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REVIEW ARTICLE

# Current research on the use of plant-derived products in farmed fish

**Chiara Bulfon, Donatella Volpatti & Marco Galeotti**

Fish Pathology Division, Department of Food Science, University of Udine, Udine, Italy

**Correspondence:** D. Volpatti, Department of Food Science, University of Udine, via Sondrio, 2, 33100 Udine, Italy. E-mail: donatella.volpatti@uniud.it

## Abstract

Over the years, aquaculture has shown increasing development in terms of production. However, due to intensive farming practices, infectious diseases represent the main problem in fish farms, causing heavy economic losses. The use of antibiotics for controlling diseases is widely criticized for its negative impact, including selection of antibiotic-resistant bacterial strains, immunosuppression, environmental pollution and accumulation of chemical residues in fish tissues. On the other hand, though vaccination is the most effective prophylactic method of preventing disease outbreaks, the development of effective formulations is often hindered by high production costs and the antigenic heterogeneity of the microbial strains. Recently, there has been increased interest in the possibility of using medicinal herbs as immunostimulants, capable of enhancing immune responses and disease resistance of cultured fish. Plant-derived products seem to represent a promising source of bioactive molecules, being at the same time readily available, inexpensive and biocompatible. The aim of this article is to provide an overview of recent research dealing with the use of medicinal plants in aquaculture. Special attention is given to the information about the effects of plant extracts/products on fish growth, haematological profiles, immune responses and resistance to infectious diseases.

**Keywords:** plants, feed additives, immunostimulants, natural antibiotics, disease resistance, aquaculture

## Introduction

Over the years, world aquaculture has grown rapidly in terms of production. However, intensive and stressful rearing conditions make farmed fish highly susceptible to different infectious diseases, which are now the most serious problem for the aquaculture industry, causing heavy economic losses. The use of antibiotics for controlling diseases is widely criticized because it is often very expensive and leads to the selection of antibiotic-resistant bacterial strains, immunosuppression, environmental pollution and the accumulation of chemical residues in fish tissues, which can be potentially harmful to public health (FAO/WHO/OIE 2006). In the United States and Europe governmental restrictions [US Food and Drug Administration (FDA) and US Environmental Protection Agency (EPA) guidance; CE Regulations Nos. 1804/1999, 37/2000, 82/2001, 178/2002, 74/2003, 28/2004, 726/2004 and 834/2007; Italian Decrees Nos. 119/1992, 47/1997, 336/1999 and 71/2003] have limited the number of drugs that can be currently used in aquaculture and even in Asian countries a strict demand for fish products free of pollutants/antibiotics is steeply increasing (Ji, Jeong, Im, Lee, Yoo & Takii 2007a). On the other hand, though vaccination is the most effective method of preventing disease outbreaks, the production of effective formulations for a number of pathogens is often hindered by high production costs and the antigenic heterogeneity of the microbial strains (Le Breton 2009; Toranzo, Romalde, Magariños & Barja 2009).

Given the problems and limitations listed above, the interest of researchers, aqua-feed mill and

pharmaceutical companies has increased in recent years with regard to the development of alternative strategies for the disease management in aquaculture, capable of strengthening the immune responses (immunocompetence) of fish and consequently their resistance to pathogens. Synthetic or natural immunostimulants (probiotics, complex carbohydrates, nutritional factors, hormones, cytokines, products derived from animal, plants and algae) are able to effectively promote fish growth, the innate/non-specific immune response (lysozyme, complement, phagocyte activity, respiratory burst and microbial activities of phagocytes) and, to a lesser extent, the adaptive/specific immune response (immunoglobulin production) (Anderson 1992; Galeotti 1998; Sakai 1999). However, the use of hormones, vitamins and chemicals is often not recommended because they may produce side effects in fish and could leave potentially dangerous residues for consumers. Set against this, natural products like plant-derived products could represent a promising approach complementary to vaccination and traditional drugs, as they provide a useful source of biologically active secondary metabolites, being at the same time easily available, inexpensive and biocompatible (Mohamad & Abasali 2010).

The use of medicinal plants in aquaculture has attracted a lot of attention globally and has become a subject of active scientific investigations (Jeney, Yin, Ardò & Jeney 2009; Chakraborty & Hancz 2011; Harikrishnan, Balasundaram & Heo 2011a). In this article, 105 scientific pubblications available in literature since 1998 up to 2011 concerning the administration of phytomedicines in fish species were examined. Such studies were performed mainly over the past 5 years. In particular, 83% were made during the period 2006–2011, 15% were performed between 2001 and 2005, relatively few in number are those conducted before 2001 (Fig. 1). The majority of these experiments were carried out in Asia [the countries with the highest percentages of documents are India (30.2%), Korea (19.8%), Thailand (7.5%) and China (5.7%)], some experiments were performed in Middle East [Iran (6.6%), Egypt (6.6%), Turkey (3.8%), Israel (2.8%)] and just a few in Europe [United Kingdom (4.7%), Greece (0.9%) and Hungary (0.9%)]. No data are available about experiments conducted in America.

The purpose of this article is to summarize and discuss the results of these studies, to provide useful information for the application of herbal products in finfish culture. This is the first detailed review in which the effects of plants on fish growth, haematological profile, immune response and disease resistance are fully described.

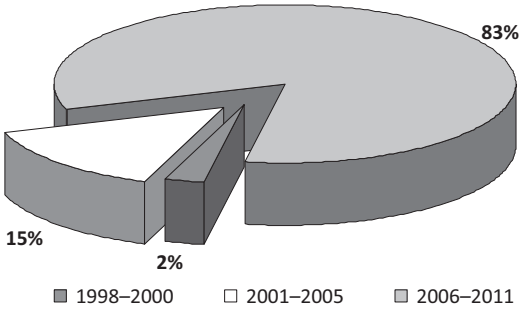


**Figure 1** Distribution between the years 1998 and 2011 of the literature concerning the use of plants and plant-derived products in fish species. Reference publications = 105.

