Herbal Extracts and Fish Gene

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Herbal bioactive components can act as immunostimulants and influence several immune-related pathways. An immunostimulant is a component or action that elevates immune responses, especially innate immunity. Herbal bioactive components can have anti-bacterial, anti-viral, and anti-fungal functions and increase resistance against infectious microorganisms.

Keywords: herbal extracts ; gene regulations ; immunity ; growth ; aquaculture

1. Introduction

The provision of food for human beings is one of the main challenges facing humanity, which has attracted the attention of different countries to increase the number of aquaculture products in their food basket. Besides, high-quality proteins derived from aquaculture products have made the aquaculture industry, with an annual growth of 8%, the highest activity in the food industry [1], and in the last decade, global aquaculture has increased by 163%, reaching 114.5 million tons in 2018 [1]. Fish, both salt and fresh water are healthy and high-quality foods because they contain valuable nutrients such as vitamins, minerals, high protein content, essential amino acids, and fatty acids. During recent years, growth performance and artificial reproduction have been considered as two primary concerns in aquaculture. Increasing stock densities in limited areas to achieve more production in line with the increasing demand has resulted in the increase of organic load, which impairs water quality in the environment, and the imbalance of water parameters such as dissolved oxygen and pH important for fish health. This has triggered the fish to get stressed and contract diseases more easily. Chemotherapeutics and antibiotics have been utilized for many years to prevent stress and combat diseases in aquatic animals [2]. However, their excessive and improper use suppressed the immune system of hosts and caused resistance against pathogenic microorganisms.

In fish, growth hormone (GH) and insulin-like growth factor, I (IGF-I) play a central role in regulating growth. Associated with reproduction, the presence of various genes including luteinizing hormone β (lhβ), follicle-stimulating hormone β (fshβ) [3], estrogen receptors (erα , erβ1 , and erβ2), androgen receptors (arα and arβ) [4], vitellogenins (Vtgs) [5], aromatase genes [6] has been confirmed. Although the number of studies is limited, Phyto-additives have been reported to affect expressions of growth genes such as GH and IGF and reproductive genes such as fshβ , lhβ , cyp19a , and vtg in fish.

The number of aquatic species for artificial reproduction and farming is on the rise owing to the development of commercial aquaculture. A prerequisite for the artificial reproduction and sustainable production of fish is the control of the reproductive process of fish in captivity and the production of high-quality sperms and eggs. Different studies showed the effect of Phyto-additives on reproductive processes.

In this review, studies related to the effects of Phyto-additives on the expressions of the genes associated with immunity, digestion, growth, and reproduction were reviewed.

2. The Effect of Phytochemicals and Their Derivatives on Growth-Related Genes

Growth is a polygenic and environmentally controlled trait that is defined as a somatic function that reflects the balance between feed composition and quality, consumption, utilization, and the physiological functions of an organism [7]. Many factors, including genetics, nutrition, and the environment can affect the growth rate of an organism. Feed additives are the substances that are used in the diet of animals in small amounts to improve the effectiveness and absorption of nutrients in the intestine [8]. In this way, these materials can increase the growth efficiency as well as the health of farmed organisms [9]. In recent years, the use of phytochemicals as natural growth promoters has been increased in animal husbandry and aquaculture [10][11][12][13]. The application of phytochemicals and their derivatives as immune stimulators

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1. FAO. Fisheries and Aquaculture Information and Statistics Branch; FAO: Rome, Italy, 2020.

and growth promotors in aquatic animals has been well-reviewed [10][11][14][15]. However, there is a lack of information on the mode of action of these components in the pathways, affecting growth-related genes in aquatic organisms. Thus, we have attempted to provide an overview of the effect of phytochemicals and their metabolic components on growth-related genes in aquatic organisms (**Table 1**).

Table 1. Selected studies regarding phytochemicals and their derivatives effects on growth-related genes.

