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Review article

Herbs/spices as feed additive in aquaculture

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ABSTRACT

Nutrition of fish is an important consideration in fish health management of farmed finfish and shellfish. The shift in some countries from extensive to semi-intensive and intensive farming of fish demands that nutritionally complete feeds be provided by the farmer. The use of nutritionally inadequate feeds can result in reduced growth and production due to stress, but more seriously, the use of such feeds can result in loss of fish from nutritional deficiency syndromes and/or from mortality brought on by increased susceptibility of nutritionally compromised fish to infectious diseases. Medicinal plants are the main sources of natural antioxidants and antimicrobial compounds. A large number of plants have been used in traditional medicine for the treatment and control of several diseases. The present article gives an idea about the use of medicinal plants in aquaculture.

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1. Introduction

Fish constitutes the fastest growing source of food in the developing world. Fish consumption will have a significant impact on the food security, nutrition, diets, and income of poor people in developing countries during the next two decades. Food quality is defined in terms of consumer acceptability: taste, aroma, and appearance characteristics. While use of antibiotics in fish feed leads to consumer concern regarding their safety has motivated to seek natural alternatives. The main cause of the decrease in fish production is the occurrence of diseases caused

by different pathogens due to intensification of fish culture. It is preferable that, in the case of commercial aquaculture, the cost of production should also be reduced. Because the cost of antibiotics used for prevention and treatment of disease, and hormones used for growth performance is high, and from the desire to search for new options, several studies have been carried out to test new compounds from plant sources, from which the concept of phytobiotics has developed.

Plants have been selected and used empirically as drug for centuries, initially as traditional preparations then as pure active principles, with this knowledge and accumulated practice passing on from generation to generation. Herbal medicines includes herbs, herbal material, herbal preparations, finished herbal products that contain as active ingredients parts of plant materials, or combinations thereof. Traditional medicines is however used when referring to Africa, Latin America, South East Asia, and/or the West Pacific, whereas, complementary or alternative medicines are used. Medicinal herbs are significant sources of synthetic and herbal drugs. Almost all medicinal herbs have active ingredients which are responsible for various biological activities they exhibit.

2. Phytobiotics

Phytobiotics can be defined as plant derived products added to feed in order to improve the performance of animal. The phytobiotics have a wide variety of properties such as: antioxidant, antimicrobial, anticarcinogenic, analgesic, insecticidal, antiparasitic, anticoccidial, growth promoters appetite enhancement, stimulant of secretion of bile and digestive enzyme activity etc. The evaluation of phytobiotics in aquaculture is a relatively new area of research showing promising results. Addition of different single herbal extracts (Massa medicata, Crataegi fructus, Artemisia capillaries, Cnidium officinale) or a mixture of all the herbs promoted growth and enhanced some non-specific immunity indicators of fish (Yin et al., 2009).

2.1. Classification of phytobiotics

Medicinal plants are generally classified on the basis of their growth habit. It may be a tree, shrub, herb, annuals, biennial, tubers, rhizomes and climbers.

The difference between tree, shrub and herb

Tree: The tree has a firm single stem. The whole tree stands on the stem.

Shrub: The shrub has multiple stems. It forms a sort of bush. That is the reason for it to be planted as a barricade.

Herb: The Herb does not have a firm stem but a flexible juicy structure which does not have the woody hard part as in a Tree or a Shrub. A shrub is a low usually several-stemmed woody plant. Herbs are (usually) small plants that not develop persistent woody tissue but die down at the end of a growing season.

Medicinal plants used as natural medicines. This practice has existed since prehistoric times. There are three ways in which plants have been found useful in medicine. First, they may be used directly as teas or in other extracted forms for their natural chemical constituents. Second, they may be used as agents in the synthesis of drugs. Finally, the organic molecules found in plants may be used as models for synthetic drugs. Historically, the medicinal value of plants was tested by trial and error, as in the Doctrine of Signatures. Modern approaches to determining the medicinal properties of plants involve collaborative efforts that can include ethnobotanists, anthropologists, pharmaceutical chemists, and physicians. Many modern medicines had their origin in medicinal plants. Examples include aspirin from willow bark (*Salix* spp.), digitalis from foxglove (*Digitalis purpurea*), and vinblastine from Madagascar periwinkle (*Vinca rosea*) for the treatment of childhood leukemia.