## Plant species and plant-derived products currently under investigation

Medicinal plants have been reported as having a broad spectrum of growth promotion, appetite stimulation, antimicrobial, immunostimulant, antinflammatory, antistress, anticancer properties and their use in traditional medicine has been known for thousands of years around the world. In some Asian countries such as China, India, Japan, Thailand, Korea and some countries in South and Central America, different herbal preparations are used in human and veterinary medicine to prevent and treat bacterial, fungal and viral diseases as well as in the therapy of several disorders (Briskin 2000; Lovkova, Buzuk, Sokolova & Kliment'eva 2001; Sher 2009; Hashemi & Davoodi 2011; Sakarkar & Deshmukh 2011; Sharma, Parihar & Parihar 2011; Wallace, Oleszek, Franz, Hanh, Baser, Mathe & Teichmann 2011). In aquaculture, the application of medicinal herbs has been reported in various Asian countries (Direkbusarakom 2004).

Recently, more than 60 different plant species have been studied for the improvement of fish health and disease management in aquaculture (Tables 1–5). The most investigated herbs are those widely used in folk medicine in China, India, Thailand and Korea, such as *Achyranthes aspera*, *Aegle marmelos*, *Andrographis paniculata*, *Angelica*

  

**Table 1** Impact of plant-derived immunostimulants used *per os* on growth, survival, haematological profile, immune response and disease resistance of cultured fish

| Immunostimulants – oral administration | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Single plant/plant mixture** | **Parts/ products** | **Concentrations** | **Exposure** | **Fish species** | **Weight (g)** | **Growth performance and survival** | **Haematological and immunological parameters** | **Disease resistance*** | **References** |
| *Achyranthes aspera* | Seeds | 0.5% | 3% BW day$^{-1}$ 9 weeks | *C. catla* | 20 ± 4 | n.a. | TP (↔), ALB (↔), GLB (↔), AST (↑), ALT (↑), LYZ (↑), AP (↑), BA (↑), RB (↑) | *A. hydrophila* | Vasudeva and Sunil (2009) |
| | Seeds | 0.5% | 2% BW day$^{-1}$ 4 weeks | *C. carpio* | 90 ± 17 | n.a. | TP (↔), ALB (↔), GLB (↑), LYZ (↑), AP (↑), RNA/DNA (↑) | n.a. | Vasudeva and Chakrabarti (2005b) |
| | Seeds | 0.01%, 0.1%, 0.5% | 3% BW day$^{-1}$ 5 weeks | *L. rohita* | 3.0 ± 0.4 | SGR (↑), FCR (↓) | TP (↔), ALB (↑), GLB (↔), AST (↓), ALT (↓), ALP (↑), LYZ (↑), BA (↑), RB (↑) | *A. hydrophila* | Vasudeva *et al.* (2006) |
| | Seeds extract (water) | 0.5% | 1% BW day$^{-1}$ 4 weeks | *C. catla* | 150 ± 20 | n.a. | TP (↔), ALB (↔), GLB (↑), AP (↑), RNA/DNA (↑) | n.a. | Vasudeva and Chakrabarti (2005a) |
| | Roots extract (water) | 0.5% | 1% BW day$^{-1}$ 4 weeks | *L. rohita* | 200 ± 17 | n.a. | TP (↔), GLB (↑), AP (↑), RNA/DNA (↑) | n.a. | Vasudeva *et al.* (2004); Vasudeva & Chakrabarti (2004) |
| *Aegle marmelos* | Leaves extract (water) | 0.5%, 1%, 1.5%, 2%, 2.5%, 3% | 30 d | *C. catla* | 16 ± 5 | n.a. | †PHAG (↑) | n.a. | Pratheepa *et al.* (2011) |
| | Leaves extract (water) | 0.5%, 1%, 2%, 2.5%, 5% | 2% BW day$^{-1}$ 50 d | *C. carpio* | 45.9 ± 1.5 | n.a. | †RBC (↑), Hb (↑), WBC (↑), ALP (↑), ACP (↑), LYZ (↑), PO (↑), PHAG (↑), RB (↑), specific Ig (↑) | *A. hydrophila* | Pratheepa *et al.* (2010) |

**Table 1** Continued

**Immunostimulants – oral administration**

| Single plant/plant mixture | Parts/ products | Concentrations | Exposure | Fish species | Weight (g) | Growth performance and survival | Haematological and immunological parameters | Disease resistance* | References |
|---|---|---|---|---|---|---|---|---|---|
| | Plant extract (acetone) | 1% | 5% BW $day^{-1}$ 45 d | *O. mossambicus* | 7.46 ± 0.11 | FBW (↑), SGR (↑) | Hct (↑), Lct (↔), TP (↑), ALB (↑), GLB (↔), GLU (↓), CHO (↓), TRIG (↓), Ca (↔), LYZ (↔), PHAG (↔) | *V. vulnificus* | Immanuel *et al.* (2009) |
| *Allium sativum* | Bulbs | 0.1%, 0.5%, 1% | 4% BW $day^{-1}$ 60 d | *L. rohita* | 10 ± 2 | SGR (↔), FCR (↔) | RBC (↑), Hb (↑), WBC (↑), TP (↑), ALB (↑), GLB (↑), GLU (↓), LYZ (↑), BA (↑), RB (↑) | *A. hydrophila* | Sahu, Das, Mishra *et al.* (2007a) |
| | Bulbs | 0.05%, 0.1%, 0.5%, 1% | To satiety 2 weeks | *O. mykiss* | 14 | WG (↑), SGR (↑), FCR (↓), PER (↑) | RBC (↑), Hct (↑), Hb (↔), MCV (↓), MCH (↓), MCHC (↑), WBC (↑), LYM (↑), MON (↓), NEU (↓), TP (↑), ALB (↔), GLB (↑), LYZ (↑), AP (↔), BA (↑), PHAG (↑), RB (↑) | *A. hydrophila* | Nya and Austin (2009a) |
| | Bulbs | 0.5%, 1% | To satiety 2 weeks | *O. mykiss* | 14 | WG (↔), SGR (↔), CF (↔) | RBC (↑), Hct (↔), Hb (↔), WBC (↑), LYM (↑), MON (↓), NEU (↓), TP (↔), Ca (↑), Mg (↑), Fe (↑), K (↑), LYZ (↑), PO (↔), RB (↑) | *A. hydrophila* | Nya and Austin (2011) |
| | Bulbs | 1%, 2% | 1–3% BW $day^{-1}$ 4/8 weeks | *O. niloticus* | 6.5 ± 1.0 | WG (↑) | Hct (↔), WBC (↓), LYM (↓), MON (↑), NEU (↔), EOS (↔), BAS (↔), PHAG (↑) | *A. hydrophila* | Aly *et al.* (2008a) |