Phytochemicals/Derivatives	Dose	Type of Administration	Duration	Enhanced Gene Expression	Fish Species	Reference
Tannin	0.05 or 0.1% diet	oral	6 weeks	*gh* and *igf-i*	beluga sturgeon (*Huso huso*)	[16]
Curcumin	0.5 and 1% diet	oral	35 days	*gh*, *igf-1*, and *igf-2*	tilapia (*Oreochromis mossambicus*)	[17]
D-limonene	400 and 800 ppm diet	oral	63 days	*igf-1*, *muc*, *pept1*, *lpl* and *alp*	Nile tilapia (*O. niloticus*)	[18]
Apple cider vinegar	3 and 4.5% diet	oral	8 weeks	*GHRL*	zebrafish (*Danio rerio*)	[19]

gh: growth hormone; IGF-I insulin-like growth factor-I; *muc*: mucin-like protein; pept1: oligo-peptide transporter I; *lpl*: lipoprotein lipase; *alp*: alkaline phosphatase.

Growth hormone (GH) and insulin-like growth factor- I (IGF-I) are considered the main genes influencing the growth that form the core of the hypothalamic–pituitary–somatotropic (HPS) axis. These genes are influenced by several factors such as the environment, genetics, and nutrition of an organism [7]. Growth hormone has direct and indirect metabolic effects. In direct mode, GH in a series of steps enhances protein syntheses, including synthesis of RNA and amino acid uptake. Indirectly, after being secreted from the pituitary gland, GH circulates through the blood to the liver, where it stimulates the synthesis and secretion of IGFs such as IGF-1 and IGF-2 [17][20]. In addition, GH may boost the local synthesis of IGF-1 that exerts paracrine or autocrine effects [20]. Subsequently, IGF-1 by acting on target cells, causes them to proliferate and differentiate and ultimately stimulates the growth of the body [21]. The previous studies on the Nile tilapia showed that the GH's transcription correlated with the level of IGF-1 mRNA levels, individual body weight gain and SGR of this fish species [22][23]. In addition to these hormones, other factors play a role in fish growth. For example, fish intestine plays the most important role in the digestion and absorption function of the gastrointestinal tract and thus show a substantial effect on fish nutrition and growth [17]. The profile and activity of an animal's digestive enzymes largely characterize its capacity to absorb nutrients from a particular food source [24]. It has been demonstrated that there is a significant correlation between increased production of digestive enzymes (mainly included a-amylase, protease, and lipase) and digestive capacity [17] and growth [25]. Moreover, the expression of several genes such as mucin-like protein (muc), oligo-peptide transporter I (pept1), and lipoprotein lipase (lpl) enhance digestion, absorption, and transport of nutrients in the intestine [18]. These functions are essential for the efficient utilization of dietary components and ultimately lead to enhanced growth of the animal. For example, muc gene plays a role in secreting the mucus needed for the efficient transport of nutrients from the intestine into the blood [26][27]. Pept1 is known as a nutrient transporter that actively transports di- and tripeptides from enterocyte cells into the bloodstream, using mucus as a mediator [28]. Diets containing di- and tripeptides have been shown to increase fish growth more efficiently than individual amino acids [28][29]. Lpl is an enzyme that breaks down plasma lipids and releases fatty acids, which in turn are transported to tissues through the bloodstream to produce energy [30].

Curcumin is a polyphenolic compound found naturally in the turmeric plant rhizome (Curcuma longa). It contains biologically active compounds such as alkaloids, triterpenoids, and reducing sugars that have immune-modulating properties and act as prebiotics [31]. Curcumin can strengthen the balance of positive and negative gut flora and increase intestinal digestion and absorption, thereby stimulating general health and increasing fish growth [31]. Midhun et al. [17] examined the modulating effects of the dietary curcumin on digestive enzymes, and the expression of gh , igf-1 , and igf-2

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genes in tilapia (Oreochromis mossambicus). They found that 0.5 and 1% curcumin inclusion significantly increased gh in the brain, and igf-1 and igf-2 gene expression in muscles. These genes, along with myostatin, are involved in fish myogenesis and have been shown to act as extrinsic regulators and control fish muscle growth [31].

