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Phytobiotics in aquaculture health management: A review

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Abstract
Use of phytobiotics or herbal extracts for increasing growth and health status is one of the major focuses in fish nutrition research. Intensification of aquaculture causes stress in fishes leading to immunosuppression which ultimately results in reduced growth and diseases. Uses of most antibiotics are banned in aquaculture as they have residual effects and can develop drug-resistant bacterial strain. Hence, stimulation of non-specific immune system is a smart choice available for enhancing the immunity as well as growth performance of cultured species. Phytobiotics are rich in various bioactive compounds that act as immunostimulants. Those compounds mainly enhance the activity of phagocytic cells and increase their bactericidal activities, stimulate the natural killer cells, complement activity, proliferate lymphocytes, lysozyme and antibody responses of fish. Use of diets containing dietary herb or plant extracts to improve growth and disease resistance in fish and shrimp are generally based upon tradition and folklore transferred through generations without knowing any biochemical properties. However, phytobiotics are a new frontier area of aquaculture and there is an underlying need to obtain a clear and direct dose-dependent stimulatory effect upon the growth, immune status and physiology of fish.

Keywords: Phytobiotics, bioactive compounds, antibiotic, growth performance, immunostimulants

Introduction
Fish is the cheapest source of easily digestible animal protein constitutes a significant share in the global food basket. The current world population is around seven billion, and by 2050 it is expected to reach nine billion [1]. Hence, animal protein consumption is expected to double by that time. A robust growth is expected in the consumption of farmed fish and chicken. Currently, the world fish production sector is facing the challenge to boost the production in order to combat the protein hunger and to ensure livelihood and nutritional security in the future years [1].

Aquaculture sector is the major contributor towards fish supply as production from marine capture fisheries is almost stagnant over recent years [2]. The rapid development of aquaculture system and growing demand of fish leads to the intensification of the culture practices, overdrawing stressors for fish and thus magnifying the risk of diseases. Until now, chemotherapy is the only option for prevention and treatment of aquaculture disease outbreaks. But the use of chemical drugs has several inherited negative impacts on the environment as well as human. Hence, in recent years, attention is given towards eco-friendly and sustainable methods of aquaculture disease management practices [3-4].

Herbs as therapeutics in aquaculture
Use of plant products for aquaculture disease management is a new and promising alternative to chemical drugs [3]. Many products from the plant origin have been reported to stimulate appetite, promote growth performance, act as immunostimulants, antibacterial, antiviral and anti-parasitic (protozoans, monogeneans) agent in aquaculture. These activities are observed due to the presence of bioactive compounds such as phenols, sulphur, terpenoids, alkaloids, flavonoids, and saponins [5-6].

Several studies have been reported to assess the effect of dietary algal derivatives; herb and plant extract on fish health. Majority of such studies are based upon tradition and folklore transferred through generations and confined to certain geographical areas. Interestingly, some of the studies clearly depict the direct dose-dependent stimulatory effect of these phytobiotics or herbal extract upon the fish immune system [7].

However, there is still a knowledge gap between the method of different herbal extract preparation, administration and the long-term effects on fish physiology. The increased consumer preferences on organic food products over recent years underlined the possibilities of phytobiotics in aquaculture.

Some useful herbs can be used as therapeutics

List of herbs can be used as therapeutics in aquaculture shown in Table 1.