**Table 1** Continued

**Immunostimulants – oral administration**

| Single plant/plant mixture | Parts/ products | Concentrations | Exposure | Fish species | Weight (g) | Growth performance and survival | Haematological and immunological parameters | Disease resistance* | References |
|---|---|---|---|---|---|---|---|---|---|
| | Bulbs | 0.5%, 1% | To satiety 4 weeks | *O. niloticus x O. aureus* | 25.5 ± 1.0 | WG (↔) | WBC (↑), LYZ (↑), COMP (↑), PHAG (↑) | n.a. | Ndong and Fall (2007) |
| | Plant powder | 2%, 3%, 4% | 30 d | *O. mykiss* | 50 ± 5 | n.a. | RBC (↔), Hct (↔), Hb (↔), MCV (↓), MCH (↔), MCHC (↔), WBC (↑), LYM (↑), NEU (↓), AST (↔), ALT (↔) | n.a. | Fazlolahzadech, Keramati, Nazifi, Shirian and Seifi (2011) |
| | Plant powder | 1%, 2%, 3%, 4% | 3% BW day$^{-1}$ 90 d | *O. niloticus* | 7 ± 1 | FBW (↑), WG (↑), SGR (↑), FCR (↓), FER (↑), PER (↑), HIS (↔), SR (↔) | RBC (↑), Hct (↑), Hb (↑), MCV (↔), MHC (↔), MCHC (↔), TP (↑), GLU (↓), TL (↓), AST (↓), ALT (↓) | *A. hydrophila* | Shalaby *et al.* (2006) |
| | Plant extract (chic. acid) | 3% | 3% BW day$^{-1}$ 12 weeks | *O. niloticus* | 0.8 ± 0.2 | WG (↑), SGR (↑), SR (↑) | Hct (↑), WBC (↔), LYM (↔), MON (↔), NEU (↓), EOS (↔), BAS (↔) | *A. hydrophila* | Aly and Mohamed (2010) |
| *Allium tuberosum* | Essential oil | 0.02%, 0.04%, 0.06%, 0.08% | 5% BW day$^{-1}$ 19 d | *O. niloticus* | 10 | WG (↔), SGR (↔), FCR (↔), SR (↔) | n.a. | *F. columnare* | Rattanachaikunsopon and Phumkhachorn (2009a) |
| *Alnus firma* | Plant extract (ethanol) | 0.01%, 0.1%, 1% | 5% BW day$^{-1}$ 8 weeks | *P. olivaceus* | 22.3 ± 1.3 | n.a. | †RBC (↑), Hct (↑), Hb (↑), WBC (↑), TP (↑), GLU (↑), CHO (↑), Ca (↑), LYZ (↑), RB (↑) | *T. maritimum* | Harikrishnan, Kim, Kim *et al.* (2011i) |
| *Aloe vera* | Plant extract (solvent n.a.) | 0.5% | 5% BW day$^{-1}$ 6 weeks | *C. carpio* | 108 ± 11.4 | n.a. | RBC (↔), WBC (↑), TP (↑), GLB (↑), LYZ (↑), COM (↔), BA (↑), specific Ig (↑) | *A. hydrophila* | Alishahi *et al.* (2010) |
| | Plant powder | 0.1%, 0.5% | To satiety 6 weeks | *S. schlegeli* | 25 | n.a. | LYZ (↔), RB (↓) | n.a. | Kim *et al.* (1999) |

**Table 1** Continued

**Immunostimulants – oral administration**

| Single plant/plant mixture | Parts/ products | Concentrations | Exposure | Fish species | Weight (g) | Growth performance and survival | Haematological and immunological parameters | Disease resistance* | References |
|---|---|---|---|---|---|---|---|---|---|
| *Andrographis paniculata* | Leaves | 5%, 10%, 15%, 20%, 25% | 5% BW day$^{-1}$ 2 weeks | *O. niloticus* | 10 | SR (↔) | n.a. | *S. agalactiae* | Rattanachaikunsopon and Phumkhachorn (2009b) |
| | Plant extract (water) | 2.5%, 5%, 7.5%, 10%, 12.5% | | | | | | | |
| | Plant extract (methanol) | 0.05%, 0.1%, 0.2%, 0.3% | *ad libitum*, 45 d | *O. mossambicus* | 20–40 | FBW (↔), WG (↑), SGR (↑) | RBC (↑), Hb (↑), MCV (↓), MHC (↓), MCHC (↔), WBC (↑), TRB (↑) | n.a. | Prasad and Mukthiraj (2011) |
| *Artemisia capillaries* | Leaves | 0.5% | To satiety, 12 weeks | *P. major* | 24.0 ± 0.2 | FBW (↑), SGR (↑), DFI (↔), FER (↔), CF (↔), SR (↑), HSI (↔), VSI (↔) | Hct (↔), Hb (↑), HDL-CHO (↔), AST (↓), ALT (↓), LYZ (↔), COM (↔) | *V. anguillarum* | Ji, Takaoka *et al.* (2007b) |
| *Artemisia cina* | Plant powder | 1%, 3%, 5% | 3% BW day$^{-1}$ 30 d | *C. gariepinus* | 22 | WG (↔), SGR (↔), FCR (↓), CF (↓), SR (↑), HSI (↑), SSI (↑) | Hct (↑), Hb (↑), WBC (↔), RB (↑) | n.a. | Abdelhadi, Saleh and Sakr (2010) |
| *Astragalus membranaceus* | Plant extract (solvent n.a.) | 0.5% | *ad libitum* 5 weeks | *C. carpio* | 62.8 ± 5.4 | n.a. | LYZ (↑), PHAG (↑), RB (↑), specific Ig (↑) | *A. hydrophila* | Yin *et al.* (2009) |
| | Plant extract (solvent n.a.) | 0.1%, 0.5%, 1% | a*d libitum* 4 weeks | *O. niloticus* | 62.8 ± 5.4 | n.a. | LYZ (↑), PHAG (↑), RB (↔) | n.a. | Yin *et al.* (2006) |
| | Plant extract (solvent n.a.) | 0.1% | 4 weeks | *O. niloticus* | n.a. | n.a. | TP (↔), LYZ (↑), PHAG (↑), RB (↑), total Ig (↔) | *A. hydrophila* | Ardò *et al.* (2008) |
| *Camellia sinensis* | Plant extract (water) | 0.01%, 0.1%, 1% | To satiety 6 weeks | *E. bruneus* | 14.5 ± 2.1 | n.a. | †LYZ (↑), COMP (↑), AP (↑), RB (↓), RNI (↑), MPO (↑) | *V. carchariae* | Harikrishnan *et al.* (2011b) |
| | Plant extract (eth. acid) | 0.002%, 0.01%, 0.05% | n.a. | *O. mykiss* | 35 ± 3 | n.a. | LYZ (↑), AP (↑), PO (↑), BA (↑) | n.a. | Sheikhzadeh *et al.* (2011) |