It seems that the mode of action of phytochemicals and their derivatives involves up-regulating the expression of growth-related genes, which activate a series of functions and eventually improve fish growth.

3. The Effect of Herbal Extracts and Plant Components on Immune-Related Genes in Fish Species

By the same token, polyphenols have been described as anti-inflammatory [32], anti-microbial [33], and anti-oxidant [34] bioactive compounds. An example in this regard is trans-cinnamic acid, which has an immunostimulant role via activation of pro-inflammatory cytokine gene expression, including IL-1β, IL-8, transforming growth factor-beta (TGF-β), tumor necrosis factor-alpha (TNF-α), IgM, and IgT [35]. The findings on head kidney specimens of rainbow trout were consistent with the previous studies in other fish species, adding 250 or 500 mg/kg feed resulted in up-regulation of gene expression levels of head kidney pro-inflammatory cytokines after 60 days [35].

Jatropha species have been used as traditional medicine for prophylaxis and treatment of various clinical disorders in tropical regions. Jatropha vernicosa is a recently registered species, and a recent screening study was conducted to investigate its antioxidant and immune-related features in longfin yellowtail fish [36]. This plant has been evaluated as a rich source of many phytochemicals including flavonoids, saponin, and coumarin [37]. The stem bark extract of J. vernicosa demonstrated antioxidant and immunostimulant effects via activation of respiratory burst, phagocytosis, nitric oxide synthesis, up-regulation of pro-inflammatory cytokines, and down-regulation of anti-inflammatory markers, resulted in control of vibriosis in spleen leukocytes [36].

Traditional herbal medicine from Southeast Asia is the Myrtaceae family. An example is Alton (Rhodomyrtus tomentosa), which has been reported to be an anti-inflammatory agent in the treatment of various diseases [38][39][40]. Adding Alton extract in 10 μg/mL doses has been shown to reduce inflammatory mediators and enhance antioxidant status and anti-inflammatory markers in rainbow trout macrophages [41].

In addition to the abovementioned components, blue-green algae or spirulina can be a potential feed supplement for the health and welfare of marine species. Pectin, the main ingredient of the cell wall in spirulina, showed an immunomodulatory effect in zebrafish via up-regulation of pro-inflammatory cytokines, chemokines, lysozyme, and mucin as a medication against E. piscicida and A. hydrophila [42]. The combination of spirulina with selenium nanoparticles can trigger immune responses via activation of liver SOD and TNF-α gene expressions and suppression of HSP70 in Nile tilapia [43].

4. The Effect of Herbal Extracts and Phytochemicals on Reproduction-Related Genes

Reproduction is a physiological procedure known to be regulated by a complex coordination of hormones at the hypothalamus, pituitary, and gonadal (HPG) levels. In vertebrates, hypothalamic gonadotropin-releasing hormone (GnRH) stimulates the synthesis and secretion of gonadotropins from pituitary cells [44]. Gonadotropins (luteinizing hormone β (lhβ) and follicle-stimulating hormone β (fshβ)), in turn, regulate gonadal function, that is, gametogenesis and hormone production. Recent studies performed in zebrafish demonstrated that GnRH isoforms locally produced in the gonads could also directly control gametogenesis via an autocrine/paracrine manner [45]. In addition to GnRH-induced gonadal function, numerous environmental factors could also affect reproduction. Many studies as discussed below have provided evidence for linkage between herbal extracts and plant secondary metabolites (PSM) with physiological or pathophysiological conditions associated with reproduction. These herbal extracts and PSMs have been shown to alter fertility by changing the levels and activities of several hormones on the HPG axis. This alteration is known to be in part regulated by induced changes in the expression of reproductive-related genes. For instance, it has been demonstrated that phytochemicals could act on fish reproductive function by decreasing estradiol concentration through inhibition of aromatase enzyme (cyp19a) or reducing the bio-conversion of testosterone to estradiol [46][47]. Besides, it has been shown that phytochemicals could affect fish reproduction and prevent the synthesis of vitellogenin (VTG) by binding to the estrogen receptor instead of estradiol [48]. Changes in the transcript levels of ERs in the liver are closely related to the regulation of vitellogenin synthesis in most teleosts [49][50]. VTGs are synthesized and secreted by the liver during estrogen stimulation and then transported to the ovary through the blood, taken up by oocytes, and converted into phospholipid-rich yolk proteins [5]. In addition to the above-mentioned genes, alteration of the expression levels of estrogen receptors (ERs) such as erα, erβ1, and erβ2 [50], and androgen receptors including arα and arβ [51], have been