2.2. Methods of extraction

Three primary extraction techniques are used for phytochemicals: solvents, solid-phase extraction, and supercritical extraction (Fig. 1). Using a Soxhlet apparatus combines percolation and immersion that increases extraction efficiency. Several extractions can be accomplished with solvents having different polarities (petrol ether, toluene, acetone, ethanol, methanol, ethyl acetate, and water). Methanol/water/HCl (70:29:1, v/v/v) has been shown to be the best among several solvents (Xu et al., 2010). Grinding in a mortar in liquid nitrogen provides uniform particle size allowing for a more consistent extraction. Ultrasound can be used to assist liquid solvent extraction. Supercritical CO₂ extraction can also be used (Schwarz et al., 2001). Hydrodistillation of plant materials has several advantages. The essential oils that carry the intrinsic flavor of a spice can be removed and polyphenols, primary antioxidant compounds, are concentrated. In addition, the hydrodistilled compounds are generally more

soluble in aqueous media than are those extracted using organic solvents. Hydrodistillation also avoids potential residues from organic solvents. Hydrodistilled extracts have also been reported to have a variety of functional effects in foods and in human health (Hinneburg et al., 2006). Optimizing the extractin process could lead to even better results (Table 1).

2.1.1. Medicinal trees (My Agriculture Information Bank)

Common name	Botanical name	Parts used
Babul	<i>Acacia nilotice Delite</i>	Pods, leaves, bark, gum
Bael	<i>Aegle marmelos L. Corr.</i>	Roots, leaves, fruit
Neerh	<i>Azafirachta indica</i>	Bark leaves, flowers, seed, oil
Palas	<i>Butea monossperma (Lam.)</i>	Bark, leaves, flowers, seed, gum
Gugul	<i>Commiphora mukulEngl J</i>	Resinous gum
Olive	<i>Olea europeae</i>	Leaves, Oil
Arjun	<i>Terminalia arjuan Roxb.</i>	Bark
Behela	<i>Terminalia bellirica Gaertu</i>	Bark, fruit
Hirda	<i>Terminalia bellirica Gaertu</i>	Fruits
Nagakesar	<i>Mesua ferrea L.</i>	Blowers, oil
Markingnut	<i>Semecarpus & anacardium L.</i>	Fruits

2.1.2. Medicinal shrubs

Common name	Botanical name	Parts used
Davana	<i>Artemisia nilagirica</i>	Leaves, flowering top
Safed musli	<i>Aparagus adscendens Roxbi</i>	Tuberous roots
Belladonna	<i>Atropa belladonna</i>	Leaves and roots
Lavender	<i>Lavandula officinalis</i>	Flowers
Sarpagandha	<i>Rauvalfia serpentina L.</i>	Roots
Chitrak	<i>Plumbage zeylanica L.</i>	Leaves, roots

2.1.3. Medicinal herbs

Common name	Botanical name	Parts used
Brahmi	<i>Bacopa monnieri L.</i>	Whole plant
Am haldi	<i>Curcuma amada Roxb.</i>	Rhizomes
Haldi	<i>Curcuma domestica Valet</i>	Rhizomes
Datura	<i>Datura metel L.</i>	Leaves, flowers
Kalazira	<i>Nigella sativa L.</i>	Seed
Afim	<i>Papaver somniferum L.</i>	Latex, seed
Pipli	<i>Piper Longum L.</i>	Fruits, roots
Babchi	<i>Psoralea corylifolia</i>	Seed, Fruit

2.1.4. Medicinal annuals

Common name	Botanical name	Parts used
Jangali muli	<i>Blumea lacera</i>	Whole plant
Cockscomb	<i>Celosia cristala L.</i>	Inflorescence
Red poppy	<i>Papaver rhoeas</i>	Flowers
Bhui amla	<i>Phyllantius niruri</i>	Whole plant

