Chapter 5

Plant-Derived Compounds as an Alternative Treatment Against Parasites in Fish Farming: A Review

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Abstract

Aquaculture has grown rapidly for food production around the world. However, outbreaks of infectious diseases have also increased in aquaculture, causing serious economic losses. For many years, fish farmers have applied conventional treatments such as anti-parasitics and chemical treatments to control fish parasites. However, previous studies have revealed an accumulation of these chemical residues in fish tissues, and a negative environmental impact from farms to aquatic organisms. As an alternative to conventional methods, many plant-derived compounds such as essential oils (e.g. *Origanum* sp. and *Lippia* spp.) and plant extracts (e.g. *Allium sativum* and *Mentha* spp.) have been used as an efficient treatment to control parasites in freshwater, brackishwater and marine aquaculture systems. Our objective with this review is to highlight the advantages of the use of plant extracts as an alternative treatment against parasites in aquaculture (e.g. protozoans, myxozoans and monogeneans) and to show the possible negative environmental impacts of conventional treatments used in fish farming systems. Finally, we also highlight the potential of discovering new plant-derived bioactive compounds that have been increased in the last year due to the use of new tools such as the application of nanotechnology and microencapsulation to control diseases in fish farming.

Keywords: plant extract, anthelmintic activity, fish parasites, fish farming

1. Introduction

Aquaculture has grown rapidly for food production around the world [1], but infection in aquaculture is an important factor affecting food production [2]. Outbreaks of the infectious diseases have caused significant economic losses in freshwater, brackish water and marine aquaculture systems [2–5]. For instance, although the salmon farming has supplied 53% of the world market [6], their losses due to attack by the salmon louse (*Lepeophtheirus salmonis*) increase farming salmon costs with a global annual cost exceeding $400 million [7].

The increase of the parasites in the farming system led to the development of several chemical treatments [8, 9]. For many years, fish farmers have applied conventional treatments such as anti-parasitics, chemotherapeutics and insecticides to prevent or control parasitic infections in aquaculture [4, 10]. Indeed, the use of traditional parasiticides is well known in the control of helminths [11], such as praziquantel [12], mebendazole [13] and trichlorphon [14]. However, previous studies have revealed side effects of chemical parasiticides, including an accumulation in fish tissues [15], and adverse consequences on the indigenous microflora of the fish [16, 17].

Also, the accumulation of anti-parasitics and chemical residues in water has caused impacts on the environment [18, 19], especially in aquaculture in open waters where drugs are not easily controlled [10]. These chemical residues may have lethal or sub-lethal effects on non-target organisms in the environment [20] (**Figure 1**). For example, when pesticides such as Neguvon and Nuvon were used to control *L. salmonis* in the salmon net-pen farming in Norway, there have been harmful effects on several crustaceans near the farms [21].

During the last years, the search for new and natural treatment to mitigate the side effects of chemicals used in aquaculture included bioactive chemicals from plants [22]. Plants are a rich source of bioactive compounds like alkaloids and glycosides, and they might be an alternative source of natural parasitic control [23]. Medicinal plants have been reported as appetite stimulation, antimicrobial, immunostimulant, anti-inflammatory, biopesticides and anti-parasitic properties and their use in traditional medicine has been known for thousands of years around the world [15, 24–26]. Nowadays, natural products are preferred because of their biodegradability in the environment [23] (**Figure 1**). As an alternative to the conventional methods, different essential oils and plant extracts have been tested and used as an efficient and alternative treatment against parasites in aquaculture [9, 15]. For example, plant-derived compounds have been used either as immunostimulants [17] or as anti-parasitic activity against fish parasites, especially monogeneans and protozoans [15, 27].

The use of the plant-derived compounds has been concentrated in protozoans and especially in monogeneans [27]. Monogeneans (e.g. *Dactylogyrus* spp. and salmon fluke *Gyrodactylus salaris*) and protozoans (e.g. Ich *Ichthyophthirius multifiliis* and *Trichodina* spp.) are very common ectoparasites living on the gills of freshwater and marine fish [28, 29]. Recently, a few studies have used these plant-derived compounds to control myxozoan species such as *Myxobolus* spp. and *Enteromyxum* spp. [30, 31]. For example, essential oil of *Origanum* has been reported to provide varying degrees of protection and therapy in fish infected with myxosporean parasites [30–32].