Table 1: List of herbs used in aquaculture

SL. No.	Herbs (Scientific Name)	Common Name	Properties	Reference
1.	*Achyranthes aspera*	Prickly Chaff Flower	Antibacterial	Rao *et al.*, 2006 [8]
2.	*Eclipta erecta*	Bhringraj	Antibacterial	Direkbusarakom, 1998 [9]
3.	*Ceramium rubrum*	Red hornweed	Antibacterial	Dubber and Harder, 2008 [10]
4.	*Ricinus communis*	Castor oil plant	Antibacterial	Immanuel *et al.*, 2004 [11]
5.	*Inonotus obliquus*	Chaga mushroom	Antibacterial	Harikrishnan *et al.*, 2012b [12]
6.	*Emblica officinalis*	Indian gooseberry	Antimicrobial	Minomol, 2005 [13]
7.	*Allium sativum*	Garlic	Antibacterial, Antiparasitic	Musa *et al.*, 2008 [14]; Militz *et al.*, 2013 [15]
8.	*Cinnamonum verum*	Ceylon cinnamon tree	Antibacterial	Ravikumar *et al.*, 2011 [16]
9.	*Aucklandia lappa*	Acklandia	Antifungal	Xue-Gang *et al.*, 2013 [17]
10.	*Solanum trilobatum*	Pea eggplant	Antibacterial	Divyagnaneswari *et al.*, 2007 [18]
11.	*Andrographis paniculata*	Maha tita, king of bitters	Antibacterial, Growth promoter	Rattanachaikunsopon and Phumkhachorn, 2009 [19]; Rani, 1999 [20]
12.	*Aloe vera*	Aloe	Antibacterial	Ahilan *et al.*, 2010 [21]
13.	*Azadirachta indica*	Neem	Antibacterial, Antifungal, Antiparasitic	Chitmanat *et al.*, 2005 [22]; Campbell *et al.*, 2001 [23]
14.	*Solanum torvum*	Sandakai fruit coat	Antibacterial	Mydeen & Haniffa, 2011 [24]
15.	*Laminaria digitata*	Oarweed	Antibacterial	Cox *et al.*, 2010 [25]; Dubber and Harder, 2008 [10]
16.	*Adhatoda vasica*	Malabar Nut	Antibacterial	Velmurugan & Citarasu, 2010 [26]
17.	*Centella asiatica*	Asiatic pennywort	Antibacterial	Purkait *et al.*, 2018 [27]
18.	*Paris polyphylla*	Himalayan paris	Antibacterial	Hufford *et al.*, 1988 [28]
19.	*Eupatorium odoratum*	Siam weed	Antibacterial	Ravikumar *et al.*, 2011 [16]
20.	*Lactuca indica*	Indian lettuce	Antibacterial	Dung, 1990 [29]
21.	*Datura metal*	Thorn apple	Antibacterial, Antifungal	Ravikumar *et al.*, 2010 [30]; Adiguzel *et al.*, 2005 [31]
22.	*Curcuma longa*	Haldi	Antibacterial	Harikrishnan *et al.*, 2010 [32]
23.	*Mastocarpus stellatus*	Carrageen moss	Antibacterial	Dubber & Harder, 2008 [10]
24.	*Withania somnifera*	Ashwagandha, Indian ginseng	Antibacterial, Growth promoter, Antistress	Sharma *et al.*, 2010 [33]; Citarasu *et al.*, 1998 [34]; Citarasu *et al.*, 2002 [35]
25.	*Phyllanthus niruri*	Stonebreaker	Growth promoter	Punitha *et al.*, 2008 [36]
26.	*Crataegi fructus*	Hawthorne	Growth promoter, Antistress	Ji *et al.*, 2007 [37]; Wang *et al.*, 2008 [38]
27.	*Piper longum*	Long pepper	Growth promoter, Antiparasitic	Punitha *et al.*, 2008 [36]; Traxler, 1971 [39]
28.	*Picrorhiza kurooa (apocynin)*	Guduchi	Growth promoter	Citarasu *et al.*, 2002 [35]
29.	*Tridax procumbens*	Coat buttons	Growth promoter, Antibacterial	Punitha *et al.*, 2008 [36]
30.	*Ocimum sanctum*	Tulsi	Appetite stimulation, Growth promoter, Antibacterial,	Pavaraj *et al.*, 2011 [40]; Logambal *et al.*, 2000 [41]; Direkbusarakom, 1998 [9]; Venkatalakshmi & Michael, 2001 [42]
31.	*Psoralea corylifolia*	Babchi	Antiprotozoan	Ling *et al.*, 2013 [43]
32.	*Eclipta alba*	False Daisy	Antistress, Growth promoter	Rani, 1999 [20]
33.	*Toona sinensis*	Chinese cedar	Antistress, Antibacterial	Hsieh *et al.*, 2008 [44]; Wu *et al.*, 2010 [45]
34.	*Zingiber officinalis*	Ginger	Antistress, Growth promoter, Antibacterial	Punitha *et al.*, 2008 [36]; Citarasu *et al.*, 1998 [34]; Citarasu *et al.*, 2002 [35]; Nya & Austin, 2009 [46]; Indu *et al.*, 2006 [47]
35.	*Tephrosia purpurea*	Sharpunkha Fish Poison, Wild Indigo	Antistress, Growth promoter	Rani, 1999 [20]
36.	*Tinospora cordifolia*	Heart leaved moonseed	Antiviral	Direkbusarakom, 1998 [9]
37.	*Cynodon dactylon*	Bermuda grass	Antiviral, Growth promoter	Balasubramanian *et al.*, 2007 [48]; Balasubramanian *et al.*, 2008 [49]; Punitha *et al.*, 2008 [36]
38.	*Cassia alata*	Candle bush	Antiviral	Direkbusarakom, 1998 [9]
39.	*Phyllanthus acidus*	Star gooseberry	Antiviral	Direkbusarakom *et al.*, 1996 [50]
40.	*Calophyllum*	Indian doomba oil	Antiviral	Direkbusarakom, 1998 [9]