2.1.5. Biennial

Common name	Botanical name	Parts used
Bankultthi	<i>Cassia abus L.</i>	Leaves, seeds
Caper spurge	<i>Euphorbia lathyris</i>	Seed latex
Catchfly	<i>Melandrium firmum</i>	Whole plant

2.1.6. Tubers and rhizomes

Common name	Botanical name	Parts used
Satavar	<i>Asparagus adscendens Roxb</i>	Tubers
Safed musli	<i>Chlorophytum borivilianum</i>	Tubers
Puskarmul	<i>Inula racemosa Hook</i>	Roots
Sakarkhand	<i>Manihot esculenta crantz</i>	Tubers

2.1.7. Biennial

Common name	Botanical name	Parts used
Chocloate vine	<i>Akebia quinata Deene</i>	Stem, fruit
Malkunki	<i>Celustrus paniculatus Wild</i>	Bark, leaves, seed
Hajodi	<i>Cissus quadrangularis L.</i>	Whole plant
Khira	<i>Cucumis sativus L.</i>	Fruit, seed
Gudmar	<i>Gymnema sylvestre Retzx</i>	Whole plant, leves
Kali mirch	<i>Piper nigrum L.</i>	Fruit

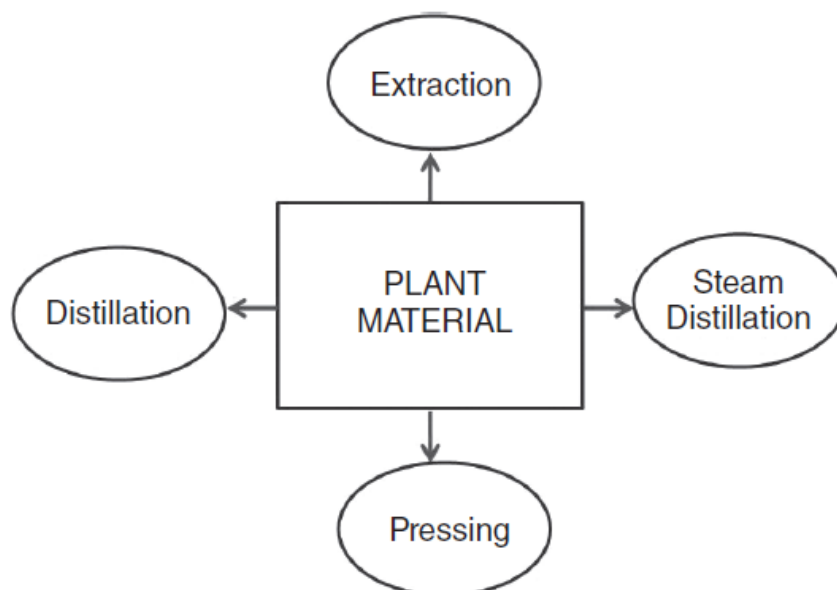


Fig. 1. Methods for recovery of secondary metabolites.

Table 1

Main herbal plant extracts and their multiple therapeutic properties in aquaculture (Coutteau et al., 2011).

English name	Scientific name	Useful part	Active substances	Therapeutic properties
Aromatic species				
Nutmeg	<i>Myristica flagrans</i>	Seed	Sabinene	Digestion stimulant, antidiarrhotic
Cinnamon	<i>Cinnamomum zeylanicum</i>	Bark	Ammameldehyde	Appetite and digestion stimulant, antiseptic
Clove	<i>Syzygium aromaticum</i>	Cloves	Eugenol	Appetite and digestion
Cardamom	<i>Elettaria caramomum</i>	Seed	Cinook	Appetite and digestion stimulant
Pungent species				
Capsicum	<i>Capsicum annum longum</i>	Fruit	Capsaicin	Antidiarrhoic, stimulant tonic, anti-inflammatory
Ginger	<i>Zingiber officinale</i>	Rhizom	Zingerole	Gastric Stimulant
Pepper	<i>Piper nigrum</i>	Gruit	Piperine	Digestion stimulant
Aromatic herbs and spices				
Garlic	<i>Allium tuberosum</i>	Bulb	Allicin	Digestions stimulant, antiseptic
Rosemary	<i>Aniba rosaeodora</i>	Leaves	Cineole	Digestion stimulant, antiseptic, antioxidant
Thyme	<i>Thymus vulgaris</i>	Whole plant	Thymol	Digestion stimulant, antiseptic, antioxidant
Sage	<i>Salvia apiana</i>	Leaves	Cineole	Digestion stimulant
Peppermint	<i>Mentha piperita</i>	Leaves	Menthol	Appetitie and digestion stimulant, antiseptic
Neem	<i>Azadirachta indica</i>	Leaves, bark	Azadirachtin, salanin, numbin	Antiviral, antiseptic, fungicidal