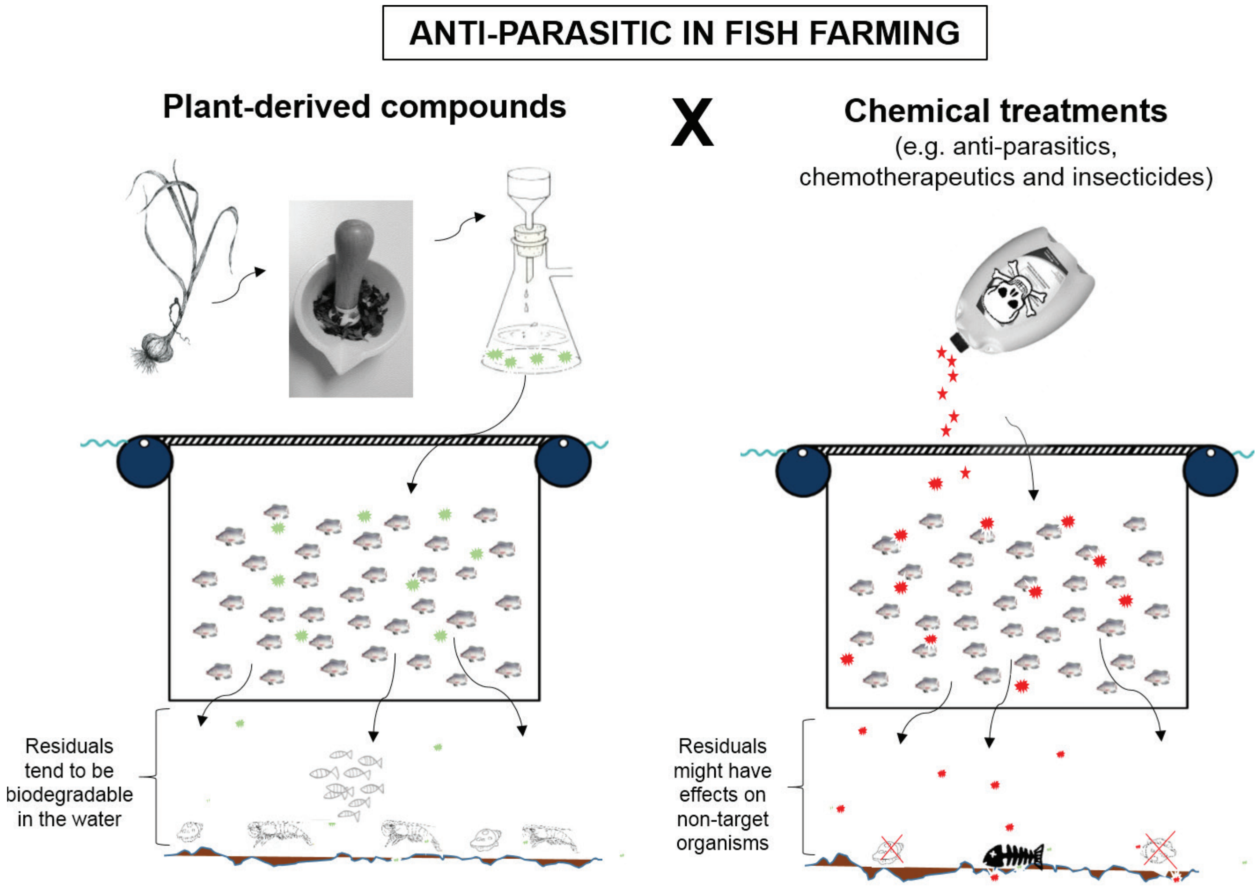

Figure 1. Effects of the residues between plant-derived compounds and conventional anti-parasitic treatments used in fish farming in the environment. Left: Residues from plant-derived compounds treatments tend to be biodegradable in the water. Right: Residues from conventional anti-parasitic treatments might have effects on non-target organisms (e.g. fishes, crustaceans).

In this review, we will begin with an overview of the use of plant-derived compounds as anthelmintic activity in fish aquaculture and identify the advances made by phytotherapy in this research field. We will also describe essential oils, plant extracts and isolated substances that have been used to control parasites in fish farming. Overall, we will illustrate the use of these compounds with several case studies for which information exists on anti-parasitic activity against protozoans, myxozoans and helminths (monogeneans), which are one of the most economically important parasite species in fish farming. Therefore, our main objective in this review is to highlight the advantages of the use of plant extracts as an alternative treatment against parasites in aquaculture and discuss the environmental impacts of conventional treatments used in fish farming systems.

2. Plant-derived compounds as fish anti-parasitics

Historically, plant-derived compounds have long been used in traditional medicine for the treatment of many diseases [33]. Numerous plants have been used to investigate the effects of their compounds to enhance the immune responses and increase the protective abilities against pathogenic agents in fish farming [17, 34].

Many studies have shown that essential oils, extracts and isolated substances from plants might be an important and alternative oral and immersion treatment against parasites in aquaculture (For a review see [27]). In addition, these plant extracts are capable of enhancing immune responses and disease resistance of cultured fish, serving as a great phytotherapeutics against infections in aquaculture [15]. To date, more than 60 plant species have been studied for the use as phytochemicals to control and prevent parasites such as protozoans (**Table 1**), myxozoans (**Table 2**) and monogeneans (**Table 3**) in freshwater and marine aquaculture [9, 15].

