*-allyl-mercapto cysteine (SAMC), the major organosulfur compounds found in aged garlic extract, showed radical scavenging activity. The allyl sulphides enhance the glutathione S-transferase enzyme system, which through their dependent biochemical pathways enhance the liver detoxification of carcinogenic substances. Garlic has also been shown the protective nature against gastrointestinal neoplasias, against blood clots (anti-platelet action). This may be due to the presence of compounds allicin and ajoene, which have fibrinolytic activity. Garlic also enhances cell membrane stabilization and protect tissues against free radical-mediated toxic damages that resulted in decreased the non-functional enzymes found in liver and kidney includes alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST) which significantly increased due to vital tissue damage and hemolysis during ammonia exposure (Yousefi *et al*., 2020).

It has been found that aqueous extract of raw garlic and dried powder scavenges hydroxyl radicals and superoxide anion in rohu infected by *Aeromonas hydrophila* (Kim *et al*., 2001). Garlic supplemented diets have shown significantly higher antioxidant enzymes like SOD and CAT activity in blood serum and liver in tilapia and decreased level of MDA level (Metwally, 2009). Garlic supplemented diet suppressed increased activities of antioxidant enzymes (SOD, CAT, GPx) under ammonia toxicity and mitigated the increment of MDA production in common carp (Yousefi *et al*. 2020). Dietary garlic administration improved the antioxidant activity in Nile tilapia (Metwally and Metwally, 2009).

## Discussion

Herbs are a rich source of bioactive phytochemicals or bio nutrients which possess biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. So, herbs have received increasing attention as spices for humans and additives in diets for animals. There are diverse of medicinal plants in India which are being used extensively in aquaculture for several purposes but the present review focuses only on the immunostimulatory and antioxidant property of certain herbs-Turmeric, Green tea, Ginger and Garlic owing to its effectiveness and availability.

The present study revealed the presence of medicinally active constituents in all the studied herbs. The phytochemical constituents of the investigated herbs (Table 1) and its physiological effects on some economically important fishes are also studied (Table 2). Flavonoids, alkaloids, phenolic compounds, terpenoids, tannins, saponins and glycosides were present in all the herbs. Study findings suggest that among the phytochemicals, curcumin (polyphenol) in turmeric, gingerol and shogoal (homologue series of phenol) in ginger, catechins (flavonoids) in green tea and ajoene (an organosulfur) in garlic are the most active component found to have both immunostimulatory and antioxidant property. The supplementation of medicinal herbs in the diet of fish elevates the innate or non-specific defence mechanisms of fish against

**Table 2.** Study of *Curcuma longa* L., *Camellia sinensis* L. *Zingiber officinale* and *Allium sativum* Linn. on physiological and immunological performances in some commercially important fishes.