	inophyllum	tree		
41.	*Cnidium monnieri*	Conidium fruit	Antifungal	Xue-Gang *et al.*, 2013 [17]
42.	*Asparagopsis taxiformis*	Limu kohu	Antifungal	Genovese *et al.*,2013 [51]
43.	*Magnolia officinalis*	Magnolia bark	Antifungal	Wang *et al.*, 2008 [52]; Xue-Gang *et al.*, 2013 [17]
44.	*Terminalia catappa*	Indian almond	Antifungal, Antibacterial	Chitmanat *et al.*, 2003 [53]; Purivirojkul, 2012 [54]
45.	*Ruta graveolens*	Common rue	Antifungal	Hashemi *et al.*, 2011 [55]
46.	*Radix bupleuri*	Bupleurum root	Antihelminthics	Wu *et al.*, 2011 [56]
47.	*Pseudolarix kaempferi*	Golden larch	Antihelminthics	Ji *et al.*, 2012 [57]
48.	*Leucaena glauca*	Sababul, River tamarind	Antiparasitic	Dung, 1990 [29]
49.	*Lindera agreggata*	Chinese spice bush	Antiparasitic	Wu *et al.*, 2011 [56]
50.	*Arica catechu*	Indian nut	Antiparasitic	Dung, 1990 [29]
51.	*Santalum album*	Indian sandalwood	Antiparasitic	Tu *et al.*, 2013 [58]
52.	*Melia azedarach*	China berry	Antiparasitic	Dung, 1990 [29]

Potential of phytobiotics in aquaculture

In aquaculture, phytobiotics or plant extracts have been reported to have various properties like anti-stress, growth promotion, appetite stimulation, immune system enhancement, broodstock maturation, aphrodisiac and anti-pathogenic due to the presence of various bioactive substances such as alkaloids, terpenoids, tannins, saponins, glycosides, flavonoids, phenolics, steroids and essential oils [5-6]. Furthermore, phytotherapies are cost effectives, environment friendly and more eco-friendly than synthetic molecules and are less likely to elicit drug resistance due to the high diversity of plant extract molecules [41, 59-60].

Phytobiotics as antibacterial agent

Bioactive compounds present in plant extracts such as polysaccharides, phenolics, proteoglycans, and flavonoids play a significant role in preventing or controlling infectious microbes [5-6]. The phytobiotics exhibit antibacterial activities by various mechanisms including bacterial cell wall disruption, lysozyme and complement activity enhancement, nucleic acid translation and transcription blockage etc. These activities eventually inhibit the enzyme secretions and interfere with the cell signalling mechanism of quorum sensing pathway. Lysozyme lyses the peptidoglycan layer of bacterial cell walls and releases polysaccharides that have been shown to promote the secretion of cytokines and antibodies, and enhance the function of natural killer cells, T and β lymphocytes [61].