2.1. Anti-protozoan activity

Plant-derived compounds to control protozoans have been recently experimented and tested [9, 27]. Research on essential oils for controlling protozoans that inhibit the growth of fingerlings is still scarce. Soares et al. [35] analysed the essential oil of *Lippia alba* (bushy matagrass) leaves at concentrations of 100 and 150 mg/L and obtained efficacies of 40.7 and 50.3% against the *I. multifiliis* protozoan, which is a parasite of *Colossoma macropomum* (tambaqui).

Recent studies of medicinal plants have also shown promising results in the treatment of protozoal diseases in aquaculture [27]. The results revealed that the exposure of methanol extract of *Magnolia officinalis* (2.45 mg/L) and *Sophora alopecuroides* (pea flowered tree) (3.43 mg/L) caused the highest mortality against *I. multifiliis*, a pathogenic ciliate that infects fresh and marine fish farming [36]. These extracts revealed the highest antiprotozoal activity against theronts, which are released from infective stages (i.e. tomites) as swarmers to seek new hosts [36] actively. Extracts of *Eclipta* prostrate (false daisy), *Lycium chinense* (Chinese matrimony vine), *Ophiopogon bodinieri* and *Trichosanthes kirilowii* (Chinese cucumber) showed high antiprotozoal activity against *I. multifiliis* in fish *Carassius auratus*, ranging from 80 to 100% mortality [36]. *Allium sativum* (garlic) and *Matricaria chamomilla* (chamomile) extracts were also active in the control of *I. multifiliis* in *Poecilia latipinna* (sailfin molly) [37]. These results suggest, therefore, that the use of essential oil and medicinal plant extracts is viable and has a significant efficacy for the control of these protozoans in fish farming.

2.2. Anti-myxozoan activity

Recently, a few studies have used the essential oils to control myxosporean species such as *Myxobolus* spp. and *Enteromyxum* spp. [30, 31]. For example, *Origanum* essential oils have exhibited differential degrees of protection against myxosporean infections in gilthead and sharpsnout sea bream tested in land-based experimental facilities [30, 32]. Athanassopoulou et al. [30] tested the essential oil of *Origanum* and found a reduction of the prevalence of *Myxobolus* sp., but with a high level of fish mortality in *Puntazzo puntazzo* (sharpsnout sea bream). This same oil showed a reduction in the prevalence of the myxozoan *Polysporoplasma sparis* in *Sparus aurata* (gilthead sea bream) from 50% to less than 4% [32]. Cojocaru et al. [38] showed a decrease from about 40 to 20% in the prevalence of the infestation of the *Enteromyxum leei* in *S. aurata* after a month of oral and bath treatments using several essential oils. The essential

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Plant	**Fish**	**Type of extract**	**Isolated substances**	**Type of administration**	**Protozoan species**	**References [number]**
Allium sativum	*Poecilia latipinna*			Bath	*Ichthyophthirius multifiliis*	Gholipour-Kanani et al. [37]
Allium sativum	*Pterophyllum scalare*	Essential oil	*E*,*Z*-Ajoene	Oral/Bath	*Spironucleus vortens*	Williams et al. [39]
Eclipta prostrate	*Carassius auratus*	Methanol		Bath	*Ichthyophthirius multifiliis*	Yi et al. [36]
Lippia alba	*Colossoma macropomum*	Essential oil (leaves)		Bath	*Ichthyophthirius multifiliis*	Soares et al. [35]
Lycium chinense	*Colossoma acropomum*	methanol		Bath	*Ichthyophthirius multifiliis*	Yi et al. [36]
Macleaya microcarpa	*Colossoma acropomum*		Dihydrosanguinarine, dihydrochelerythrine	Bath	*Ichthyophthirius multifiliis*	Yao et al. [40]
Magnolia officinalis	*Carassius auratus*	methanol		Bath	*Ichthyophthirius multifiliis*	Yi et al. [36]
Matricaria chamomilla	*Poecilia latipinna*			Bath	*Ichthyophthirius multifiliis*	Gholipour-Kanani et al. [37]
Ophiopogon bodinieri	*Carassius auratus*	methanol		Bath	*Ichthyophthirius multifiliis*	Yi et al. [36]
Psoralea corylifolia	*Carassius auratus*	methanol	Isopsoralen, psoralidin	Bath	*Ichthyophthirius multifiliis*	Song et al. [41]
Sophora alopecuroides	*Carassius auratus*	methanol		Bath	*Ichthyophthirius multifiliis*	Yi et al. [36]
Toddalia asiatica	*Carassius auratus*	Methanolic (leaves)	Chelerythrine and chloroxylonine		*Ichthyophthirius multifiliis*	Xiao-feng et al. [42]
Trichosanthes kirilowii	*Carassius auratus*	methanol		Bath	*Ichthyophthirius multifiliis*	Yi et al. [36]

Table 1. Medicinal plants with activity against protozoan species in fish farming.