2.3. In vitro screening

The purpose of the disk diffusion susceptibility test is to determine the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria to various antimicrobial compounds. The pathogenic organism is grown on specific agar in the presence of various antimicrobial impregnated filter paper disks. The presence or absence of growth around the disks is an indirect measure of the ability of that compound to inhibit that organism.

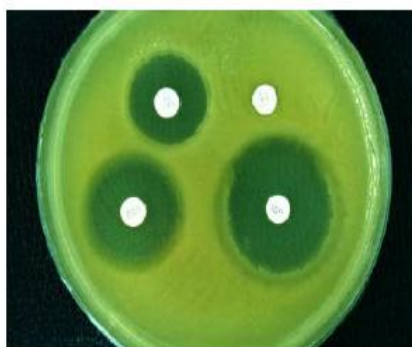


Fig. 2. Disk diffusion susceptibility test.

2.4. In Vivo study

To improve the health and growth of commercially important aquatic species (Shizothoracids, trout, mahaseer and carps), and to improve the economics of fish production by improving the efficiency of feeds and by lowering the cost of feeds, in vivo studies has been done. Recently in aquaculture, scores of plant extracts have

been tested and used with good results in the control of bacterial and viral diseases. Fourteen herbs have been tested against *Aeromonas hydrophila* infection in fishes. These findings suggest that herbs and their derivatives such as extracts could be an alternative to the chemotherapeutics in aquaculture.

3. Mode of action

Wenk (2003) reported that herbs, spices and their extracts can stimulate appetite and endogenous secretions such as enzymes or have antimicrobial, coccidiostatic or anthelmintic activities in monogastric animals. Oligosaccharides and polysaccharides such as inulin (fructan), fructo– oligosaccharides (FOS) and arabinogalactans, which are extracted from plants, and sulfated fucans, which are extracted from seaweeds, are potential substitutes for currently used antibiotic growth promoting compounds (Tringali, 1997) (Table 2).

4. Antimicrobial effects

Some bioactive substances from plants, like most antimicrobial agents, exert their effects by modulating the cellular membrane of microbes (Kamel, 2001). *In vitro* studies of (Kamel, 2001) indicate that the minimum inhibitory concentration (MIC50) and minimum bactericidal concentration (MBC50) are linked to the level of active substance and purity of the plant extract. Furthermore, a strong increase in hydrophobicity of the microbial species in the presence of some plant extracts may influence the surface characteristics of microbial cells and thereby affect the virulence properties of the microbes (Kamel 2001). This may be an important antimicrobial mechanism of some plant extracts. This concept may have implications for the gut, in which adhesion of microbes to intestinal mucosal cells is of vital importance for some pathogenic microflora and is strongly influenced by the hydrophobic surface properties of microbial cells (Pusztai et al., 1990), (Kamel, 2001). Various essential oil mixtures, which contain natural polyphenolic compounds or flavonoids as major active ingredients, have been identified as potential antimicrobial and antioxidant agents (Friedman et al., 2004). Thus, supplementation of broiler diets with essential oil mixtures can create a healthier gut microflora, aiding optimum digestion and improving bird performance (Cruickshank, 2001).

5. Competitive blocking of bacterial adhesion

Lectin–carbohydrate receptor interactions are the main mechanism in adhesion of pathogens to the brush border of the gut mucosal epithelium. Many prebiotic and phytogetic bioactive substances can have a direct effect on certain pathogenic bacteria either by specific adhesion of pathogens through the ‘lectin–receptor’ mechanism (agglutination) by blocking the adhesion of pathogens onto the mucosal layer of the intestine (Guo et al., 2003).