**Table 1** Continued

**Immunostimulants – oral administration**

| Single plant/plant mixture | Parts/ products | Concentrations | Exposure | Fish species | Weight (g) | Growth performance and survival | Haematological and immunological parameters | Disease resistance* | References |
|---|---|---|---|---|---|---|---|---|---|
| *Cinnamomum verum* | Barks<br>Barks extract (water) | 4.8%, 10%, 15.8%<br>3.2%, 6.7%, 10.3% | 3% BW day$^{-1}$ 2 weeks | *Oreochromis* sp. | 11 ± 2 | n.a. | n.a. | *S. agalactiae* | Alsaid, Daud, Bejo and Abuseliana (2010) |
| | Essential oil | 0.1%, 0.2%, 0.3%, 0.4% | 5% BW day$^{-1}$ 19 d | *O. niloticus* | 10 ± 1 | WG (↔), SGR (↔), FCR (↔), SR (↔) | n.a. | *S. iniae* | Rattanachaikunsopon and Phumkhachorn (2010a) |
| *Cnidium officinale* | Roots | 0.5% | To satiety 12 weeks | *P. major* | 24.0 ± 0.2 | FBW (↑), SGR (↑), DFI (↔), FER (↑), CF (↔), SR (↑), HSI (↔), VSI (↔) | Hct (↔), Hb (↑), HDL-CHO (↑), AST (↓), ALT (↓), LYZ (↑), COMP (↔) | *V. anguillarum* | Ji, Takaoka *et al.* (2007b) |
| *Cotinus coggyria* | Plant powder | 0.5%, 1% | *ad libitum* 3 weeks | *O. mykiss* | 89.25 ± 0.12 | SGR (↔) | TP (↑), LYZ (↑), PHAG (↑), RB (↑) | n.a. | Bilen *et al.* (2011) |
| *Crataegi fructus* | Fruits | 0.5% | To satiety 12 weeks | *P. major* | 24.0 ± 0.2 | FBW (↑), SGR (↑), DFI (↔), FER (↔) CF (↔), SR (↑), HSI (↔), VSI (↔) | Hct (↔), Hb (↔), HDL-CHO (↔), AST (↓), ALT (↓), LYZ (↔), COMP (↔) | *V. anguillarum* | Ji, Takaoka *et al.* (2007b) |
| *Cratoxylum formosum* | Plant extract (water) | 0.1%, 1%, 1.5% | 2% BW day$^{-1}$ 30 d | *O. niloticus* | 30 ± 2 | WG (↔), SGR (↔), FCR (↔), SR (↔) | LYZ (↑), PHAG (↑), RB (↑) | *S. agalactiae* | Rattanachaikunsopon and Phumkhachorn (2010c) |
| *Curcuma longa* | Plant powder | 0.01%, 0.05%, 0.1%, 0.5% | 60 d | *L. rohita* | 10 ± 2 | SGR (↔), FCR (↔), SR (↑) | RBC (↑), Hb (↔), WBC (↑), TP (↑), ALB (↑), GLB (↑), AST (↓), ALT (↓), ALP (↑), LYZ (↑), BA (↑), RB (↑) | *A. hydrophila* | Sahu *et al.* (2008) |
| | Rhizomes extract (ethanol) | 0.0005%, 0.01%, 0.002%, 0.004%, 0.008% | 30 d | *O. niloticus* | 40 ± 5 | n.a. | IL-1β (↑), IL-8 (↑), TGF-β (↑) | n.a. | Panprommin, Vanichkul, Panprommin and Areechon (2011) |