Plant	Fish	Type of extract	Type of administration	Myxozoan species	References [number]
Achillea millefolium	*Sparus aurata*	essential oil/ water/ Ethanol	Oral/Bath	*Enteromyxum leei*	Cojocaru et al. [38]
Betula alba					
Calendula officinalis					
Cerasus sativa					
Crategus monogyna					
Equissetum arvensis					
Hypericum perforatum					
Matricaria chamomilla					
Mentha piperita					
Origanum spp.	*Diplodus puntazzo*	essential oil	Oral	Myxobolus sp.	Karagouni et al. [31]
Origanum minutiflorum	*Puntazzo puntazzo*	essential oil	Oral	*Myxobolus* sp.	Athanassopoulou et al. [30]
Origanum spp.	*Sparus aurata*	essential oil	Oral	*Polysporoplasma sparis*	Athanassopoulou et al. [32]
Ocinum basilicum	*Sparus aurata*	essential oil/ water/ethanol	Oral/Bath	*Enteromyxum leei*	Cojocaru et al. [38]
Prunus spinosus					
Rosa canina					
Sambucus nigra					
Thymus serpillum					
Tilia sp.					
Vaccinium myrtilus					
Viola tricolor					

Table 2. Medicinal plants with activity against myxozoan species in fish farming.

oil of *Origanum minutiflorum* (spartan oregano) decreased the prevalence of *Myxobolus* sp. in *P. puntazzo* from 37 to 39% in all oral treatments in comparison to untreated fish [31].

Also, medicinal plant extracts have also shown good results as anti-myxozoan agents. Aqueous and methanol extracts of the species *Achillea millefolium* (milenrama milfoil), *Betula alba* (silver birch), *Calendula officinalis* (marigold), *Cerasus sativa* (sweet chestnuts), *Crategus monogyna*

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Plant	Fish	Type of extract	Isolated substances	Type of administration	Anthelmintic activity	References [number]
Allium sativum	*Poecilia reticulata*	Water		Oral/Bath	*G. turnbulli*, *Dactylogyrus* sp.	Fridman et al. [43]
Allium sativum	*Cyprinus carpio*	Hexane		Bath	*Capillaria* sp.,	Peña et al. [44]
Artemisia annua	*Heterobranchus longifilis*	Ethanol (leaves)		Bath	Monogenean	Ekanem and Brisibe [45]
Bixa orellana	*Colossoma macropomum*	Acetone (seeds)	Bixin and geraniol	Bath	*A. spathulatus*	Andrade et al. [46]
Brucea javanica	*Carassius auratus*	Methanolic (fruits)	bruceine A and bruceine D	Bath	*D. intermedius*	Wang et al. [47]
Bupleurum chinense	*Carassius auratus*	PE, CHL, EA, ME, water		Bath	*D. intermedius*	Wu et al. [48]
Caulis spatholobi,	*Carassius auratus*	PE, CHL, EA, ME, water		Bath	*D. intermedius*	Liu et al. [49]
Cimicifuga foetida L.	*Carassius auratus*	PE, CHL, EA, ME, water		Bath	*D. intermedius*	Wu et al. [48]
Cinnamomum cassia	*Carassius auratus*	PE, CHL, EA, ME, water		Bath	*D. intermedius*	Ji et al. [50]
Dioscorea zingiberensis	*Carassius auratus*				*Dactylogyrus* sp.	Jiang et al. [51]
Dryopteris crassirhizoma	*Carassius auratus*	PE, CHL, EA, ME, acetone		Bath	*D. intermedius*	Lu et al. [52]
Dryopteris crassirhizoma	*Carassius auratus*	PE, EA, ME (roots)	Protocatechuic acid, sutchuenoside A, and kaempferitrin	Bath	*D. intermedius*	Jiang et al. [53]
Euphorbia fischeriana	*Carassius uratus,*	PE, EA, ME, n-butanol, water		Bath	*D. vastator*	Zhang et al. [54]
Fructus bruceae	*Carassius auratus*	PE, CHL, EA, ME, water		Bath	*D. intermedius*	Liu et al. [49]
Fructus cnidii	*Carassius auratus*	Ethanol (fruits)	Osthol and isopimpinellin	Bath	*D. intermedius*	Wang et al. [55]