Plant products with antibacterial properties are widely studied with potential application in aquaculture systems. Previous authors have reported [10] that hexane extracts of oarweed (*Laminaria digitata*) (31 mg dry weight/mL) and methanolic extracts of red hornweed (*Ceramium rubrum*) (10 mg dry weight/mL) have strong antibacterial activities against 16 different marine bacteria and fish pathogenic bacteria. They concluded that Gram-positive marine Bacillaceae were more susceptible whereas Gram-negative marine Vibrionaceae were the least. Scientific study [14] have found that garlic extracts were effective against the two pathogenic gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus agalactiae*), four gram-negative bacteria (*Escherichia coli, Citrobacter freundii, Vibrio vulnificus and Vibrioparahaemolyticus*) and 18 isolates of *Edwardsiella tarda* to different concentration of aqueous extract (500, 250, 125, 62.5 mg/mL). Some authors [62] also reported that methanolic extracts of 31 Brazilian plants showed antibacterial activity (agar diffusion method) against the fish pathogenic bacteria *A. hydrophila, Streptococcus agalactiae* and *Flavobacterium columnare*. Similar studies reported by several other researchers [63-65] reveal that seaweed and algae are also a potential source of antimicrobial products. Researchers [66] have observed that ethanolic extract of chaga mushroom (*Inonotus obliquus*) resulted a lower cumulative mortality in kelp grouper (*Epinephelus bruneus*) against *Vibrio harveyi* infection (enriched diet with 20% and 15% for 1% and 2% respectively) compared to control group (90% mortality). A study [67] found that ethanolic extract of algae limu kohu (*Asparagopsis taxiformis*) (100 mg/mL) inhibits nine pathogenic fish bacteria includes *Vibrio vulnificus, Vibrio alginolyticus*, and *Aeromonas salmonicida*. Indian major carp (*Labeo rohita*) fed with prickly chaff flower (*Withania somnifera*) (0.5%) and Indian ginseng (*Achyrantes aspera*) (0.2%) showed reduced mortality (49% and 41% respectively) when challenged against *A. hydrophila* [8, 33]. Similar findings reported by other authors [18, 45] has also shown that tilapia (*Oreochromis mossambicus*) intraperitoneally injected with water extracts of purple fruited pea eggplant (*Solanum trilobatum*) (400 mg/kg) and Chinese cedar (*Toona sinensis*) (8 mg/kg) reduces mortality (27% and 57% respectively) when challenged against *A. hydrophila*.

Phytobiotics as antiviral agent

Different ethanolic and methanolic herbal extracts are rich in several bioactive compounds that can inhibit or block the viral mRNA synthesis to reduce the replication in the host cells and enhance the non-specific immunity. Researchers have [50] found that 18 traditional Thai herbs presented antiviral activity against Oncorhynchus Mason Virus (OMV) and Infectious Hematopoietic Necrosis Virus (IHNV), while star gooseberry (*Phyllanthus acidus*) and *Orchocarpus siamensis* inhibited the replication of OMV and IPNV (Infectious Pancreatic Necrosis Virus) in cells. A study [68] showed that ethanolic extract of *Clinacanthus nutans* inactivated Yellow-Head Virus (YHV) *in vitro* at low concentrations (1 µg/mL). When black tiger shrimp (*Penaeus monodon*) were fed with 1% extract (*C. nutans*) inclusion, the cumulative mortality decreased from 75% (in control group) to 33% [9]. In other studies, intramuscularly injected, or orally administered aqueous extract of Bermuda grass (*C. dactylon*) to *P. monodon* displayed no mortality against WSSV whereas 100% mortality was observed in control group [48-49]. The extracts of olive tree leaf (*Olea europaea*) had antiviral

properties against VHSV infected carp EPC (Epithelioma papulosam cyprini) cell lines [69]. Indian traditional medicinal plants such as *A. marmelos, C. dactylon, L. camara, M. charantia* and *P. amarus* showed strong antiviral activity against WSSV in *P. monodon* [48-49].

Phytobiotics as antifungal agent

The herbal extracts or phytobiotics can lyse the fungal cell wall, alter the permeability, and affect the metabolism and protein synthesis. A molecule called 2-(3, 4-dimethyl- 2, 5-dihydro-1H-pyrrol-2-yl)-1-methyl ethyl pentanoate (DHP) from plant *Datura metel* showed antifungal properties against 19 species of *Aspergillus* and 10 species of *Candida* [31, 70]. Extracts from *O. basilicum* and dried ground leaves of Indian almond (*Terminalia catappa*) reduced the fungal infection in tilapia eggs [22]. Several plant-derived compounds such as coconut diethanol-amide (2.5 ppm), Neem (*Azadirachta siamensis*) (5 ppm) and tea seed oil (*Melaleuca alternifloria*) (20 ppm) are reported to have strong antifungal properties against *Aphanomyces invadans*. Toxicity studies of these compounds on silver barb (*Barbonymus gonionotus*) and rainbow trout (*Oncorhynchus mykiss*) showed no mortalities and behavioural changes [23]. A study [17] showed that 10 plant species used in traditional Chinese medicine have strong inhibitory effect on fungal species like *Saprolegnia* and *Achlya klebsiana*. The petroleum ether extracts of conidium fruit (*Cnidium monnieri*), magnolia bark (*Magnolia officinalis*) and aucklandia root (*Aucklandia lappa*) displayed best antifungal activity. Another study [55] found that ethanol extracts of common rue (*Ruta graveolens*) had antifungal effects and prevented the growth of *Saprolegnia sp.* while the red algae *Asparagopsis taxiformis* showed antifungal activity against *Aspergillus species* [51].