**Table 1** Continued

**Immunostimulants – oral administration**

| Single plant/plant mixture | Parts/ products | Concentrations | Exposure | Fish species | Weight (g) | Growth performance and survival | Haematological and immunological parameters | Disease resistance* | References |
|---|---|---|---|---|---|---|---|---|---|
| *Cynodon dactylon* | Plant extract (ethanol) | 0.05%, 0.5%, 5% | 2% BW day$^{-1}$ 45-60 d | *C. catla* | 88.05 ± 4.75 | FBW (↔), WG (↔), SGR (↑), FCR (↑), SR (↔) | RBC (↑), Hb (↑), WBC (↑), TP (↑), GLB (↑), ALB/GLB (↑), GLU (↑), CHO (↑), LYZ (↑), COMP (↑), AP (↑), RB (↑), RNI (↑), MPO (↑), RNA/DNA (↑), specific Ig (↑), APC (↑), MMC (↑) | *A. hydrophila* | Kaleeswaran *et al.* (2010, 2011a,b) |
| | Plant extract (acetone) | 1% | 5% BW day$^{-1}$ 45 d | *O. mossambicus* | 7.46 ± 0.11 | FBW (↑), SGR (↑) | Hct (↑), Lct (↑), TP (↑), ALB (↑), GLB (↑), GLU (↓), CHO (↓), TRIG (↓), Ca (↔), LYZ (↑), PHAG (↑) | *V. vulnificus* | Immanuel *et al.* (2009) |
| *Echinacea purpurea* | Plant extract (chic. acid) | 0.025% | 3% BW day$^{-1}$ 24 weeks | *O. niloticus* | 4.5 ± 0.2 | WG (↑), SGR (↑), CF (↔) | Hct (↑), WBC (↑), LYM (↑), MON (↔), NEU (↔), EOS (↑), BAS (↔), LYZ (↑), RB (↔) | *P. fluorescens* | Aly *et al.* (2008b) |
| | Plant extract (chic. acid) | 0.1% | 3% BW day$^{-1}$ 12 weeks | *O. niloticus* | 0.8 ± 0.2 | WG (↑), SGR (↑), SR (↑) | Hct (↑), WBC (↑), LYM (↑), MON (↔), NEU (↓), EOS (↔), BAS (↔) | *A. hydrophila* | Aly and Mohamed (2010) |
| *Eclipta alba* | Leaves extract (water) | 0.01%, 0.1%, 1% | 2% BW day$^{-1}$ 3 weeks | *O. mossambicus* | 50 ± 5 | n.a. | LYZ (↑), COMP (↑), AP (↑), RB (↑), RNI (↑), MPO (↑) | *A. hydrophila* | Christybapita *et al.* (2007) |
| *Epilobium hirsutum* | Plant extract (ethanol) | 0.5%, 1%, 3% | 2% BW day$^{-1}$ 8 weeks | *C. carpio* | 20 ± 2 | SGR (↔), FCR (↔), CF (↔), SR (↔) | †RBC (↔), Hct (↔), Hb (↔), WBC (↑), LYM (↔), MON (↔), NEU (↔) | *A. hydrophila* | Pakravan *et al.* (2011) |

**Table 1** Continued

**Immunostimulants – oral administration**

| Single plant/plant mixture | Parts/ products | Concentrations | Exposure | Fish species | Weight (g) | Growth performance and survival | Haematological and immunological parameters | Disease resistance* | References |
|---|---|---|---|---|---|---|---|---|---|
| *Eriobotrya japonica* | Plant extract (ethanol) | 0.1%, 1%, 2% | 5% BW day$^{-1}$ 8 weeks | *E. bruneus* | 25.4 ± 1.2 | n.a. | †WBC (↑), TP (↑), ALB (↑), GLB (↑), GLU (↓), LYZ (↑), COMP (↑), BA (↑), PHAG (↑), RB (↑), LYMK (↑) | *V. carchariae* | Kim *et al.* (2011) |
| *Euphorbia hirta* | Leaves extract (water) | 0.5%, 1%, 2%, 2.5%, 5% | 2% BW day$^{-1}$ 50 d | *C. carpio* | 45.9 ± 1.5 | n.a. | †RBC (↑), Hb (↑), WBC (↑), LYZ (↑), PHAG (↑), RB (↑), specific Ig (↑) | n.a. | Pratheepa and Sukumaran (2011) |
| *Garcinia kola* | Seeds extract (ethanol) | 0.025%, 0.05%, 0.1%, 0.2% | 3% BW day$^{-1}$ 56 d | *C. gariepinus* | 245.20–255.00 | FBW (↑), WG (↑), SGR (↑), FCR (↑) | RBC (↔), Hb (↔), WBC (↑) | n.a. | Dada and Ikuerowo (2009) |
| *Ginseng* | Roots extract (solvent n.a.) | 0.005%, 0.01%, 0.015%, 0.02%, 0.025% | 3% BW day$^{-1}$ 6 weeks | *O. niloticus* | 24.4 ± 0.2 | FBW (↑), WG (↑), SGR (↑), CF (↔), FCR (↓), DFI (↑), PER (↑), PPV (↑), FR (↑), ER (↑) | RBC (↑), Hct (↑), Hb (↑), MCV (↓), MCH (↓), MCHC (↔), WBC (↔), LYM (↔), MON (↔), NEU+EOS+BAS (↔), TP (↑), ALB (↑), GLB (↑) | n.a. | Goda (2008) |
| *Kalopanax pictus* | Plant extract (ethanol) | 0.1%, 1%, 2% | 5% BW day$^{-1}$ 30 d | *E. bruneus* | 26.1 ± 1.4 | n.a. | †RBC (↑), Hct (↑), Hb (↑), MCV (↔), MCH (↔), MCHC (↔), WBC (↑), LYM (↑), MON (↑), NEU (↔), TRB (↔), TP (↑), LYZ (↑), COMP (↑), AP (↑), PO (↑), BA (↑), PHAG (↑), RB (↑) | *V. alginolyticus P. dicentrarchi* | Harikrishnan *et al.* (2011f) |
| *Lactuca indica* | Plant extract (ethanol) | 0.1%, 1%, 2% | 5% BW day$^{-1}$ 30 d | *E. bruneus* | 27.7 ± 1.4 | n.a. | †LYZ (↔), PHAG (↑), RB (↑), total Ig (↑) | *S. inieae* | Harikrishnan *et al.* (2011d) |
| *Laurus nobilis* | Plant powder | 0.5%, 1% | To satiety 3 weeks | *O. mykiss* | 89.25 ± 0.12 | n.a. | TP (↔), LYZ (↔), PHAG (↑), RB (↑) | n.a. | Bilen and Bulut (2010) |