Plant	Fish	Type of extract	Isolated substances	Type of administration	Anthelmintic activity	References [number]
Ginkgo biloba	*Anguilla anguilla*	PE (exopleura)	Ginkgolic acid C13:0 and C15:1	Bath	*Pseudodactylogyrus* sp.	Wang et al. [56]
Ginkgo biloba	*Carassius auratus*				*Dactylogyrus*	Jiang et al. [51]
Kochia scoparia	*Carassius auratus*	PE, CHL, EA, ME, acetone		Bath	*D. intermedius*	Lu et al. [52]
Lippia alba	*Colossoma macropomum*	Essential oil (leaves)		Bath	*A. spathulatus, N. janauachensis, M. boegeri*	Soares et al. [35]
Lindera aggregata	*Carassius auratus*	PE, CHL, EA, ME, water		Bath	*D. intermedius*	Ji et al. [50]
Lippia sidoides	*Oreochromis niloticus*	Essential oil (leaves)		Bath	*C. tilapiae; C. thurstonae; C. halli; S. longicornis*	Hashimoto et al. [57]
Macleaya Microcarpa	*Carassius auratus*	Ethanol (aerial parts)	Sanguinarine, cryptopine, β-allocryptopine, protopine, 6-methoxyl-dihydro-chelerythrine	Bath	*D. intermedius*	Wang et al. [58]
Mentha piperita	*Arapaima gigas*	Essential oil (Leaves and inflorescences)		Bath	*Dawestrema* spp.	Malheiros et al. [59]
Mentha piperita	*Oreochromis niloticus*	Essential oil (leaves)		Bath	*C. tilapiae; C. thurstonae; C. halli; S. longicornis*	Hashimoto et al. [57]
Momordica cochinchinensis Spreng	*Carassius auratus*	PE, CHL, EA, ME, water		Bath	*D. intermedius*	Wu et al. [48]
Ocimum gratissimum	*Colossoma macropomum*	Essential oil (leaves)		Bath	Monogenean	Boijink et al. [60]
Paris polyphylla			polyphyllin D and dioscin		*D. intermedius*	Wang et al. [61]
Peucedanum decursivum (Miq.)	*Carassius auratus*	PE, CHL, EA, ME, water		Bath	*D. intermedius*	Wu et al. [48]

Plant	Fish	Type of extract	Isolated substances	Type of administration	Anthelmintic activity	References [number]
Piper guineense	*Carassius auratus auratus*	Methanolic (seeds)	Piperanine, *N*-isobutyl (E,E)-2,4 decadienamide, Δα-β-dihydrowasanine	Oral	*G. elegans, D. extensus*	Ekanem et al. [62]
Polygala tenuifolia	*Carassius auratus*	PE, CHL, EA, ME, acetone		Bath	*D. intermedius*	Lu et al. [52]
Prunus amygdalus Batsch	*Carassius auratus*	PE, CHL, EA, ME, water		Bath	*D. intermedius*	Wu et al. [48]
Pseudolarix kaempferi	*Carassius auratus*	PE, CHL, EA, ME, water		Bath	*D. intermedius*	Ji et al. [50]
Radix angelicae pubescentis	*Carassius auratus*	PE, CHL, EA, ME, water		Bath	*D. intermedius*	Liu et al. [49]
Radix angelicae pubescentis	*Carassius auratus)*	Ethanol	Osthol	Bath	*D. intermedius*	Wang et al. [22]
Santalum album	*Carassius auratus*	CHL, EA, ME, water		Bath	*Dactylogyrus* sp., *Gyrodactylus* spp.	Tu et al. [63]
Semen aesculi	*Carassius auratus*	PE, CHL, EA, ME, water		Bath	*D. intermedius*	Liu et al. [49]
Semen pharbitidis	*Carassius auratus*	PE, CHL, EA, ME, water		Bath	*D. intermedius*	Liu et al. [49]

PE, petroleum ether; CHL, chloroform; EA, ethyl acetate; ME, methanol.

Table 3. Medicinal plants with anthelmintic activity in fish farming.

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(hawthorn), *Equissetum arvensis* (horsetail), *Hypericum perforatum* (st. johnswort), *M. chamomilla*, *Mentha piperita*(peppermint), *Ocinum basilicum*, *Prunus spinosus* (blackthorn), *Rosacanina* (dogrose), *Sambucus nigra* (elder), *Thymus serpillum* (wild thyme), *Tilia* sp., *Vaccinium myrtilus* (bilberry) and *Viola tricolor* (johnny Jump up) were evaluated for 1 month of oral and bath treatments against *Enteromyxum leei* infection in cultured gilthead sea bream, *S. aurata* [38]. They decreased the infection of *E. leei* in *S. aurata*, from approximately 40 to 20% compared with the control [38]. Also, these extracts decreased the spore's level from the water, suggesting that the extract might eliminate some stages that are released into water [38].