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reported in fish. Other important genes having changes in the expressions of reproductive functions include aromatase (cyp19a and cyp19b). cyp19a plays a role in female gender determination and differentiation, while cyp19b shows high expression in both sexes in adult fish [6]. In addition, the presence of other genes associated with steroidogenesis such as star , cyp11a1 , cyp17a1 , 3β-hsd , 11β-hsd2 , 17β-hsd3 , and ftz , androgen receptor (ar), sex-determining region Y-box 9a (sox9a), and double-sex and mab-3 related transcription factor 1 (dmrt1) have been reported in fish. Other than the referred reproduction-related genes affected by herbal extracts and PSMs in fish species, there are considerable numbers of genes affected in higher vertebrates. It is also acknowledged that the integrated regulation of reproduction can be altered through changes in apoptotic mechanisms, at different levels of the HPG axis. Here, we summarized the effects of a number of herbal extracts and PSMs on reproduction-related genes at different levels of the reproductive axis (**Table 2**) in different species of fish.

Table 2. The effect of herbal extracts and phytochemicals on reproduction-related genes.

Species/Source	Compound(s)	Species/Organ	Affected Gene(s)	References
Genistein	Isoflavone Angiogenesis inhibitor Phytoestrogen	Common carp (*Cyprinus carpio*) Ovary European bass (*Dicentrarchus labrax*) Scales and liver Zebrafish and Medaka (embryos)	*cyp19a1a* liver *vtgb2, erβ* *vtg2, chgl* Zebrafish: *cyp19a1b, vtg1* Medaka: *cyp19a1a, vtg, cyp19a1b*	[51] [52] [53]
Genistein and daidzein	Genistein: Isoflavone Angiogenesis inhibitor Phytoestrogen Daidzein: naturally occurring compound in soybeans and other legumes Isoflavone	Zebrafish Ovary Testis Zebrafish Embryos-larvae Rainbow trout (*Oncorhynchus mykiss*) Juvenile	Genistein exposure: Ovary: *erβ* Ovary and testis: *HE1* Daidzein exposure: Testis: *BRDT, esrrb, cyp1a* Liver *vitellogenin, erα1, erb1*	[54] [55] [56]
Eurycomanone and chitosan conjugated eurycomanone	Eurycomanone: the major quassinoid in Eurycoma longifolia root extract Chitosan: a linear polysaccharide composed obtained from the outer skeleton of shellfish including lobster, crab, and shrimp	Walking catfish (*Clarias magur*) Male Walking catfish (*Clarias magur*) Female	Brain: *fshβ* and *lhβ* Testes: *cyp11a1, star, cyp17a1, 3β-hsd, 17β-hsd, cyp19a1, ftz, ar, sox9a, dmrt1* Brain: *fshβ, lhβ, cyp19a2* Ovary: *ftz, star, cyp19a1, 3β-hsd, 17β-hsd, cyp17a1*	[57] [58]
Equol	isoflavandiol estrogen metabolized from daidzein	Japanese medaka *Larvae, Liver, Gonads*	*vtg1* *17β-hsd3, cyp11b, 11β-hsd2*	[59]

