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# Exploring the Roles of Dietary Herbal Essential Oils in Aquaculture: A Review

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**Simple Summary:** Essential oils, also known as aetheroleum, have a variety of therapeutic properties, including analgesic, adaptogen, and astringents, among others. Essential oils have potential growth-promoting, antibacterial, and immunostimulant effects for several fish species. Furthermore, they potentiate the antioxidative capacity and the resistance of aquatic animals against infectious diseases. This article spotlights on the essential oils derived from selected medicinal plants, and their roles in the improvement of the performances of aquatic animals.

**Abstract:** The aquaculture sector is one of the main activities contributing to food security for humanity around the globe. However, aquatic animals are susceptible to several farming stressors involved in deteriorated growth performance, reduced productivity, and eventually high mortality rates. In some countries still, antibiotics and chemotherapies are comprehensively applied to control biotic stressors. Aside from the apparent benefits, the continuous usage of antibiotics develops bacterial resistance, deteriorates bacterial populations, and accumulates these compounds in the aquatic environment. Alternatively, environmentally friendly additives were used to avoid the direct and indirect impacts on the aquatic ecosystem and human health. In aquaculture, medicinal herbs and extracts are extensively used and approved for their growth-promoting, anti-inflammatory, and antioxidative properties. Herbal essential oils contain many bioactive components with powerful antibacterial, antioxidative, and immunostimulant potentials, suggesting their application for aquatic animals. Essential oils can be provided via diet and can benefit aquatic animals by improving their well-being and health status. The use of essential oils in aquafeed has been studied in a variety of aquatic animals to determine their beneficial roles and optimum doses. The outputs illustrated that herbal essential oils are exciting alternatives to antibiotics with prominent growth promotion, antioxidative, and immunostimulant roles. Herein, we reviewed the beneficial roles of essential

oils in aquaculture. This review also aims to describe trends in herbal essential oils use, mainly in commercial fish species, and to analyze different factors that affect essential oils' efficacy on the growth performance, antioxidative, and immune responses of finfish species.

**Keywords:** alternative medication; herbs; aquaculture; antioxidants; immunity; essential oils

## 1. Introduction

The expansion of the population requires sustainable and safe food resources. The aquaculture sector provides humanity with secure food and profitable income [1]. Nevertheless, the aquaculture sector is confronted with serious challenges related to immunocompromised, deteriorating health and well-being, substantially resulting in a high mortality of farmed aquatic animals [2]. Intensive and super-intensive farming practices induce stress to the aquatic animals, thereby increasing the possibility of infection with pathogenic invaders [3]. Synthetic antibiotics and chemotherapies are commonly used to relieve the negative impacts of infection, and enhance the immunological response and overall well-being of aquatic animals. The continuous application of antibiotics has resulted in several negative impacts on aquatic animals (e.g., bacterial resistance developed against antibiotics, suppressed host immunity, imbalance of microbial populations, and hazardous environmental features) [4]. Accordingly, safer alternative methods, such as organic feed additives, are highly suggested to mitigate and control pathogenic infections in aquatic animals [5]. Feed additive inclusion is an appropriate strategy that can be used in aquafeed at specific doses and can be orally administered to aquatic animals [6]. Aquafeed is commonly supplemented with various additives such as probiotics, prebiotics, and herbal substances to stimulate the health and well-being of aquatic animals [7,8]. Specifically, the inclusion of medicinal herbs and their extracts in aquafeed is involved in multiple functional roles associated with active metabolites and functional components [9].

Medicinal herbs, also known as phytochemicals, and the essence and oil derivatives of these plants, play significant roles as appetite enhancers, growth promoters, and immunostimulators for aquatic animals [10]. Essential oils (EOs) have gained special focus as natural antioxidant and immunostimulant agents. The chemical structural compound of EOs is resistant to gastric acid, ensuring its efficacy and effect [11,12]. Furthermore, these volatile oils enhance palatability and regulate appetite control genes in the hypothalamic–pituitary glands of fish [11]. EOs show a strong antibacterial effect against pathogenic bacteria by impairing their activity and damaging the bacterial cell walls [13]. Then, beneficial bacteria play influential roles in food digestion mediated by the secretion of digestive enzymes, along with improved local intestinal immunity and high resistance to the challenged pathogens [14]. In addition, EOs enhance the permeability of intestinal barriers and increase intestinal nutrient absorption. The immune system-boosting effects of EOs are attributed to the increase in intestinal immunity [3]. The balanced intestinal microbiota (increased abundance of beneficial microorganisms and decreased abundance of pathogenic microorganisms), the inhibition of pathogenic bacteria's adhesion sites, and the modification of the intestinal pH are the primary actions of EOs to relieve the pathogenic effects of bacteria on aquatic animals [15,16]. In fish challenged with pathogenic bacteria, EOs have been shown to improve the survival rate of many species [17–22]. Further, EOs contain high concentrations of polyphenols and natural antioxidants that remove the free radicals that cause lipid peroxidation and immune cell damage [23,24].

The ultimate beneficial effects of herbal EOs on fish and their mechanisms of action are not well described. This review aims to illustrate the effects of EOs and their modes of action on fish and to highlight knowledge gaps for future studies.