2.3. Anthelmintic activity

Essential oils have been used against helminths, especially to control and prevent monogeneans [9]. Studies of essential oils from various plant species have shown the oils to have excellent biological activity when tested against various fish parasites [27]. For instance, essential oils of *Lippia sidoides* (pepper rosemary) and *M. piperita* have shown to be active at a concentration of 40 mg/L when tested in vivo against monogenean species (*Cichlidogyrus tilapiae*, *C. thurstonae*, *C. halli* and *Scutogyrus longicornis*). In that case, a therapeutic bath was recommended as an alternative treatment against monogeneans in Nile tilapia *Oreochromis niloticus*, due to a decrease of 70% of the parasite prevalence in Nile tilapia culture [57]. Moreover,in a therapeutic bath with the essential oil of *Ocimum gratissimum* (clove basil), the authors found an anti-parasite efficacy (percentage reduction in parasite count) around of 100% on the gills of juvenile tambaquis C. macropomum in concentrations of 10 and 15 mg/L^{-1} [60]. Soares *et al*. [35] demonstrated an anthelmintic activity against monogeneans species (*Anacanthorus spathulatus*, *Notozothecium janauachensis* and *Mymarothecium boegeri*) using essential oil of *L. alba* on the gills of *C. macropomum* after 20 minutes of exposure at concentrations of 1280 and 2560 mg/L. Similar results were found by Malheiros et al. [59] using the essential oil of *M. piperita*, yielding an anti-parasitic effect in the in vitro assay against *Dawestrema cycloancistrium* and *D. cycloancistrioides*, while in the in vivo test to evaluate the toxicity, the result was not satisfactory and caused changes in fish gill tissues. Thus, it is necessary to create therapeutic strategies capable of increasing the efficacy of the use of essential oils as phytotherapeutic agents to reduce their toxicity in *Arapaima gigas* (pirarucu).

Furthermore, recent reviews also have shown that plant extracts indicated efficient anthelmintic properties in numerous fish species [9, 15, 27]. Alcoholic or organic solvents have a greater efficiency in the isolation of bioactive substances. For example, the ethanol extract of the leaves of *Artemisia annua* (sweet wormwood), in 1 hour of exposure, at a concentration of 200 mg/L, killed 85% of the parasites without any mortality of juvenile *Heterobranchus longifilis* (vundu) [45]. The aqueous and methanol extract of *Semen aesculi* (buckeye seed) [49]; ethyl acetate, methanol and chloroform extracts of *Radix Bupleuri chinensis* (schisandra fruit) [48]; methanol extract of *Dryopteris crassirhizoma* (thick stemmed wood fern), *Kochia scoparia* (kochia) and *Polygala tenuifolia* (yuan zhi) [52] and methanol extracts of *Cinnamomum cassia* (cinnamon), *Lindera aggregata* (evergreen lindera) and *Pseudolarix kaempferi* [50] proved to be efficient against monogeneans *Dactylogyrus intermedius* in gold fish *C. auratus* (goldfish). Among the ethyl acetate, petroleum ether, n-butanol and water extracts from *Euphorbia fischeriana* (Lang-Du), only the ethyl acetate extract showed a killing effect in the in vitro and in

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vivo test on *D. vastator*, a monogenean of *C. auratus*. Moreover, the extract showed anthelmintic activity 40% higher than mebendazole or phoxim and had effects similar to those observed for praziquantel and trichlorfon, chemicals often used against *Dactylogyrus* spp. These results suggest that this extract can serve as a potent anti-parasitic agent in the aquaculture industry [54].

Fridman et al. [43] used an aqueous extract of garlic *A. sativum* in an in vivo assay (30 mL/L), and it caused the separation and decreased movement of two species of monogeneans (*Gyrodactylus turnbulli* and *Dactylogyrus* sp.). In the oral (10 and 20%) and bath (7.5 and 12 mL/L) test, the extract showed a significant reduction of parasites when compared to the control group [43]. Previous studies have shown 75% of the anthelmintic activity of the hexane extract of *A. sativum* against *Capillaria* sp., a nematode of *Cyprinus carpio* (common carp) [44]. The extracts of *Ginkgo biloba* (ginkgo) and *Dioscorea zingiberensis* (yellow ginger) showed potent, synergistic, anti-parasitic effects when combined against *Dactylogyrus* spp. in *C. auratus* under in vivo conditions [51].

2.4. Isolated substances from plants with anthelmintic activity

Chemicals of different classes such as alkaloids, flavonoids, saponins, coumarins, quinones, quassinoids, phenolics, lignans and terpenoids have been isolated. Andrade et al. [46] evaluated the efficacy of the extract of *Bixa orellana* (achiote) seeds against monogenean *A.spathulatus*, a parasite of *C. macropomum* in an in vivo test and achieved 100% efficacy. This activity may be related to the bixin and geranylgeraniol terpenoids present in the ketone extract. Studies indicated that the parasiticidal activity is due to the presence of these lipophilic substances since they can cross the surfaces of the membranes, causing a rupture and killing the parasites [64].

Wang et al. [55] isolated the osthol and isopimpinellin coumarins of the *Fructus cnidii* fruit (cnidium), which were 100% effective at concentrations of 1.6 and 9.5 mg/L, respectively, against *D. intermedius*, a parasite of goldfish *C. auratus*. Osthol is an important coumarin with extensive medical activity, including anti-tumour [65, 66], prevention of atherosclerosis [67], anti-aging and anti-proliferative [68]. However, there are few reports of anti-parasitic effects. Osthol was also isolated from *Radix angelicae pubescent* (pubescent angelica root) and exhibited excellent activity against *Dactylogyrus intermedius* achieving 100% mortality at a concentration of 1.6 mg/L and did not show any toxicity to *C. auratus* at a dose of up to 6.2 mg/L [22].