Phytobiotics as antihelminthic and antiparasitic agent

Monogenean parasites are flatworms which infect skin, gills and eyes of fish. Monogeneans from the genus *Dactylogyrus*, *Gyrodactylus*, and *Neobenedenia* are widespread parasites that affect a large variety of cultured fish resulting significant economic losses worldwide [71-73]. Till date, there are no effective methods to prevent monogenean infections in open aquaculture systems. The only available technique is to remove attached parasite stages through different bath treatments. Moreover, monogenean eggs are highly resistant to physical and chemical treatments due to their sclerotized protein shell that protects the developing embryo [74-75]. Several studies have been performed recently to assess the anthelminthic activity of plant extracts to treat monogenean infections. Plant extracts like methanolic extract of bupleurum root (*Radix bupleuri* chinensis), aqueous and methanolic extracts of cinnamon (*Cinnamomum cassia*), methanolic extract of Chinese spice bush (*Lindera agreggata*) and methanolic and ethyl acetate extracts of golden larch (*Pseudolarix kaempferi*) have shown 100% *in vivo* efficacy against monogenean (*Dactylogyrus intermedius*) when added to water with infected goldfish (*Carassius auratus*) [56-57]. Earlier study [58] reported the effect of Indian sandalwood (*Santalum album*) extract against *D. intermedius* and *Gyrodactylus elegans* on goldfish. They concluded that chloroform extract was the most effective as well as safest for the fishes. They also observed that bath treatment with longer duration and multiple administrations could eliminate a considerable proportion of monogeneans infections. Some authors have [76] assessed the effect of aqueous extracts from different algae on the lifecycle of the parasite *Neobenedenia* sp. They observed that infection on *Lates calcarifer* was lower in the presence of *Asparagopsis taxiformis* (51%) and *Ulva* sp. (54%) extracts in seawater compared to the control (71%). *A. taxiformis* extract inhibited the embryonic development of *Neobenedenia* sp. and reduced the hatching rate to 3% from 99% (in seawater control). Studies have [15] shown the effect of enriched garlic diets against *Neobenedenia* sp. infection in farmed barramundi (*L. calcarifer*). They also observed that long-term supplementation (30 days) displayed 70% reduced infection compared to control and short-term supplementation (10 days). Researchers [77] have also found that garlic extract killed theronts and tomocysts stage of the ciliate *Ichthyophthirius multifilis* (responsible for freshwater white spot disease) at 62.5 mg/L and 570 mg/L respectively. Another study [12] showed that, the mortality of ciliate (*Miamiensis avidus*) infected olive flounder decreased from 80% to 40% when fed with a diet supplemented with *Suaeda maxima* extract. Some researchers [78-79] also suggested use of piperine and azadirachtin at a concentration of 9 mg L^{-1} and 15 mg L^{-1} for controlling Argulus infestation in goldfish (*Carassius auratus*) without any effects on its physiology.

Phytobiotics as antistress agent

Bio-active compounds present in herbal extracts inhibit the generation of oxygen anions and scavenge free radicals. The herb *Picrorhiza kurroa* is used as an antistress compound for shrimps and its effect is similar to that of superoxide dismutase, metal-ion chelators and xanthine oxidase inhibitors [34]. A bioflavonoid compound extracted from *Toona sinensis* (named Rutin) showed strong antioxidant and antistress activity in *V. alginolyticus* infected *L. vannamei* at 10, 20, or 50 μg/g [44]. Common carp (*Cyprinus carpio*) fed with diet containing 0.3 g/kg Qompsell extract reduced the stress and induce the immunological parameters such as serum lysozyme activity, superoxide dismutase (SOD), nitric oxide synthase (NOS) and levels of total serum protein, globulin, and albumin [80]. Chinese medicinal herbs *A. membranaceus* and *L. japonica*, at 0.1% separately and together with and without boron 0.05% in diet has improved the non-specific immune response in Nile tilapia (*O. niloticus*) when challenged with *A. hydrophila* [81].