## 2. Natural Sources of Essential Oils

Essential oils (EOs) are a lipophilic mixture of organic compounds from the secondary metabolism of aromatic plants, which mostly are limpid liquid (colorless) with an obvious fragrance [25,26]. Plant EO constituents include two main groups of terpene-derived compounds: the first consists of hydrocarbon terpenes/terpenoids, and the second group includes oxygenated molecules, which in hydrocarbon terpenes derivatives (e.g., alcohols, aldehydes, ketones, phenols, acids, and esters) represent the common form, while phenylpropanoids and their derivatives are another class of oxygenated compounds. Rarely, one or more sulfur or nitrogen molecules are found in a few compounds of EOs [26,27]. EOs may contain dozens of ingredients in a trace proportion of the wet weight of the plant origin source [26]. In most cases, EOs are distinguished by two or three major components in relatively high ratios (major constituents of 20–95%) compared with other components that occur in small amounts (secondary constituents of 1–20% and trace components of below 1%) [28,29]. The chemical composition of EOs may vary according to plant species, the development stage, cultivation and environmental condition (i.e., soil and climate) [30], oil extraction, and processing methods [31,32]. Hence, various types of EOs are extracted, and their chemical composition and organoleptic properties are verified as follows.

### 2.1. Menthol

Menthol (mint camphor) is a covalent organic cyclic monoterpene alcohol ( $C_{10}H_{20}O$ : 2-Isopropyl-5-methyl-cyclohexanol) that exists in eight optically active isomers with different organoleptic properties from four stereoisomers (menthol, isomenthol, neomenthol, and neoisomenthol) in two optic forms (levogirous and dextroirous) [33]. Menthol is a colorless or white waxy crystalline solid substance at room temperature that melts slightly above it, and was first isolated in 1771 by the Dutch botanist Gambius [34]. The L- or (–)-menthol from the natural sources (plant origin) or synthesized is the most stable and preferred isomer [35]. Natural menthol is primarily derived from aromatic plants as it is the main constituent of EOs of the genus *Mentha* sp. [36]. *Mentha arvensis* L., (syn. *M. canadensis* L., Japanese mint) cornmint-MA, and *M. piperita* L. (Hudson) peppermint-MP are two well-known menthol mints in cultivation [35]. Menthol represents 70–90% of cornmint oil and about 20–60% peppermint oil [37,38]. The main supply of the world's menthol production (19,170 tones) is obtained naturally (67.14%) exclusively from *M. canadensis*, and about 6300 tones (32.86%) are produced synthetically [39].

### 2.2. Linalool

Linalool is an odorant non-cyclic monoterpene alcohol ( $C_{10}H_{18}O$ : 3,7-Dimethyl-1,6-octadien-3-ol) found in nature in two stereoisomers: Licareol (R)- or (–)-linalool, and Coriandrol (S)- or (+)-linalool [40,41]. Linalool represents up to 90.6% of the oil constituents of over 200 aromatic plant species of different families (Supplementary Materials, Table S1) [38].

### 2.3. Myrcene

Myrcene is an alkene natural hydrocarbon acyclic monoterpene compound ( $C_{10}H_{16}$ : 7-Methyl-3-methylene-octa-1,6-diene) that exists in two isomers, the first of which is  $\beta$ -myrcene (the natural form), and the second form ( $\alpha$ -myrcene) is not available naturally, but can be prepared industrially [42]. Myrcene is a colorless liquid found naturally in different sources (Supplementary Materials, Table S2) [38,43–45].

### 2.4. Eucalyptol

Eucalyptol is a cyclic monoterpene ether alcohol ( $C_{10}H_{18}O$ : 1,3,3-Trimethyl-2-oxabicyclo 2.2.2 octane) that is a colorless liquid that exists plentifully in nature [46]. Eucalyptol, also known as 1,8-cineole, and its isomer (1,4-cineole), occur naturally in the same plant species, although at much lower concentrations of 1,4-cineole than 1,8-cineole [47]. Eucalyptol is the main component (up to 80%) of EOs of *Eucalyptus* leaves

(*Eucalyptus* spp.) [48,49]. It is present in varying proportions in the essential oils of some other aromatic plants (Supplementary Materials, Table S3) [38].

### 2.5. Globulol (Ledol)

Globulol is known as 5,10-cycloaromadendrane sesquiterpenoids alcohol ( $C_{15}H_{26}O$ : 1,1,4,7-tetramethyl-2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa-e-azulen-4-ol), and exhibits a potent antimicrobial effect [50]. Globulol is also found in varying concentrations in EOs of various plants that belong to different families such as Myrtaceae, including *Eucalyptus* spp., with a rate of 5.3% extracted from the leaves and white kunzea (*Kunzea ambigua* (Sm.) Druce) by 11.2% from leaf oil; the family of Asteraceae, including Vassoura (*Baccharis dracunculifolia* DC), with a rate of 2.5–14.5% from leaf oil; the family of Cyperaceae such as Piri-piri (*Cyperus articulatus* L.) by 3.2–4.6% from rhizomes oil; and the family of Valerianaceae, e.g., Valerian (*Valeriana officinalis* L.) at a rate of 2.1% from root oil [38,50,51].