In addition to the aforementioned compounds, genistein, and daidzein, two natural Phyto-estrogens found in plants, affect reproductive processes depending on the dosage used, fish species, and age [60]. Schiller and colleagues exposed zebrafish embryos to genistein at 2.4 mg/L (EC10) for 48 h. They also exposed medaka embryos to genistein at 6 mg/L (EC10) and 10 mg/L (EC20) for 7 days [53]. Results showed that in both zebrafish and medaka cyp19a1b and vtg1 gene expressions increased, while a decrease in the expression level of the cyp19a1a gene was only found in medaka. In a different study, genistein was injected intraperitoneally to Dicentrarchus labrax (immature; 59.4 g ± 0.7) fish at a dose of 5 mg/kg, and after 24 h, scale and liver vtg2 and chgl gene expressions increased. At the end of the 5th day, similar results were found only in the liver tissue [52]. In a very recent study using the Cyprinus carpio fish model it was found that ovary

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cyp19a1a , and liver vtgb2 , and erβ gene expressions decreased after feeding female Cyprinus carpio fish with 0.01, 0.03, 0.06, and 0.09 g/kg genistein supplements for 60 days [51]. Moreover, other studies in zebrafish (embryos-larvae) showed that exposure of fish to genistein and daidzein at a concentration of 1.25, 2.5, 5, 10, and 20 mg/L for 96 h, increased expressions of esrrb and cyp1a [55]. Adult male and female zebrafish were also exposed to 10 mg/L genistein and daidzein concentrations for 10 days [54]. Results showed that in genistein exposure, HE1 gene expression increased in both ovary and testis, while only the ovary showed a decrease in erβ . Moreover, only testicular BRDT gene expression changed in the daidzein exposure [54]. Apart from the above-mentioned studies, another research performed in Oncorhynchus mykiss juveniles showed that injection of 5 μg/g body weights of genistein and daidzein along with 50 μg/g body weight genistein to fish, for 24 h, liver vtg, and era1 gene expressions increases [56]. Equol, on the other hand, is a nonsteroidal estrogen, metabolized from daidzein. It has been shown that this compound when tested on Japanese medaka larvae for 2 days in 2, 4, 8, 16, 40, 200, and 1000 ng/L, increased liver vtg1 gene expression and decreased 17s-hsd3 , cyp11b , and 11β-hsd2 gene expression in gonads [59].

Eurycomanone, found in Eurycoma longifolia plant extract, is a quassinoid that increases the reproductive processes of male animals. Studies report that eurycomanone increases testosterone production in rat testicular Leydig cell-rich interstitial cells by blocking aromatase and phosphodiesterase enzymes [46] and 25 mg/kg orally administered eurycomanone rich E. longifolia extract increases female fertility index, fecundity index, and the pup litter size [47]. Bahat and coworkers injected 0.059 and 0.118 μg eurycomanone/kg body weight and chitosan-conjugated eurycomanone to male Clarias maggot fish. Brain fshβ and lhβ expressions and testis cyp11a1, star, cyp17a1, 3β-hsd, 17β-hsd, cyp19a1, ftz, ar, sox9a, and dmrt1 increased depending on time, dosage, or mode of application [57]. In another study conducted by the same researchers, eurycomanone and chitosan-conjugated eurycomanone was injected in female Clarias magur fish 3 times in 21 days, and brain fshβ, lhβ ve cyp19a2 and ovary ftz, star, cyp19a1, 3β-hsd , 17β-hsd, and cyp17a1 gene expressions increased depending on dosage or mode of application [58].

Findings from all of these different studies demonstrated interactions between herbal extracts and PSMs with the regulation of different levels of the HPG axis. The changes observed in reproductive-related gene expression appear to be variable, depending on the species, mode, and duration of administration of herbal extracts and PSMs. However, herbal extracts and PSMs can influence reproduction either directly or indirectly by affecting the hormones of the HPG axis and/or by influencing apoptotic or steroidogenic pathways. The availability of sufficient steroidogenic enzymes is particularly important to support ovarian and testicular development and function, and the observed changes in gene expression of these enzymes would likely have important effects on the reproduction.