6. Immunostimulatory effects

It is also known that certain plant polysaccharides act as immunostimulatory (adjuvant effect) substances (Hosono et al., 2003). The gut–associated lymphoid tissue (GALT) plays a key role in immunomodulation in farm animals. Recent data from several animal models clearly indicate that prebiotic compounds can exert beneficial effects on gut health by enhancing GALT responses directly (systemic and local immunological effects) or indirectly by the mediation of short chain fatty acids such as butyrate and lactic acid producing bacteria (Xu et al., 2003).

7. Stimulation of digestive enzymes

Another possible mode of action of phytogetic bioactive compounds on growth performance of farm animals could be their effects on the activities of digestive enzymes. Xu et al. (2003) reported that dietary supplementation of fructooligosaccharides improved daily body weight gain of animals by increasing the activities of amylase and protease. Furthermore, a study with these animals indicated that feeding a diet containing a commercial blend of essential oils (CRINAR) in combination with lactic acid induced a significant increase in activities of digestive enzymes of the pancreas and intestinal mucosa of animals, leading to a significant increase in growth (Jang et al., 2004).

Table 2

Use of herbal phytochemicals as immunostimulants in fish culture (Chakraborty and Hancz, 2011).

Medicinal plant	Major phytochemical	Species	Dose & length of administrati-on	Results	References
<i>Astragalus radix</i> , root extract	Astragalus polysaccharide	<i>O. niloticus</i>	0.1,0.5,1.0% 4 weeks	↑Leucocytic phagocytosis ↑Lysozyme activity →Respiratory burst activity	Yin <i>et al</i> 2006
<i>Astragalus membranaceus</i> root extract and <i>Lonicera japonica</i> flower extract	Astragalus polysaccharide and chlorogenic acid	<i>O. niloticus</i>	0.1 % 4 weeks	↑Leucocytic phagocytosis →Total protein, total immunoglobulin ↑Respiratory burst activity	Ardo <i>et al</i> 2008
<i>Ganoderma lucidum</i> extract	polysaccharide	<i>C. carpio</i>	0.5%- 5 weeks	↑Leucocytic phagocytosis ↑Lysozyme activity ↑Respiratory burst activity	Roa and Chakrabarti 2004; Roa <i>et a.</i> 2004; Roa and Chakrabarti 2005; Chakrabarti and Rao 2006
<i>Achyranthes aspera</i> root extract	Triterpenoid saponins	<i>L. rohita</i> and <i>C. datla</i>	0.5% 4 weeks	↑Antigen-specific serum antibody ↑Antigen clearance ↑RNA/DNA ratio of spleen, kidney ↑Serum globulin,anti-protease	Roa <i>et al</i> 2006
<i>Allium sativum</i>	Alliin,allicin, ajoene, ally propyl disulphide, ally trisulphide, S-ally cystein, vinylidithiins, S-allylmercaptocystein	<i>L. rohita</i>	0.1. 0.5 , 1.0% 60 days	↑Superoxide anion production ↑Lysozyme activity ↑Serum bactericidal activity, serum protein, serum albumin	Sahu <i>et al</i> 2007
<i>Withania somnifer</i> root extract	Alkaloids, withanolids with a glucose at carbon 27, steroidal lactones, saponins containing an additional acyl group	<i>L. rohita</i>	1.0, 2.0, 3.0g/kg 42 days	↑Nitroblue tetrazolium level ↑Phagocytic cell activity ↑Lysozyme actibvity ↑Total immunoglobulin level	Sharma <i>et all</i> 2010
Sibarian ginseng <i>Eleutherococcus senticosus</i> residuum extract	Lignin, iridoid glycoside	<i>P. olivaceus</i>	3.0% 8 weeks	↑Non-specific immunity	Won <i>et al</i> 2008

Symbols represent an increase (↑) or no effect (→) on the specified response.

8. Present status of phytobiotics in aquaculture

In aquaculture one of the most promising methods of strengthening the defense mechanism and disease management is through prophylactic administration of immunostimulants (Raa et al., 1992). Recent advancement in immuno-nutrition studies revealed that some nutrients are linked to the immunological status of fish (Kumar et al., 2005). This has drawn the attention of fish nutritionists to the immunoprotection of fish besides the growth.