### 2.6. Spathulenol

Spathulenol is a viscous colorless tricyclic sesquiterpene alcohol with antileishmanial impacts ( $C_{15}H_{24}O$ : 1aR, 4aR, 7S, 7aR, 7bR)–1, 1, 7-Trimethyl-4-methylidene-1a, 2, 3, 4a, 5, 6, 7a, 7b-octahydrocyclopropa -h- azulene-7-ol) that is a major constituent of EOs extracted from the fresh leaves of croton species (*C. argyrophyllodes*, *C. jacobinensis*, and *C. sincorensis*) at 42.54%, 15.41%, and 9.58%, respectively [51]. It occurs as the main component in the oil originating from the male and female specimen leaves of *B. semiserrata* DC, achieving 50.75 and 42.65%, respectively [52]. Among other sources, the oil is extracted from the leaves of *E. polybractea* R. T. Baker (14.3%), *B. dracunculifolia* DC (2.6–10.0%), *Pilocarpus jaborandi* Holmes, and *P. microphyllus* Stapf. (7.6%) [38]. Spathulenol is one of the main constituents of leaf EOs of *Myrciaria tenella* (DC.) Berg (9.7%) [53]. In addition, it is the main component (20.7%) of the essential oil of air-dried herb of *Origanum vulgare* L. ssp. [54].

### 2.7. Guaiol (Champacol)

Guaiol is a sesquiterpenoid alcohol ( $C_{15}H_{26}O$ : 2-(3S,5R,8S)-3,8-Dimethyl-1,2,3,4,5,6,7,8-octahydro-5-azulenyl-2-propanol) that has anti-cancer, anti-anxiety, anti-inflammatory, anti-bacterial, and antioxidant properties [53,55–57]. Guaiol is one of many terpenes found in the oil of several aromatic plants, especially oils of a wood origin, e.g., guaiacwood (*Bulnesia sarmientoi* Lorentz ex Griseb.) at 26.8%, cypress emerald (*Callitris columellaris* F. Muell.) at 20.0%, cypress jade (*C. glaucophylla* Joy Thomps. and L.A.S.F. Muell.) at 14.7%, cypress blue (*C. intratropica* R.T. Baker and H.B. Sm.) at 13.7%, and araucaria (*Neocallitropsis pancheri* (Carriere) de Laub.) at 6% [38]. In addition, guaiol represents 13.1% of the leaf oil constituent of *Calycorectes sellowianus* O. Berg [53].

### 2.8. Caryophyllene Oxide

Caryophyllene oxide ( $\beta$ -caryophyllene) is the oxidized form of caryophyllene ( $C_{15}H_{24}O$ : 1R, 4R, 6R, 10S-4, 12, 12-trimethyl-9-methylidene-5-oxatricyclo-8.2.0.0 (4,6)-dodecane) with therapeutic applications [45]. There is a sesquiterpenoid compound called caryophyllene found in the essential oils of common eucalyptus *Melaleuca stylpheloides* in concentrations as high as 43.8% [58]. According to Tisserand and Young [38],  $\beta$ -caryophyllene is one of the major terpenes found in EOs originating from various plant species (Supplementary Materials, Table S4).

### 2.9. Thymol

Thymol is a monoterpene phenolic compound ( $C_{10}H_{14}O$ : 5-Methyl-2-(propan-2-yl)phenol) that exists naturally along with its isomer carvacrol [59,60]. Thymol occurs in varying percentages in the EOs of thyme species leaves (*T. zygis* (30.9–74.0%), *T. vulgaris* (48.3–62.5%), *T. serpyllum* L. (16.7–25.9%), *T. zygis* (25.5%), and *T. satuireioides* Coss. and Bal. (10.0%); dried aerial parts of oregano flowering plant (*Lippia graveolens*) HBK (60.6 %) and ajowan seeds (*Trachyspermum ammi* L. (36.9–53.8%) [38].

### 2.10. Carvacrol (CVC)

Carvacrol (cymophenol) is a natural monoterpene phenol (C<sub>10</sub>H<sub>14</sub>O: 2-Methyl-5-(propan-2-yl) phenol) that occurs along with its isomer thymol [46]. Carvacrol exhibits a distinct set of biological activities including antioxidant, antitumor, antibacterial, antifungal, and insecticidal properties [25,61]. Carvacrol is the primary compound of EO constituents of Lamiaceae species, including oils from aerial parts of oregano plants, including *O. onites* (66.5–80.4%), *O. majorana* L. (23.3–81.0%), *O. vulgare* (61.6–83.4%), *L. graveolens* HBK (0.5–24.8%), savory (*Satureia hortensis* L.) (43.6–70.7%), and *S. montana* L. (46.5–75.0%), thyme (*Thymbra spicata* L.) (70.0%), *Thymus vulgaris* L. (20.5%), and *T. satureioides* Coss. and Bal. Aerial parts (20.0%) [38,46].