**Table 1** Continued

**Immunostimulants – oral administration**

| Single plant/plant mixture | Parts/ products | Concentrations | Exposure | Fish species | Weight (g) | Growth performance and survival | Haematological and immunological parameters | Disease resistance* | References |
|---|---|---|---|---|---|---|---|---|---|
| *Lonicera japonica* | Plant extract (solvent n.a.) | 0.1% | 4 weeks | *O. niloticus* | n.a. | n.a. | TP (↔), LYZ (↑), PHAG (↑), RB (↑), total Ig (↔) | *A. hydrophila* | Ardò *et al.* (2008) |
| *Lupinus perennis* | Seeds | 1% | 3% BW day$^{-1}$ 2 weeks | *O. mykiss* | 15 | n.a. | RBC (↑), Hct (↑), Hb (↔), MCV (↔), MCH (↔), MCHC (↔), WBC (↑), LYM (↔), MON (↔), NEU (↔), TRB (↑), TP (↔), LYZ (↑), COMP (↔), AP (↔), PO (↔), BA (↓), PHAG (↔), RB (↑) | n.a. | Awad and Austin (2010) |
| | Seeds | 1%, 2% | n.a. | *O. mykiss* | 18.0 ± 0.2 | n.a. | IL-1β (↑), IL-8 (↑), TGF-β (↑) | n.a. | Awad *et al.* (2011) |
| *Mangifera indica* | Kernels | 0.1%, 0.5%, 1% | 60 d | *L. rohita* | 10 ± 2 | SGR (↔), FCR (↔) | RBC (↑), Hb (↑), WBC (↑), TP (↑), ALB (↑), GLB (↑), GLU (↓), LYZ (↑), BA (↑), RB (↑) | *A. hydrophila* | Sahu, Das, Pradhan *et al.* (2007b) |
| | Fruits | 1% | 3% BW day$^{-1}$ 2 weeks | *O. mykiss* | 15 | n.a. | RBC (↑), Hct (↑), Hb (↔), MCV (↔), MCH (↔), MCHC (↔), WBC (↑), LYM (↔), MON (↔), NEU (↔), TRB (↔), TP (↑), LYZ (↑), COMP (↔), AP (↔), PO (↔), BA (↓), PHAG (↔), RB (↑) | n.a. | Awad and Austin (2010) |
| | Fruits | 1%, 2% | n.a. | *O. mykiss* | 18.0 ± 0.2 | n.a. | IL-1β (↔), IL-8 (↔), TGF-β (↑) | n.a. | Awad *et al.* (2011) |

**Table 1** Continued

**Immunostimulants – oral administration**

| Single plant/plant mixture | Parts/ products | Concentrations | Exposure | Fish species | Weight (g) | Growth performance and survival | Haematological and immunological parameters | Disease resistance* | References |
|---|---|---|---|---|---|---|---|---|---|
| *Massa medicata* | Fruits | 0.5% | to satiety 12 weeks | *P. major* | 24.0 ± 0.2 | FBW (↔), SGR (↔), DFI (↔), FER (↔), CF (↔), SR (↑), HSI (↔), VSI (↔) | Hct (↔), Hb (↑), HDL-CHO (↔), AST (↓), ALT (↔), LYZ (↔), COMP (↔) | *V. anguillarum* | Ji, Takaoka *et al.* (2007b) |
| *Matricaria chamomilla* | Plant powder | 1%, 3%, 5% | 3% BW day$^{-1}$ 30 d | *C. gariepinus* | 22 | WG (↔), SGR (↔), FCR (↓), CF (↓), SR (↑), HSI (↔), SSI (↔) | Hct (↔), Hb (↔), WBC (↑), RB (↔) | n.a. | Abdelhadi *et al.* (2010) |
| *Myristica fragans* | Leaves extract (ethanol) | 0.01%, 0.02%, 0.04%, 0.08% | 5% BW day$^{-1}$ 12 weeks | *E. tauvina* | 30.0 ± 0.5 | WG (↔), SGR (↔), FCR (↔) | Lct (↔), ALB (↔), GLB (↔), LYZ (↔), BA (↔), PHAG (↔) | No effect against *V. harveyi* infection | Sivaram *et al.* (2004) |
| *Nigella sativa* | Seeds | 1%, 2.5%, 5% | 2% BW day$^{-1}$ 3 weeks | *O. mykiss* | 34.43 ± 3.56 | n.a. | Hct (↑), WBC (↔), Lct (↑), TP (↑), RB (↔), total Ig (↑) | n.a. | Dorucu *et al.* (2009) |
| *Nyctanthes arbortristis* | Seeds extract (chloroform) | 0.01%, 0.1%, 1% | 2% BW day$^{-1}$ 3 weeks | *O. mossambicus* | 30 ± 5 50 ± 5 | n.a. | LYZ (↑), COMP (↑), RB (↑), RNI (↑), MPO (↑) | *A. hydrophila* | Kirubakaran *et al.* (2010) |
| *Ocimum sanctum* | Leaves | 0.00005%, 0.0005%, 0.005%, 0.05%, 0.25% | 4 d | *O. mossambicus* | 25 | n.a. | specific Ig (↑) | *A. hydrophila* | Logambal *et al.* (2000) |
| | Leaves extract (ethanol) | 0.01%, 0.02%, 0.04%, 0.08% | 5% BW day 12 weeks | *E. tauvina* | 30.0 ± 0.5 | WG (↑), SGR (↑), FCR (↑) | Lct (↑), ALB (↔), GLB (↑), LYZ (↔), BA (↑), PHAG (↑) | *V. harveyi* | Sivaram *et al.* (2004) |
| *Origanum vulgare* | Essential oil | 0.05% | To satiety 8 weeks | *I. punctatus* | 50 | WG (↑), SGR (↑), CF (↑), FCR (↓), PER (↑), HSI (↓), VSI (↓), SR (↑) | LYZ (↑), SOD (↑), CAT (↑) | *A. hydrophila* | Zheng *et al.* (2009) |
| *Prunella vulgaris* | Plant extract (ethanol) | 0.01%, 0.1%, 1% | *ad libitum* 8 weeks | *P. olivaceus* | 28.2 ± 1.3 | n.a. | †LYZ (↑), COMP (↑), PHAG (↑), RB (↑) | *U. marinum* | Harikrishnan *et al.* (2011e) |
| *Psidium guajava* | Leaves | 25% | 5% BW day$^{-1}$ 5 d | *O. niloticus* | 10 ± 1 | n.a. | n.a. | *A. hydrophila* | Pachanawan *et al.* (2008) |
| | Leaves extract (ethanol) | 4% | | | | | | | |