Wang et al. [47] isolated the bruceina A and bruceina D quassinoids from the methanol extract of *Bruceajavanica* fruits (macassar kernels). There was strong anthelmintic activity against *D. intermedius* with EC50 (i.e. defined as the concentration of the sample leading to 50% reduction of *D. intermedius*) values of 0.49 and 0.57 mg/L after 48 hours, respectively. The substances were twice as efficient as mebendazole, which is often used to control *Dactylogyrus* spp. In the toxicity test, these substances proved to be safe for use in goldfish in concentrations of up to 5 mg/L. Bruceina A and D are similar in structure compared to the C-20 type quassinoids. This indicates that the mode of action of these substances may be similar to quassinoids. Several studies discuss the quassinoid action in different parasite species, emphasising that the primary targets of these molecules are the proteins of the cell [69–72]. Fukamiya et al. [71] demonstrated that the C-8-to-C-13 epoxymethano bridge and the hydroxyl group at C-11 and C-12 of the quassinoids are important to inhibit protein synthesis. In a previous study, quassinoids showed anti-malarial activity by inhibiting protein synthesis [72]. Therefore, the anti-parasitic activity of bruceina A and D can be related to the action mechanism that inhibits protein synthesis [47].

Sanguinarine, criptopine, β-allocriptopine, protopine and 6-methoxy-dihydro-chelerythrine alkaloids were isolated from the aerial parts of *Macleaya microcarpa* (kelway's coral plume) and were 100% efficient in monogenean *D. intermedius*, a parasite of *C. auratus* [58]. Ekanem et al. [62] showed that the methanol extract from *Piper guineense* (English West African black pepper) seeds was active against *G. elegans* and *D. extensus* in concentrations of 0.5 to 2.0 mg/L in vitro and in vivo assays. The substances identified in the extracts were piperanine, N- isobutyl (E,E)-2,4-decadienamide and $\Delta\alpha$, β-dihydrowasanine.

Wang et al. [61] isolated the steroidal saponins dioscin and polyphyllin D from the crude extract of the rhizome of *Paris polyphylla* (ginseng) and achieved excellent results for the monogenean *D. intermedius*. Wang et al. [56] isolated ginkgolic acid C13:0 (M1) and C15:1 (M2) from *G. biloba* and were 100% effective at concentrations of 2.5 and 6.0 mg/L, with ED50 values of 0.72 and 2.88 mg/L, respectively, for *Pseudodactylogyrus* sp., a parasite of juvenile eels (*Anguilla anguilla*). The flavonoids sutchuenoside A and kaempferitrin, isolated from the rhizome of *D. rhamnosides*, had satisfactory anthelmintic activity in the in vivo test against *D. intermedius* and were safe for the *C. auratus* host [53]. These studies reveal the potential of these isolated substances as anthelmintic activity in fish farming.

2.5. Isolated substances from plants with anti-protozoan activity

Several species of medicinal plants have shown efficiency in the control of protozoans in aquaculture, but there are few reports describing the isolation of bioactive molecules responsible for the anti-protozoan activity. For example, the alkaloids dihydrosanguinarine and dihydrocheleritrine, isolated from *M. microcarpa* were active against the protozoan *I. multifiliis*, a parasite of *C. macropomum* with EC50 values of 5.18 and 9.43 mg/L, respectively, which points to strong anti-parasitic possibilities for fish [40]. Xiao-Feng et al. [42] demonstrated that the alkaloids cheleritrine and chloroxylonine, isolated from the leaves of *Toddalia asiatica* (orange climber) were 100% effective against *I. multifiliis*, a parasite of *C. auratus*, in concentrations of 1.2 and 3.5 mg/L, with average effective concentrations (EC50) of 0.55 and 1.90 mg/L, respectively. In the in vivo test, the fish treated with cheleritrine and chloroxylonine at concentrations of 1.8 and 8.0 mg/L had fewer parasites than the control. The acute toxicity (LC50) was 3.3 mg/L for chelerythrine for goldfish. Direct action in the mitochondria may be involved in the eradication of the parasites since this organelle is responsible for controlling and regulating cell apoptosis, but further studies are still required to detail the action mechanism of these substances [42, 73]. Song et al. [41] isolated isopsoralene and psoralidin, which showed potent anti-protozoan activity. In the in vitro assay with psoralidin, 100% mortality of the protozoan *I. multifiliis* was observed at a concentration of 0.8 mg/L in 4 hours of exposure, which was more active than isopsoralene. Ajoene components (*Allium sativum*) showed inhibition of *Spironucleus vortens*, a protozoan fish parasite of *Pterophyllum scalare* (angelfish) with a minimum inhibitory concentration of

40 ug/mL, while the substance (Z)-ajoene (minimum inhibitory concentration = 16 ug/L) isolated from the essential oils proved to be more active that its isomer (E)-ajoene [39]. When compared with metronidazole (MTZ), the ajoene components were 10-fold greater than that of MTZ (4g/ml), the drug of choice for treatment of *S. vortens* infections [39].