### 2.11. Terpinen-4-ol

Terpinen-4-ol is a natural monoterpene isomer of terpineol (C<sub>10</sub>H<sub>18</sub>O: 4-methyl-1-propan-2-ylcyclohex-3-en-1-ol) [62] that is a promising potent therapeutic agent as it has antiviral, bactericidal, antifungal, anti-tumoral, anti-inflammatory, analgesic, insecticidal, and acaricidal activities [63–66]. Terpinen-4-ol is the primary component (30–48%) of tea tree oil (*M. alternifolia*), originating from the leaves [38,67]. Terpinen-4-ol occurs in varying percentages in EOs of plairrhizomes (*Zingiber montanum* Theilade: 41.7%), marjoram freshly dried flowering plant (*O. majorana* L.: 16.4–31.6%), basil leaves (*Ocimum canum* Sims.: 7.5–26.8%), Kewda flowers (*Pandanus fascicularis* Lam.: 0–21.0%), sugandh mantri rhizomes (*Homalomena aromatica* Schott.: 17.2%), juniper berries (*Juniperus communis* L.: 1.5–17.0%), mace pericarp (*Myristica fragrans* Houtt.: 4.4–14.0%), and nutmeg kernels (*M. fragrans* Houtt.: 1.0–10.9%) [38].

### 2.12. Dehydrofukinone

Dehydrofukinone (DHF) or 9,10-Dehydrofukinone is a sesquiterpene ketonic compound (C<sub>15</sub>H<sub>22</sub>O: (4aR,5S)-4a,5-dimethyl-3-propan-2-ylidene-5,6,7,8-tetrahydro-4H-naphthalen-2-one) that possesses sedative properties [68–70]. DHF represents the main component (22%) of canela-amarela leaf essential oil (*Nectandra grandiflora* Nees) [69]. In addition, DHF isolated from the aerial parts of *Senecio* spp. (*S. punae*, *S. humillimus*, *S. aureus*, and *S. viridis*) shows a high antifungal activity (92.7 ± 0.2%) [71] and has a beneficial effect on non-pathogenic bacteria (*L. plantarum*) [72].

## 3. Effects of Essential Oils on Growth and Gut Bacterial Communities

Eco-friendly natural alternatives to antibiotics as growth stimulators in aquaculture are very trendy [73,74]. The compounds of essential oils are an area of interest in many perspectives due to their distinctive biological properties [75]. Botanical products, including essential oils, have been shown to improve a variety of biological activities in aquatic animals, including growth, appetite stimulation, anesthetic, anti-stress, antimicrobial, tonic, and immunomodulatory effects [76–81]. The biological properties of EOs are determined by their major bioactive constituents with their additive or/and synergistic effects with each other or/and with the biological system at a cellular level or below it (electron flow) [82–85]. The positive aspects of EOs on growth are similar to the effects of prebiotic (prebiotic-like effect) and can be linked to intestine morphological and physiological changes, as well as to modulation of the gut microbiota [75,86].

Bacterial communities are influenced by environmental, nutritional, microbiological, and genetic factors [87–90]. Under normal conditions, the microbiota of GIT surfaces contains a dynamic microbial equilibrium of pathogenic and saprophytic bacteria [87,91]. The maintenance of a healthy GIT microbiota has an impact on the host body's performance and activities, such as nutrient utilization, digestibility, and immune modulation, because it can modulate the gene expression involved in epithelial proliferation, nutrient metabolism, and immune responses, as well as prevent the development of intestinal disorders and disrupting intestinal homeostasis [87,92–94]. The main role of EOs in modifying the gut microflora is the inhibition of pathogenic (harmful) bacterial groups and providing the op-



portunity for other groups (beneficial microflora) to dominate the gut [92,93]. The indirect effects of EOs on the intestinal microbiota can occur through changes in the intestinal environment, including changes in pH, and the type and amount of secretions of the intestinal mucosa [12,79,94–97]. The hydrophobicity of EOs is markedly affected by the pH value, which will control their antibacterial effect on the bacterial cell membrane. In this regard, rainbow trout (*Oncorhynchus mykiss*) provided with dietary *Thymus vulgaris* EO showed a marked antibacterial response against *Vibrio anguillarum* in the GIT [98]. Furthermore, Zhang et al. [99] reported that common carp (*Cyprinus carpio*) treated with Origanum EO had an increased count of *Propionibacterium*, *Brevinema*, and *Corynebacterium\_1*, while decreasing *Vibrio* genera. Nile tilapia (*Oreochromis niloticus*) fed diets supplemented with essential oil from lemongrass (*Cymbopogon citratus*) and geranium (*Pelargonium graveolens*) had decreased counts of total bacteria, coliforms, *Escherichia coli*, and *Aeromonas* spp. in their intestine [100].

Reports of the effect of EOs on the intestinal microflora of aquatic organisms are scarce. In this respect, Giannenas et al. [101] assessed the dietary supplementation with carvacrol or thymol derived from *T. vulgaris* EO on rainbow trout intestinal microbiota. These authors showed a significant modulation in the gut microbiota characterized by a reduction of the total anaerobic bacteria. The efficacy of carvacrol and thymol incorporation in the diet for 6 weeks was also observed as modulating the intestinal microflora in red hybrid tilapia (*O. niloticus* × *O. aureus*) [102]. In contrast, no significant impacts of EOs on the intestinal microflora were reported in red drum (*Sciaenops ocellatus*) fed diets enriched with *O. americanum* EO [97] and in rainbow trout fed a diet supplemented with *T. vulgaris* EO [98]. Nonetheless, Zhang et al. [99] showed that oregano EO could alter the intestinal microbiota of the koi carp intestine by increasing the bacterial communities of *Propionibacterium*, *Brevinema*, and *Corynebacterium*.