Yuan et al. (2007), fed common carp (*Cyprinus carpio*) diets containing a mixture of *Astragalus membranaceus* (root and stem), *Polygonum multiflorum*, *Isatis tinctoria* and *Glycyrrhiza glabra* (0.5 and 1%) for 30 days and observed that both concentrations significantly increased ($P < 0.05$) macrophage phagocytic activity, respiratory burst and levels of total protein, albumin, globulin and nitric oxide synthetase activity in the serum; no significant difference ($P > 0.05$) was found in superoxide dismutase (SOD), lysozyme activities and triglyceride level. Root extracts of the Chinese herb *Astragalus* contain polysaccharides, organic acids, alkaloids, glucosides and volatile oil as major active components that have been found to enhance immune function in fish (Jeney et al., 2009). The *Astragalus* polysaccharide (APS) from *A. membranaceus* is reported to halt reactive oxygen species (ROS) production, stimulate humoral and cellular immunity, and thus possess anticancer and immunostimulating effects (Yuan et al., 2008). The oriental medicinal herb *G. glabra* (liquorice) comprises flavonoids and pentacyclic triterpene saponin, including liquiritin, liquiritigenin, isoliquiritigenin, liquiritin apioside, glycyrrhizin and glycyrrhizic acid as major constituents and is reported to have anti-oxidant effects (Yin et al., 2011).

Kim et al. (2007), demonstrated 80% higher lysozyme activity and 66% higher leukocyte phagocytic activity in olive flounder (*Paralichthys olivaceus*) fed a diet containing a mixed culture of extracts from the mushrooms *Phellinus linteus* and *Coriolus versicolor*. Kelp grouper, *Epinephelus bruneus*, fed a diet supplemented with ethanol extract of the mushroom *P. linteus* for 30 days showed significantly higher ($P < 0.05$) alternative complement activity, serum lysozyme activity, phagocytic activity, phagocytic index, respiratory burst activity, superoxide dismutase activity and glutathione peroxidase activity compared with fish fed the control diet without mushroom extract (Harikrishnan et al., 2011a). This edible mushroom contains a large number of biologically active compounds, such as polysaccharides and triterpenes that may exhibit immunomodulating properties and stimulate proliferation of T lymphocytes, polyclonal activation of B cells.

9. Conclusion

In conclusion, defense mechanisms against fish pathogens as viruses, bacteria, and fungi that enter fishes by means of injury and the mucosa can be stimulated by using phytobiotics. May be this, can be related to either the plant species, the extraction procedure, the percentage of extract in the diet and the effect genetics of the experimental animal. The article provides a new perspective for the use of phytobiotics as adjuvant therapy added to fish food to prevent diseases.

References

- Chakraborty, S.B., Hancz, C., 2011. Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture. *Reviews in Aquaculture*. 3, 103-119.
- Coutteau, P., Ceulemans, S., Alexander, H. V., 2011. Botanical extracts improve productivity and economics in aquaculture. *Nutriad International*, Belgium.
- Cruickshank, G., 2001. Botanical growth enhancers offer natural option for broiler growers. *Poultry World*. 10, 19–22.
- Friedman, M., Buick, R., Elliott, C. T., 2004. Antibacterial activities of naturally occurring compounds against antibiotic-resistant *Bacillus cereus* vegetative cells and spores, *Escherichia coli*, and *Staphylococcus aureus*. *J Food Protec*. 67, 1774–1778.
- Guo, F.C., Savelkoul, H.F.J., Kwakkel, R.P., Williams, B.A., Verstegen, M.W.A., 2003. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. *World's Poultry Sci. J.* 59, 427–440.