| Sl. No | Herbs | Plant parts | Test fish species | Administration & doses | Physiological & immunological effects | References |
|---|---|---|---|---|---|---|
| 1 | *Curcuma longa* L.(Turmeric) | Rhizomes | *Labeo rohita* juvenile | Intraperitoneal injection of 1.15 and 1.5µg of curcumin in 0.1 mL of PBS. | Increased non-specific immune response activities such as respiratory burst, myeloperoxidase, haemagglutiation, haemolytic and bacterial agglutination activities in *L. rohita* challenged by *Aeromonas hydrophila*. | Behera *et al*., 2011 |
| 2 | *Curcuma longa* L. (Turmeric) | Rhizomes | *Oreochromis Niloticus* | Orally fed with 0.5g turmeric powder/ kg diet. | Improved growth performance in and significantly protect against *Pseudomonas fluorescens.* | Mahmoud *et al*., 2014 |
| 3 | *Curcuma longa* L. (Turmeric) | Rhizomes | *Cyprinus carpio* L. | Orally fed with 2.0g turmeric powder/ kg diet. | Improved performances and innate immunity challenged with *Aeromonas hydrophila*. | Abdel-Tawwab *et al*., 2016 |
| 4 | *Curcuma longa* L. (Turmeric) | Rhizomes | *Oreochromis Niloticus* | Orally fed 0, 2, 4 and 8g/kg dietary turmeric. | Improved Nitric oxide, leukocytes, Lysozyme,IL-4, lymphocyte proliferation index in turmeric fed *Oreochromis Niloticus* | Abdelrazek *et al*., 2017 |
| 5 | *Curcuma longa* L. (Turmeric) | Rhizomes | *Cyprinus carpio* | Orally fed 5, 10, 15g/kg dietary curcumin powder | Improved growth performances, feed conversion ratio, lysozyme, ALP, ACP, SOD, CAT activity | Giri *et al*., 2019 |
| 6 | *Curcuma longa* L. (Turmeric) | Rhizomes | *Labeo rohita* | Orally fed 0, 5, 10, 15, 20g/kg dietary turmeric. | Improved disease resistance and survival rate against *Aeromonas veronii* | Kaur *et al*., 2020 |
| 7 | *Camellia sinensis* L. (Green tea) | Seed | *Oncorhynchus mykiss* | Orally fed with watered tea seed (10 and 20 % of the fish diet). | Watered tea seed and its secondary metabolite saponin are effective agents against *L. anguillarum in in vivo* and *in vitro* showing antibacterial activity in Rainbow Trout. | Boran *et al*., 2015 |
| 8 | *Camellia sinensis* L. (Green tea) | Leaves | *Oncorhynchus mykiss* | Fed orally with 100mg decaffeinated leaves/kg diet. | Increased the activity of superoxide dismutase and improved the egg quality of rainbow trout. | Sheikhzadeh *et al*., 2011 |
| 9 | *Zingiber officinale* (Ginger) | Rhizome | *Oncorhynchus mykiss* | Orally fed with 1g powdered ginger/100 g diet. | Observed increased in respiratory burst activity, lysozyme activity, RBC and WBC count in rainbow trout. | Haghigi *et al*., 2013 |

**Table 2.** *Continued ...*

| Sl. No | Herbs | Plant parts | Test fish species | Administration & doses | Physiological & immunological effects | References |
|---|---|---|---|---|---|---|
| 10 | *Zingiber officinale* (Ginger) | Rhizome | *Cirrhinhus mrigala* | Orally fed with 2g of dry ginger powder along with 2g of turmeric powder/100 g diet. | Increased in total lymphocyte count & number of neutrophil cells thereby showing immunostimulatory efficiency of ginger infected by *Pseudomonas aeroginosa.* | Sivagurunathan *et al*., 2011 |
| 11 | *Zingiber officinale* (Ginger) | Rhizome | *Oreochromis niloticus* | Orally fed with 0.5g and 1g powdered ginger with distilled water /100g diet. | Observed increased level of SOD, CAT and total numbers of lymphocytes, monocytes, neutrophils, eosinophils while MDA levels found to be decreased in liver and gut tissues in *Oreochromis niloticus* infected with *Aeromonas hydrophila.* Decreased ortality was found recorded in the studied fish. | Sahan *et al*., 2016 |
| 12 | *Zingiber officinale* (Ginger) | Rhizome | *Clarias gariepinus* | Orally fed 0, 0.5, 1, 1.5and 2.0g/2kg dietary ginger. | Better growth performances and haematological profile in *Clarias gariepinus.* | Iheanacho *et al*., 2018 |
| 13 | *Zingiber officinale* (Ginger) | Rhizome | *Cyprinus carpio* . | Orally fed 0, 5 and 10g/kg dietary ginger | Ginger treatment suppressed stress, oxidative stress and immunosuppression caused by high stocking density. | Fazelan *et al*., 2019 |
| 14 | *Allium sativum* Linn. (Garlic) | Peel | *Clarius gariepinus* | Orally with fed 5g of garlic peel/ kg diet. | Enhanced serum proteins, RBC and survival rates in African catfish challenged with *Aeromonas hydrophila.* | Thanikachalam *et al*., 2010 |
| 15 | *Allium sativum* Linn. (Garlic) | Bulb | *Oreochromis Niloticus* | Orally fed with 40g natural garlic, 150 mg garlic oil and 32g garlic powder/ kg diet. | Significantly increased growth performance, antioxidant enzyme activities in Nile tilapia fed with garlic supplemented diet. | Metwally *et al*., 2009 |
| 16 | *Allium sativum* Linn. (Garlic) | Bulb | *Oncorhynchus mykiss* | Orally fed 0, 1, 1.5 and 2g/100g dietary garlic. | Dietary garlic supplementation promotes growth and induces changes in intestinal microbiota plays role in nutrient digestion, immune responses and disease resistance. | Büyükdevec *et al*., 2018 |
| 17 | *Allium sativum* Linn. (Garlic) | Bulb | *Cyprinus carpio* | Orally fed 0.5, 1, and 1.5g of garlic powder/kg diet. | Increased in lysozyme activity, ACH50, CAT, bactericidal activities, Immunoglobulin level and decreased plasma glucose, cortisol and MDA . | Yousefi *et al*., 2020 |