The direct and indirect impacts of EOs on the gut microbiota enhance nutrient digestion and absorption, which positively affect fish growth by increasing amino acid array for protein synthesis and through deposition in the musculature [75]. In addition, EOs can directly enhance the appetite, digestion, absorption, anti-inflammatory, and antioxidant activities through maintaining intestinal health [103–105]. Maintaining a healthy intestine supports the continuity of its vital role in digestion and absorption, which significantly affects growth [106]. The improvement indicator of intestine health is an increase in the absorption area due to an increase in the intestinal secretion sources (submucosal tissues, goblet cells and crypt, and tunica muscularis), free of inflammatory and/or degenerative alterations, as well as the villus number, height, and width [107,108]. In this regard, dietary oregano EOs positively impact the intestine histomorphometry of the common carp fingerlings, including an improvement in the morphological structure of the intestinal villus [108]. Likewise, thymol dietary incorporation increased the length of the intestinal villus in Nile tilapia [109]. Moreover, Ferreira et al. [110] concluded that dietary oregano EO increased the intestinal absorptive area of yellow tail tetra fish alongside a significant glycogen accumulation in the liver. Furthermore, the positive influences of EOs on the growth performance may be due to the increased secretion and activity of GIT protease, amylase, and lipase, as reported by Zhang et al. [99].

The impacts of the dietary supplement of EOs on fish growth performance is described in Table 1. The growth of Nile tilapia responded in a positive way with the dietary supplementation of oregano EO [111,112], cinnamaldehyde and thymol [113], limonene and thymol [114], clove basil EO [115], encapsulated oregano oil containing 7.5% of carvacrol, and 2.5% of thymol (Silaacid®) [116]. Likewise, Hassaan and Soltan [117] found that EOs of fennel and garlic, alone or in combination with *Bacillus licheniformis*, had a positive effect on Nile tilapia fry growth performance and feed efficiency. In the case of silver catfish (*Rhamdia quelen*), lemon verbena (*Aloysia triphylla*) EO has been shown to promote better growth [118]. In the case of Mozambique tilapia (*O. mossambicus*), curcumin (*Curcuma longa*) EO upregulated the mRNA expression of the growth factor (IGF-1 and IGF-2) genes in

the muscle [119]. In addition, sweet orange peel (*Citrus sinensis*) EO [17] and lemon peel (*C. limon*) EO [18] improved the growth performance of *O. mossambicus*.

Sönmez, et al. [120] demonstrated a favorable growth performance and feed efficiency of rainbow trout juveniles who consumed diets containing sage (*Salvia officinalis*) and thyme (*T. vulgaris*) oils, and the lowest performance was found with mint oil (*M. spicata*) supplementation. Oregano EO (*O. heracleoticum* L.) with its main constituents (carvacrol and thymol) exhibited affirmative growth in common carp [20], great sturgeon (*Huso huso*) [121], yellowtail tetra (*Astyanax altiparanae*) [122], rainbow trout [22,123], and channel catfish (*Ictalurus punctatus*) [21]. Carvacrol- and thymol-based diet supplements had a positive influence on trout growth and feed utilization, similar to Nile tilapia [124]. In addition, Giannenas et al. [101] confirmed that the use of carvacrol or thymol extracted from *T. vulgaris* as a dietary supplement improved the growth of rainbow trout. Gonçalves et al. [125] reported an improvement in the intestinal villus, nutrient utilization, and the growth of European sea bass (*Dicentrarchus labrax*) with dietary inclusion of a commercial EO product (Biomin® Digestarom PEP MGE 150).

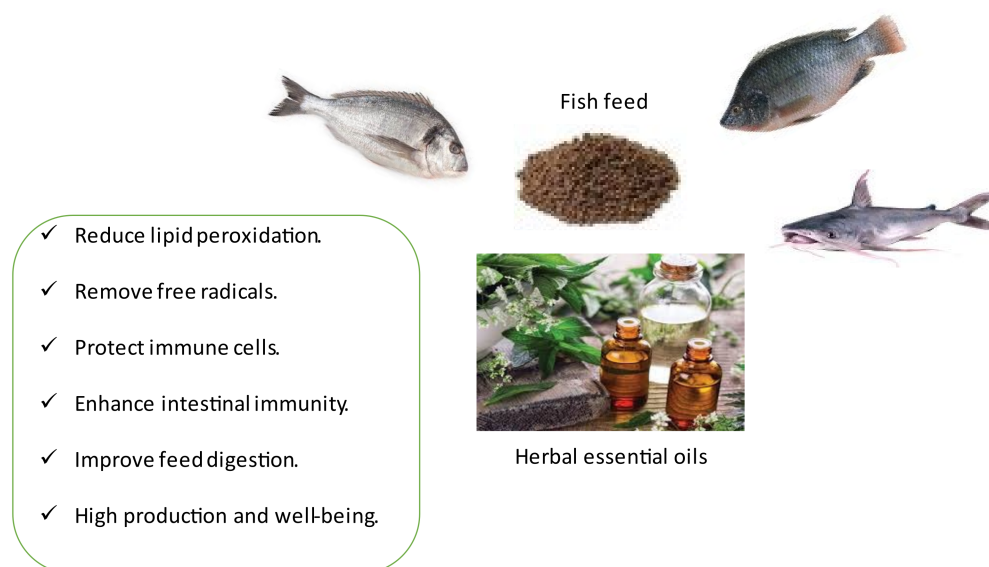
#### 4. Essential Oils as Natural Antioxidants