**Table 1** Continued

**Immunostimulants – oral administration**

| Single plant/plant mixture | Parts/ products | Concentrations | Exposure | Fish species | Weight (g) | Growth performance and survival | Haematological and immunological parameters | Disease resistance* | References |
|---|---|---|---|---|---|---|---|---|---|
| *Rheum officinale* | Plant extract (n.a.) | 0.5%, 1%, 2%, 4% | 2-4% BW day$^{-1}$ 10 weeks | *C. carpio* | 5.39 ± 0.72 | SGR (↑), FCR (↓) | GLU (↔), COR (↓), LYZ (↑), SOD (↑), CAT (↔), MDA (↓) | *A. hydrophila* | Xie *et al.* (2008) |
| *Scutellaria baicalensis* | Plant extract (solvent n.a.) | 0.1%, 0.5%, 1% | *ad libitum* 4 weeks | *O. niloticus* | 62.8 ± 5.4 | n.a. | LYZ (↔), PHAG (↓), RB (↓) | n.a. | Yin *et al.* (2006) |
| *Stryrax japonica* | Flowers extract (ethanol) | 0.1%, 1%, 2% | 5% BW day$^{-1}$ 30 d | *E. bruneus* | 29.6 ± 1.1 | n.a. | †TP (↑), LYZ (↑), COMP (↑), AP (↑), PO (↑), BA (↑), PHAG (↑), RB (↑) | *V. harveyi U. marinum* | Harikrishnan *et al.* (2011g) |
| *Urtica dioica* | Leaves | 1% | 3% BW day$^{-1}$ 2 weeks | *O. mykiss* | 15 | n.a. | RBC (↑), Hct (↑), Hb (↔), MCV (↔), MCH (↔), MCHC (↔), WBC (↑), LYM (↔), MON (↔), NEU (↔), TRB (↔), TP (↔), LYZ (↑), COMP (↔), AP (↔), PO (↔), BA (↓), PHAG (↔), RB (↑) | n.a. | Awad and Austin (2010) |
| | Leaves | 1%, 2% | n.a. | *O. mykiss* | 18.0 ± 0.2 | n.a. | IL-1β (↑), IL-8 (↑), TGF-β (↑) | n.a. | Awad *et al.* (2011) |
| | Leaves extract (water) | 0.1%, 1% | 2% BW day$^{-1}$ 3 weeks | *O. mykiss* | 41 | SGR (↔), CF (↔) | TP (↑), PHAG (↔), RB (↔) | n.a. | Dügenci *et al.* (2003) |
| *Viscum album* | Leaves, fruits, stems extract (water) | 0.1%, 0.5%, 1% | 1% BW day$^{-1}$ 4 weeks | *A. japonica* | 200 | n.a. | LYZ (↑), PHAG (↑), RB (↑) | *A. hydrophila* | Choi *et al.* (2008) |
| | Leaves extract (water) | 0.5%, 1%, 2% | 5% BW day$^{-1}$ 30 d | *E. bruneus* | 28.7 ± 1.3 | n.a. | TP (↑), LYZ (↑), PHAG (↑), RB (↑) | *P. dicentrarchi* | Harikrishnan, Balasundaram & Heo (2011c) |
| | Leaves extract (water) | 0.1%, 1% | 2% BW day$^{-1}$ 3 weeks | *O. mykiss* | 41 | SGR (↔), CF (↔) | TP (↑), PHAG (↔), RB (↔) | n.a. | Dügenci *et al.* (2003) |
| *Withania somnifera* | Roots | 1%, 2%, 3% | 3% BW day$^{-1}$ 6 weeks | *L. rohita* | 18.1 ± 0.5 | n.a. | LYZ (↑), PHAG (↑), RB (↑), Ig (↑) | *A. hydrophila* | Sharma *et al.* (2010) |

**Table 1** Continued

**Immunostimulants – oral administration**

| Single plant/plant mixture | Parts/ products | Concentrations | Exposure | Fish species | Weight (g) | Growth performance and survival | Haematological and immunological parameters | Disease resistance* | References |
|---|---|---|---|---|---|---|---|---|---|
| | Leaves extract (methanol) | 0.01%, 0.02%, 0.04%, 0.08% | 5% BW day 12 weeks | *E. tauvina* | 30.0 ± 0.5 | WG (↑), SGR (↑), FCR (↑) | Lct (↑), ALB (↔), GLB (↑), LYZ (↔), BA (↔), PHAG (↑) | *V. harveyi* | Sivaram *et al.* (2004) |
| | Plant extract (acetone) | 1% | 5% BW $day^{-1}$ 45 d | *O. mossambicus* | 7.46 ± 0.11 | FBW (↑), SGR (↑) | Hct (↑), Lct (↑), TP (↑), ALB (↑), GLB (↑), GLU (↓), CHO (↓), TRIG (↓), Ca (↔), LYZ (↑), PHAG (↑) | *V. vulnificus* | Immanuel *et al.* (2009) |
| *Zataria multiflora* | Essential oil | 30, 60, 120 ppm | 1% BW $day^{-1}$ 8 d | *C. carpio* | 30–35 | n.a. | WBC (↑), TP (↔), ALB (↔), GLB (↔), LYZ (↔), BA (↑), specific Ig (↑) | n.a. | Soltani *et al.* (2010) |
| *Zingiber officinale* | Rhizomes | 0.05%, 0.1%, 0.5%, 1% | To satiety 2 weeks | *O. mykiss* | 14 | WG (↑), SGR (↑), FCR (↓), PER (↑) | RBC (↑), Hct (↑), Hb (↔), MCV (↔), MCH (↔), MCHC (↔), WBC (↑), LYM (↑), MON (↑), NEU (↑), TRB (↔), TP (↑), ALB (↔), GLB (↑), LYZ (↑), COMP (↓), AP (↑), BA (↓), PHAG (↑), RB (↑) | *A. hydrophila* | Nya and Austin (2009b) |
| | Leaves extract (water) | 0.1%, 1% | 2% BW $day^{-1}$ 3 weeks | *O. mykiss* | 41 | SGR (↔), CF (↔) | TP (↑), PHAG (↑), RB (↑) | n.a. | Dügenci *et al.* (2003) |
| | Plant extract (acetone) | 1% | 5% BW $day^{-1}$ 45 d | *O. mossambicus* | 7.46 ± 0.11 | FBW (↑), SGR (↑) | Hct (↑), Lct (↑), TP (↑), ALB (↑), GLB (↑), GLU (↓), CHO (↓), TRIG (↓), Ca (↔), LYZ (↑), PHAG (↑) | *V. vulnificus* | Immanuel *et al.* (2009) |