- Harikrishnan, R., Balasundaram, C., Heo, M.S., 2011a. Diet enriched with mushroom *Phellinus linteus* extract enhances the growth, innate immune response, and disease resistance of kelp grouper, *Epinephelus bruneus* against vibriosis. *Fish and Shellfish Immunol.* 30, 128–134.
- . Hinneburg, I., Damien, H. J., Dorman, Hiltunen, R., 2006. Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chem.* 97.
- Hosono, A., Ozawa, A., Kato, R., Ohnishi, Y., Nakanishi, Y., Kimura, T., Nakamura, R., 2003. Dietary fructooligosaccharides induce immunoregulation of intestinal IgA secretion by Murine Peyer's patch cells. *Biosci Biotech and Biochem.* 67, 758–764.
- Jang, I.S., Ko, Y.H., Yang, H.Y., Ha, J. S., Kim, J.Y., Kim, J.Y., Kang, S.Y., Yoo, D.H., Nam, D.S., Kim, D.H, Lee, C.Y., 2004. Influence of essential oil components on growth performance and the functional activity of the pancreas and small intestine in broiler chickens. *Asian–Australasian J Anim Sci.* 17, 394–400.
- Jeney, G., Yin, G., Ardo, L., Jeney, Z., 2009. The use of immunostimulating herbs in fish. An overview of research. *Fish Physiol and Biochem.* 35, 669–676.
- Kumar, S., Sahu, N.P., Pal, A.K., Choudhury, D., Yengkokpam, S., Mukherjee, S.C., 2005. Effect of dietary carbohydrate on haematology, respiratory burst activity and histological changes in *L. rohita* juveniles. *Fish & Shellfish Immunol.* 19, 331–344.
- Kamel, C., 2001. Tracing modes of action and the roles of plant extracts in non–ruminants. *Rec Adv in Anim Nutri.* 135–150.
- Kim, M.C., Kim, M.J., Kim, J.S., Heo, M.S., 2007. Effect of culture broth from mushroom mycelium on growth and nonspecific immune parameters in flounder (*Paralichthys olivaceus*) by oral administration. *Korean J Life Sci.* 17, 1434–1440.
- Pusztai, A., Grant, G., King, T.P., Clarke, E.M.W., 1990. *Chemical Probiosis.* (eds. W. Haresign and D.J.A. Cole). Butterworths, London, UK. *Adv in Anim Nutri.*, 47–60.
- Raa, J., Roerstad, G., Ingested, R., Robertson, B., 1992. The use of immunostimulants to increase resistance of aquatic organisms to microbial infections. *Dis in Asian aqua.* 39-50.
- Schwarz, K., Bertelsen, G., Nissen, L.R., Gardner, P.T., 2001. Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant assays based on radical scavenging, lipid oxidation and analysis of the principal antioxidant compounds. *Euro Food Res and Technol.* 212, 319–328.
- Tringali, C., 1997. Bioactive metabolites from marine algae, recent results. *Current Organic Chemistry.* 1, 375–394.
- Wenk, C., 2003. Herbs and botanicals as feed additive in monogastric animals. *Asian–Australasian J Anim Sci.* 16, 282–289.
- Xu, Z.R., Hu, C.H., Xia, M.S., Zhan, X.A., Wang, M.Q., 2003 Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poultry Sci.* 82, 1030–1036.
- Yin, G., Ardo, L., Thompson, K.D., Adams, A., Jeney, Z., Jeney, G., 2009. Chinese herbs (*Astragalus radix* and *Ganoderma lucidum*) enhance immune response of carp, *Cyprinus carpio*, and protection against *Aeromonas hydrophila*. *Fish and Shellfish Immunol.* 26, 140–145.
- Yin, G., Cao, L., Xu, P., Jeney, G., Nakao, M., Lu, C., 2011. Administration of a herbal immunoregulation mixture enhances some immune parameters in carp (*Cyprinus carpio*). *Fish Physiol and Biochem.* 37, 209–216.
- Yuan, C., Li, D., Chen, W., Sun, F., Wu, G., Gong, Y., 2007. Administration of a herbal immunoregulation mixture enhances some immune parameters in carp (*Cyprinus carpio*). *Fish Physiol and Biochem.* 33, 93–101.
- Yuan, C., Pan, X., Gong, Y., Xia, A., Wu, G., Tang, J., 2008. Effects of *Astragalus polysaccharides* (APS) on the expression of immune response genes in head kidney, gill and spleen of the common carp, *Cyprinus carpio* L. *International Immunopharma.* 8, 51–58.