Oxygen reactive species (ROS) are pro-oxidant compounds that generate by the partial reduction of oxygen in the mitochondria during the oxidative metabolism as second messengers for various growth factors, as well as in cellular response to bacterial invasion, enzymic deficiency, xenobiotics, and cytokines [126,127]. A few ROS examples include superoxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), and hydroxyl radical ( $HO\bullet$ ). Essential molecules such as DNA, proteins, and lipids are particularly vulnerable to ROS, whereas antioxidants protect these molecules from the negative effects of oxidation [128]. Oxidative stress is caused by an imbalance in antioxidant supply and oxidant component disposal (ROS) [129,130]. The antioxidative defense system is composed of antioxidant enzymes (catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPx), and superoxide dismutase (SOD)) and non-enzymatic antioxidants (non-protein thiols (NPT)) (NPSH) [131–133]. The antioxidant activity is mediated by the reductive structure of the compound, which contains aromatic rings, phenolic compounds, and a high concentration of hydroxyl groups [134–136].

Several studies have indicated that natural antioxidants can improve the health status and performance of aquatic organisms [75,137–146] (Figure 1). In this context, an increase in antioxidant activity was found in koi carp fed diets with oregano EO (carvacrol and thymol) by Zhang et al. [99]. The same improvement in antioxidant activity with oregano EO (carvacrol and thymol) dietary supplements was determined in Nile tilapia [137,138], in rainbow trout [101], and in channel catfish [21,139]. The dietary incorporation of *A. triphylla* EO boosted the antioxidant status of silver catfish [140]. In addition, Sönmez et al. [120] declared a marked alteration in the antioxidant activity of rainbow trout juveniles fed diets containing sage, thyme, and mint EOs. Saccol et al. [80] reported that dietary supplementation of *L. alba* EO decreased lipid peroxidation and increased the tissue antioxidant response of silver catfish. Hsieh et al. [141] reported a strong antioxidant and anti-stress activity of rutin (bioflavonoid extracted from *Toona sinensis*) in white shrimp (*Litopenaeus vannamei*).

Moreover, research has indicated the role of EOs as an anesthetic in improving antioxidant activity. de Freitas Souza et al. [142] reported that anesthetics containing citral and linalool chemotypes of *L. alba* EO reduced lipid peroxidation while increasing the antioxidant activity in silver catfish. Similarly, Saccol et al. [143] found that rapid and extended sedation using Myrcia oil (*Myrcia sylvatica*) and turmeric oil (*Curcuma longa*) decreased lipid peroxidation and increased SOD, CAT, and GST in matrinxã (*Brycon amazonicus*). Barbas et al. [68] found that the use of *N. grandiflora* and *Spilanthes acmella* EOs as anesthetics boosted protection against muscular and gills oxidative damage of juvenile tambaqui (*Colossoma macropomum*). Silver catfish sedated with *A. triphylla* EO exhibited a lower level of lipid peroxides in the liver and higher CAT and GST activities [144]. Baldis-

sera et al. [145] found that *M. alternifolia* EO helps to protect against oxidative damage in *R. quelen* infected with *Aeromonas hydrophila*.



**Figure 1.** The roles of herbal essential oils on the performances of aquatic animals.

## 5. Essential Oils as Immunostimulants

Herbal remedies include aromatherapy as a complementary medicine area, which has existed since ancient times, in which all or part of the plant/herb, extracts, or other herbal products are used via various administration methods (orally, topically, massaged, or inhaled) [146,147] (Figure 1). EOs extracted from plant sources possess distinctive antimicrobial, antioxidant, anti-inflammatory, anti-stress, appetite stimulators, analgesic, and aphrodisiac activities [73,148]. With the diversification of EO extraction methods (steam distillation, hydro diffusion, or pressure) and availability, applications and studies have increased [148,149] (Table 1). However, traditional extraction methods are worth close attention because these techniques take a long time, resulting in a reduction and degradation of specific volatile compounds [150]. Microwave-assisted, supercritical fluid, solvent extraction under pressure, and ultrasound-assisted extraction methods are more advanced to produce high-quality EOs with a low energy, cost, and less time [151].

Two categories of immune responses, namely the natural (innate) and the acquired (adaptive) immune responses, where immune stimulation is associated with the non-specific activation of both, enhance certain immune functions and thus the defense against various pathogens [152,153]. The innate response represents the first defensive action and a considerable part of the immunity system, which includes functions of monocytes, macrophages, basophil granulocytes, neutrophil, eosinophil mast cells, natural killer (NK) cells, and dendritic cells, and these functions involve phagocytosis, cytokine production, the release of inflammatory mediators, and antigen production [154]. Phagocytosis is a defensive line in fish that employs bactericidal and lysozyme activities as non-specific immune lines to tolerate pathogens [155,156]. The acquired response employs the production of antibodies/immunoglobulins (Ig), B cells (plasma cells), and T-cells ( $CD_4^+$  T helper cells and  $CD_8^+$  cytotoxic T cells) [148]. In fish, lymphocytes mediate cellular and humoral immune responses, and the primary lymph organs in fish are the kidney, spleen, thymus, and anterior [10].