Phytobiotics as appetite stimulators and growth promoters

Phytobiotics generally stimulate secretion of the digestive enzymes and have a direct effect on gut micro-flora. Plant extracts have been shown to increase digestibility and bioavailability of nutrients that ultimately results in an increase of feed conversion, higher protein synthesis and growth of fish [6, 46, 82]. Hot spices from peppers (e.g., capsaicin and piperine) and cinnamon (provides cinnamaldehyde) are known to stimulate salivation [83]. Shrimp post larvae (PL) have shown improved digestive enzyme activity (amylase, protease, and lipase) when fed with *Artemia* enriched with herbal appetizer (*Z. officinalis*) [84]. A study [85] reported that papaya leaf meal enhances protein digestion, food conversion ratio (FCR), specific growth rate (SGR) and weight gain in *P. monodon* PL. Several plant extracts are reported to stimulate appetite and promote weight gain when they are administered to cultured species [40 67, 86]. Researchers have [36] reported that grouper (*Epinephelus tauvina*) with 41% more weight gain compared to control

when fed with diet containing a mixture of methanolic herb extracts [Bermuda grass (*Cynodon dactylon*), Long pepper (*Piper longum*), Stonebreaker (*Phyllanthus niruri*), coat buttons (*Tridax procumbens*) and ginger (*Zingiber officinalis*)]. Another study [87] showed that food intake, specific growth rate and final weight of Nile tilapia (*O. niloticus*) increased when fed with garlic incorporated diet. Authors have [37] showed that olive flounder (*Paralichthys olivaceus*) obtained higher weight and showed improved fatty acid utilization when fed with a herbal mixture of medicated leaven (*Massa medicata fermentata*), hawthorne (*Crataegi fructus*), virgate wormwood (*Artemisia capillaries*) and *Cnidium officiale* (2:2:1:1). A study [88] reported that diet supplemented with 1% of ethanolic katuk extract (*Sauropus androgynous*) resulted in increased appetite, growth and improved food utilization (lower feed conversion ratio) in grouper *Epinephelus coioides*. However, a higher concentration of katuk extract (2.5 and 5%) elicited lower growth in *E. Coioides*. These results clearly indicate the importance of exact concentration of extract to obtain the desired effects. Future studies should concentrate more on chemical characterization of the extracts in order to identify and quantify active bio molecules and establish adequate doses of extract.

Phytobiotics as aphrodisiac

Reprotism, a herbal product have shown increased reproductive performance in *Artemia franciscana* [6]. A researcher [89] has observed that *Asparagus racemosus* in combination with 5% rice bran promoted the reproduction and other sexual parameters in *A. franciscana*. The ayurvedic products are reported to promote the reproduction and cyst production in *Artemia* sp. [90]. Another researcher [91] has observed increased fecundity (42%) and gonad weight (38%), reduced intermoult period, and improved quality of the hatched *P. monodon* nauplii when fed with *Artemia* previously enriched with a herbal maturation diet containing *W. somnifera, Ferula asafoetida, Mucuna prurita* and *Piper longum.*

Conclusion and perspectives

Different parts of herbs, the extraction method and the concentration of the extract significantly influence the health, growth and reproductive performances of the cultured species. Although there are minor difficulties in preparation, concentration and administration of herbal extracts, several studies have reported the multiple activities and potential application of herbal extracts in aquaculture. Among all methods of administration, intra-peritoneal injection has been proven to be the most efficient way of administration. However, it is much expensive and can also elicit stress responses in fish. Thus administration through oral route seems to be the most suitable way in aquaculture practices [92-94]. Furthermore, the effect of different herbal products on fish is dose-dependent; hence, determination of suitable dose of extract concentration is of much importance before application [95-96]. Therefore, it is necessary to identify, quantify and characterize the bioactive molecules present in different herbal extracts to formulate a standardized protocol including different extraction method, exact and potent dose of herbal extracts besides the method of administration.

It is evident that the application of herbal extract is promising in different aspects of aquaculture including disease prevention, treatment and stimulating the growth performance in fishes. However, it is necessary to conduct various *in vitro* and *in vivo* experiments to have a clear knowledge on efficacy. Furthermore, the dose-dependent action against different pathogens, physiological regulation and mode of action of different bioactive compounds and possible residual action is also needs to be explored.

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