pathogens and increase in the levels of antioxidant enzymes SOD and CAT. In aquaculture, diseases of microbial origin cause high mortality rates and lesions on fish, with consequent economic losses worldwide (Toranzo *et al.*, 2005). Bacteria, mainly of *Yersinia ruckeri, Pseudomonas putida, Pseudomonas luteola, Aeromonas hydrophila* and *Listonella anguillarum* (formerly *Vibrio anguillarum*) have been identified as the etiological agents responsible for the disease outbreaks in fish and shellfish (Radjasa *et al.*, 2009). The potential use of these natural extracts could offer the aquaculture industry an alternative solution to synthetic chemical antimicrobials. However, the concentrations of plant secondary metabolites and their activities in biological systems vary with the maturity of the plant and plant parts, in addition to soil conditions, water and light availability and other environmental conditions in which the plant is growing. Levels of secondary metabolites are both environmentally induced as well as genetically controlled. Besides, the structure and activity of active moieties in a group of one class of plant secondary metabolites, for example, tannins, saponins or alkaloids also change with the environmental condition and maturity stages of the plant. This poses an enormous problem in the use of plants or plant products in aquaculture because of batch-to-batch variation in the product quality (Makkar *et al*, 2009). Moreover, most of the herbs and herbal extracts can be given orally, which is the most convenient method of immunostimulation. However, the effect is dose-dependent and there is always a potential for overdosing (Yin *et al.*, 2008), which might some time give a negative effect on fish health. So, dosage optimization is strongly recommended. More study on herbs and their biochemical metabolites, its effect on gene expression could help know their vital role in fish health. Recently, researchers were approaching towards the next-generation sequencing technique comprises RNA sequencing and transcriptomic analysis, new DNA based marker for the discovery of differential gene expression of RNAs involved in the various phenomenon of biological processes in fish which will provide more insight knowledge regarding the mode of action of herbal components in fish health. Therefore, it is evident that there is a need for further research to determine the ideal treatment regime and functional mechanism of these herbs for application in intensive fish culture.

## Conclusion

Natural plant products present a viable alternative to antibiotics and other banned drugs being safer for the reared organism and environment. Use of chemicals to control diseases leads to effluent remittance in the fish tissues and may cause harmful effects to the consumers. The presence of active compounds of herbs and plants are well known documented and in use against wide variety of pathogens in aquaculture industry showing potentiality to improve aquatic health management. Herbal supplementation increases immunocompetency of fish by augmenting the non-specific defence mechanism and prevent disease and stress. However, there is still need of further systematic scientific research in precise herbal mode, dosage, duration, and route of administration on fish to avoid immune suppression and toxic effects of improper use of herbs. These herbs are abundantly available and can procure at low price and therefore this herbal approach needed to be rapidly disseminated among the farmers for useful, sustainable and healthy aquaculture. The evolving technologies and advancement in aquatic science could help to decipher the effects of herbal action at the molecular level and its gene regulation that will impact to improve fish health and achieving sustainable fish productions for the coming generations. It is believed that in near future, herbal medicine with their beneficial capabilities of improving the health status of fish and other aquatic animal will be going to play an important role to bloom the blue economy.

## Acknowledgements

Authors are thankful to Dibrugarh University for providing support and facility.

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