2.6. Environmental impacts of anti-parasitics used in fish farming

The use of anti-parasitics, insecticides, pesticides and antibiotics has been used in several freshwater, brackishwater and marine farming fish systems to control parasites and pathogens [8, 9]. Although the use of these chemical treatments reduces infection rates in fish farming systems, their excessive use might lead to a build-up of drug resistance in the pathogen or parasite [8, 17]. For example, the loss of salmon stock to sea lice infestation (*L. salmonis*) led to the use of two chemical treatments in a marine aquaculture system. One insecticide called dichlorvos and one chemical (i.e. hydrogen peroxide), with the germicidal property. The frequent and widespread use of these chemicals might lead to reduced efficacy caused by the resistance that developed the parasite [8]. Umeda et al. [74] also observed a drug resistance in the use of an organophosphate insecticide (e.g. trichlorfon) and praziquantel in bath treatments for ectoparasites such as monogeneans.

Moreover, the bioaccumulation of the chemicals or the presence of residual antibiotic in the final fish product might have potential consequences on human health [9, 75]. An important issue is the transfer of resistant pathogens from fish farming to humans. As the resistance to antibiotics is transmitted from one bacterium to another, it might have a risk of transference of antibiotic resistance to healthy bacteria in the human gut [20].

Chemical and biocides used in fish farming might also have lethal or sub-lethal effects on non-target organisms in the environment [20]. The encapsulated antibiotics of the uneaten feed accumulated on the seabed beneath fish cages can affect microbial communities in the immediate vicinity, leading to a reduction in their diversity [8]. For example, the release of antibiotics into the environment can negatively affect the biodiversity of planktonic, algae, microcrustaceans and benthic communities [19].

According to Kemper [76], little is known about the effects of anti-parasitics and chemical compounds pollution to either humans or the environment, but the increasing resistance to antibiotics by bacteria and the diminishing effectiveness of therapeutic drugs have been considered a global concern. The anti-parasitics and antibiotics might remain in the water until degraded by natural processes or are accumulated in the sediment. Some chemical treatments used in fish farming may deteriorate most rapidly, but most are persistent [76].

Therefore, the use of the plant-derived compounds as an alternative treatment against parasites in fish farming has been representing few or no adverse impact on the environment because its residuals are usually biodegradable in the water [23]. Differently, of the traditional chemotherapeutics, the administration of the plant-derived compounds in fish has been associated with few or no side effects [15]. Although the persistence of plant-derived compounds in the environment and their side effects to human health have been still little emphasised, more studies are necessary to verify the real impact of these plant-derived compounds into the environment and their effects on human.

3. Conclusions and future perspectives

This review showed that the plant-derived compounds have a great potential to prevent and control parasites in fish farming, especially about protozoans, myxozoans and monogeneans. Many compounds isolated from plant extracts, for example, osthol, geraniol and bruceina A and D may have a useful role for controlling parasites in fish farming, although more studies are necessary to determine the sufficient concentration during the administration, seems that oral administration has been the most suitable for aquaculture [9]. Also, the potential for discovering new essential oils, plant extracts and bioactive compounds is increasing each year due to the use of new tools of analysis and the interest of the researchers in their pharmacological activities to control fish diseases. The use of these plant-derived compounds may become a powerful phytotherapy, although more studies are necessary to prove the efficiency of these plant-derived compounds as a natural parasitic control.

Moreover, novel applications of nanotechnology and microencapsulation are growing rapidly in agriculture, food and aquaculture sector industries [77–79]. The synthesis of the plant-based materials for the production of nanomaterials can be used to enhance the ability of fish to absorb the bioactive from the plants in the control of fish diseases in aquaculture and at the same time its products are safe for the environment [80, 81]. Another application is the microencapsulation that has been used for the incorporation of numerous compounds such as proteins, lipids, carbohydrates, vitamins, minerals, hormones, probiotics and plant extracts necessary for the growth and health of fishes [79]. Both applications might help the growing of aquaculture and enhance the treatment against parasites in fish farming.

Furthermore, there is a need to look for alternative treatments to control and prevent fish parasites in aquaculture, which are at the same time environmentally friendly and highly efficient. Studies of essential oils, crude extracts and chemicals of medicinal plants have shown them to be viable and cheap. Thus, conventional parasiticides might be replaced by the use of phytotherapeutic agents in aquaculture.

Fish health is a challenging task in the search for a sustainable aquaculture, for which the plant-derived compounds offer viable alternatives to deal with the outbreaks of infectious diseases in fish farming. Therefore, plant-derived compounds seem to represent a promising alternative to control fish diseases in aquaculture.

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