Functional and nutritional supplements, as well as balanced diets, can stimulate immune responses in fish [157]. EOs have shown immunostimulant properties in several aquatic animals. In this regard, EOs from basil (*O. gratissimum*) and ginger (*Z. officinale*) in the diet boost the Nile tilapia's immune system, increasing resistance to *S. agalactiae* and the phagocytic activity through increased thrombocytes, total leucocytes, lymphocytes, and



neutrophils (THN) [115]. dos Santos et al. [158] reported that the inclusion of cinnamon oil (*Cinnamomum* sp.) in Nile tilapia diets subjected to acute hypoxic stress resulted in an increase of  $\alpha$ 1-,  $\alpha$ 2-globulins, and maintained the homeostasis of blood after hypoxic stress. In addition, the use of cinnamon powder elevated  $\gamma$ -globulin. Baba et al. [18] found an enhancement in the immune response of *O. mossambicus* fed *C. limon* peel EO and increasing resistance against the *Edwardsiella tarda* pathogen, highlighted by the enhancement in the nitro blue tetrazolium (NBT), total white blood cell (WBC), total protein (TP), lysozyme, and myeloperoxidase activities in the blood serum. Consistently, EOs originated from sweet orange peel boosted *O. mossambicus* defensive parameters, including activities of lysozyme and myeloperoxidase, and blood hematological and biochemical indices (i.e., serum total protein, hemoglobin, hematocrit levels, and erythrocyte) [17]. Sutli et al. [97] observed a significant improvement in the lysozyme activity in red drum fed a diet enhanced with *O. americanum* EO. In addition, the dietary addition of carvacrol improved some non-specific immune (lysozyme and myeloperoxidase activities) and serum biochemical statuses (total protein, globulin, triglyceride, and lower cholesterol) in rainbow trout [159]. Carvacrol and thymol stimulated the lymphocytes cell count in great sturgeon (*H. huso*) [121]. Moreover, *D. labrax* fed carvacrol exhibited remarkable resistance to the *Vibrio* pathogen and higher survival rates [160]. Dietary supplementation of carvacrol or thymol originated from *T. vulgaris* EO showed significant modulations in the lysozyme, the total amount of complement concentrations, and the catalase activity of rainbow trout [101]. Sheikhzadeh et al. [161] indicated that the inclusion of zaatar (*Zataria multiflora*) and blue gum (*Eucalyptus globolus*) EOs in the diet is suggested to elevate the general wellbeing of common carp during thermal stress in terms of respiratory burst activity and blood hematological parameters (RBCs and haematocrit). Rattanachaikunsopon and Phumkha-chorn [162] indicated that cinnamon oil, which consists of 90.24% cinnamaldehyde, 2.42% limonene, 2.03% cinnamyl acetate, 1.16% linalool, and 0.87%  $\alpha$ -terpineol, had a protective potent effect on experimental *Streptococcus iniae* infection in Nile tilapia. The incorporation of *Z. multiflora* EO in common carp diets enhanced immunity during low temperatures.

## 6. Concluding Remarks and Future Outlook

Herbal EOs provide enormous beneficial effects in aquaculture by improving appetite, microbial balance, immune responses, antioxidative capacity, and disease resistance of aquatic animals. At the same time, EOs provide growth-promoting and feed utilization effects. A comprehensive review indicates that the primary determinants of EO efficacy in aquatic animals are the oil's source, concentration, and duration of administration. This review article clearly illustrates that herbal EOs have beneficial effects on aquatic animals' performances, and can feasibly replace antibiotics and chemotherapies for clean, healthy, and sustainable aquaculture.

The gut microbiome, metabolomics, and proteomic tools should be taken into consideration to determine the potential impacts of EOs and their mechanisms on the immune system, gut microbiota, and growth performance. Hence, further studies on fish transcriptomic profiles are also required to determine and quantify the effects of botanical EO concentrations on adaptive immune response, antioxidative status, and disease resilience. Furthermore, further research plans are needed in this direction, coupled with comprehensive studies using advanced methods to characterize the gut microbiota of targeted fish species. Additional research is also required to investigate the possibility of combining EOs with other feed additives (e.g., probiotics and prebiotics) and comparing their effects to antibiotics.

**Table 1.** Herbal essential oils and their impacts on the physical performances and physiological responses of aquatic animals.

Aquatic Species	Essential Oil	Dose and Duration	Influence	Reference
Common carp ( <i>Cyprinus carpio</i> )	<i>Zataria multiflora</i>	30–120 ppm/kg diet for 22 days	- Antibody titer, WBCs, and bactericidal activity (↑) - Resistance against heat stress (↑)	[163]
Silver catfish ( <i>Rhamdia quelen</i> )	<i>Lippia alba</i>	10 µL/L for 7 h	- LPO, CAT, SOD, and GST in the liver, gills, and brain (↓) - Resistance against transport stress (↑)	[164]
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	Black cumin seed oil	1, 2, and 3 for 14 days	- Lysozyme, total protein, antiprotease, total serum IgM, and bactericidal activity (↑)	[165]
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	Carvacrol and thymol	1 g/kg for 8 weeks	- Feed efficiency, lysozyme, total complement concentrations, and CAT (↑) - Growth performance (↔) - MDA, total anaerobe counts, and <i>Lactobacillus</i> loads (↓)	[101]
Silver catfish ( <i>Rhamdia quelen</i> )	<i>Lippia alba</i>	0.25, 0.5, 1.0, or 2.0 mL/kg diet for 60 days	- Growth performance and blood indices (↔) - SOD, CAT, GPx, and GST (brain, gills, liver, kidney, and muscle) (↑)	[80]
Red drum ( <i>Sciaenops ocellatus</i> )	Lime basil	0, 0.25, 0.5, 1.0, and 2.0 g/kg diet for 7 weeks	- Growth performance and intestinal microbial community (↔) - Intraperitoneal fat deposition and stomach lysozyme activity (↑)	[97]
Nile tilapia ( <i>Oreochromis niloticus</i> )	Limonene and thymol	0, 200, 400, and 600 mg/kg for 63 days	- Growth performance, IGF-I, MUC, PEPT1, LPL, ALP and CAT (↑)	[114]
Common carp ( <i>Cyprinus carpio</i> )	Blue gum	30, 60, and 120 µL/L or mg/kg feed for 8 days	- Antibody titers and total white blood cells (↑) - Resistance against low water temperature (↑)	[166]
Nile tilapia ( <i>Oreochromis niloticus</i> )	Pepper rosemary and peppermint	20–40 mg/L (3 baths for 10 min each)	- The monogenean parasite prevalence (↓) - RBC and thrombocytes with <i>L. sidoides</i> (↓) - Glucose concentration and neutrophil count with <i>L. sidoides</i> (↑)	[76]
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	Carvacrol	0, 1, 3, or 5 g/kg for 60 days	- Lysozyme and myeloperoxidase activities (↑) - The serum total protein and globulin (↑) - Glucose and triglyceride (↓)	[159]
Common carp ( <i>Cyprinus carpio</i> L.)	Oregano	0, 5, 10, 15, and 20 g/kg diet for 2 months	- Growth indices and feed utilization (↑) - Total protein, albumin, and globulin, AST, ALP, ALT, and renal markers (creatinine and urea) (↔) - Intestinal morphometric measurements (↑)	[108]

Table 1. Cont.

Aquatic Species	Essential Oil	Dose and Duration	Influence	Reference
Nile tilapia ( <i>Oreochromis niloticus</i> )	Peppermint and tea tree	100 and 250 mg/kg for 60 days	- The haematological and biochemical parameters (↔) - The complement system (↑) - Intestinal morphology (↑)	[167]
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	1,8-cineole, carvacrol or pulegone	0.5, 1, and 1.5% for 60 days	- Growth indices and feed utilization (↑) - Liver or kidney histological alterations (↔)	[168]
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	Oregano	6 and 10 g/kg diet	- TAC, SOD, CAT, and GPX (↑) - MDA, ALT, AST, and LHD (↔) - Resistance against diazinon toxicity (↑)	[169]
Yellowtail Tetra ( <i>Astyanax altiparanae</i> )	Oregano	0.0, 0.5, 1.0, 1.5, 2, and 2.5 g/kg for 90 days	- Growth indices and feed utilization (↑)	[170]
Nile tilapia ( <i>Oreochromis niloticus</i> )	Oregano	0.0, 1.0, and 2 mL/kg for 10 weeks	- Growth indices and feed utilization (↑) - SOD, GR, and NO (↑) - Resistance against stocking density (↑)	[138]
Nile tilapia ( <i>Oreochromis niloticus</i> )	Cinnamaldehyde and thymol	1 and 2 mL/kg diet for 75 days	- Growth indices and feed utilization (↑) - GR, lysozyme activity, IgM, IgG levels, and CAT (↑) - MDA (↓)	[113]
Great sturgeon ( <i>Huso huso</i> Linnaeus, 1758)	Thymol–carvacrol	0, 1.0, 2.0, and 3.0 g/kg for 60 days	- Growth indices and feed utilization (↑) - Haematological indices (↔)	[121]
Nile tilapia ( <i>Oreochromis niloticus</i> )	Menthol	0.25% for 30 days	- Growth indices and feed utilization (↑) - Antioxidative capacity (↑) - Immune response, anti-inflammatory, and proinflammatory related genes (↑) - Resistance against chlorpyrifos toxicity (↑)	[171]

(↑): significantly increases; (↓): significantly decreased; (↔): no significant change; WBCs: white blood cells; LPO: lipoperoxidation; CAT: catalase; SOD: superoxide dismutase; GST: glutathione-S-transferase; IgM: immunoglobulin; MDA: malondialdehyde; IGF-I: insulin growth factor I; MUC: mucin-like protein; PEPT1: oligo-peptide transporter I; LPL: lipoprotein lipase; ALP: alkaline phosphatase; RBC: red blood cells; TAC: total antioxidant capacity; LHD: lactate dehydrogenase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GR: glutathione reductase; NO: nitric oxide.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ani12070823/s1>. Table S1: Linalool in the essential oil constituents of its natural sources (>10%). Table S2: Myrcene in the essential oil constituents of its natural sources (>10%). Table S3: Eucalyptol (1,8-cineole) in the essential oil constituents of its natural sources (>10%). Table S4:  $\beta$ -caryophyllene in the essential oil constituents of its natural sources (>10%).

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