**Table 1** Continued

**Immunostimulants – oral administration**

| Single plant/plant mixture | Parts/ products | Concentrations | Exposure | Fish species | Weight (g) | Growth performance and survival | Haematological and immunological parameters | Disease resistance* | References |
|---|---|---|---|---|---|---|---|---|---|
| *Angelica sinensis* + *Astragalus membranaceus* | Roots | 1%, 1.5% | To satiety 30 d | *C. carpio* var. *Jian* | 101 ± 7.38 | FBW (↑) | LYZ (↑), COMP (↑), PHAG (↑) | n.a. | Jian and Wu (2004) |
| | Roots | 0.5%, 1%, 1.5% | To satiety 30 d | *P. crocea* | 120 ± 3.4 | n.a. | LYZ (↑), COMP (↑), PHAG (↑) | *V. alginolyticus* | Jian and Wu (2003) |
| *Chrysanthemum cinerariaefolium* + *Punica granatum* + *Zanthoxylum schinifolium* | leaves extracts (ethanol) | 0.0005%, 0.005%, 0.01% | *ad libitum* 4 weeks | *P. olivaceus* | 68.3 ± 2.9 | n.a. | LYZ (↑), COMP (↑), PHAG (↑), RB (↑) | *P. dicentrarchi* | Harikrishnan, Balasundaram, Kim *et al.* (2010d) |
| *Artemisia capillaries* + *Cnidium officinale* + *Crataegi fructus* + *Massa medicata* | Leaves/roots/fruits | 0.5% | To satiety 12 weeks | *P. major* | 24.0 ± 0.2 | FBW (↑), SGR (↑), DFI (↔), FER (↑), CF (↑), SR (↑), HSI (↔), VSI (↔) | Hct (↔), Hb (↑), HDL-CHO (↓), AST (↓), ALT (↓), LYZ (↓), COMP (↓) | *V. anguillarum* | Ji, Takaoka *et al.* (2007b) |
| *Artemisia capillaries* + *Cnidium officinale* + *Crataegi fructus* + *Massa medicata* | Plant powder | 0.1%, 0.3%, 0.5%, 1% | To satiety 8 weeks | *P. olivaceus* | 15.2 ± 0.4 | FBW (↑), WG (↑), SGR (↑), DFI (↔), FER (↑), CF (↔), SR (↑), HSI (↔), VSI (↔) | Hct (↔), Hb (↔), TP (↔), CHO (↔), TRIG (↔), HDL-CHO (↑), GLU (↔), AST (↓), ALT (↔) | n.a. | Ji, Jeong *et al.* (2007a) |

**Table 1** Continued

**Immunostimulants – oral administration**

| Single plant/plant mixture | Parts/ products | Concentrations | Exposure | Fish species | Weight (g) | Growth performance and survival | Haematological and immunological parameters | Disease resistance* | References |
|---|---|---|---|---|---|---|---|---|---|
| *Cinnamomum zeylanicum* + *Juglans regia* + *Mentha piperita* + *Ocimum basilicum* | Barks/leaves extracts (ethanol) | 0.025%, 0.05%, 0.075%, 0.1%, 0.125% | 3% BW day$^{-1}$ 45 d | *C. carpio* | n.a. | n.a. | RBC (↑), Hb (↑), WBC (↑), TP (↑), ALB (↑), GLB (↑), GLU (↓), LYZ (↑), BA (↑), RB (↑) | *A. hydrophila* | Abasali and Mohamad (2010) |
| *Brussica nigra* + *Chelidoniume majus* + *Echinacea purpurea* + *Inula helenium* + *Tussilago farfara* | Whole plants/seeds/ roots/rhizomes/flowers extracts (ethanol) | 0.1%, 0.25%, 0.5%, 0.75%, 1% | 3% BW day$^{-1}$ 60 d | *C. carpio* | n.a. | n.a. | RBC (↑), Hb (↑), WBC (↑), TP (↑), ALB (↑), GLB (↑), GLU (↓), LYZ (↑), BA (↑), RB (↑) | *A. hydrophila* | Mohamad and Abasali (2010) |
| *Cynodon dactylon* + *Piper longum* + *Phyllanthus niruri* + *Tridax procumbens* + *Zingiber officinale* | Whole plants/seeds/ rhizomes extracts (petroleum ether) | 0.01%, 0.02%, 0.04%, 0.08% | 5% BW day$^{-1}$ 60 d | *E. tauvina* | 20 ± 2 | SGR (↑) | ALB/GLB (↑), BA (↑), PHAG (↑) | *V. harveyi* | Punitha *et al.* (2008) |

ACP, acid phosphatase; ALB, albumin; ALP, alkaline phosphatase; ALT, alaninaminotransferase; AP, antiprotease activity; APC, antibody producing cells; AST, aspartate aminotransferase; BA, serum bactericidal activity; BAS, basophils; BW, body weight; Ca, calcium; CAT, catalase activity; CF, condition factor; CHO, cholesterol; COMP, complement activity; COR, cortisol; d, days; DFI, daily feed intake; EOS, eosinophils; ER, energy retention; FBW, final body weight; FCR, feed conversion ratio; Fe, iron; FER, feed efficiency ratio; FR, fat retention; GLB, globulins; GLU, glucose; Hb, haemoglobin concentration; Hct, haematocrit value; HDL-CHO, high density lipoprotein cholesterol; HSI, hepatosomatic index; Ig, immunoglobulins; IL-1β, interleukin 1β; IL-8, interleukin 8; K, potassium; Lct, leucocrit value; LYM, lymphocytes; LYMK, lymphokines; LYZ, lysozyme activity; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MDA, hepatic malondialdehyde content; Mg, magnesium; MMC, spleen melanomacrophage centres; MON, monocytes; MPO, myeloperoxidase of leucocytes; NEU, neutrophils; PHAG, phagocytosis; PER, protein efficiency ratio; PO, serum peroxidase activity; PPV, protein productive value; RBC, red blood cell count; RB, respiratory burst activity; RNI, nitrogen reactive intermediates; SGR, specific growth rate; SOD, superoxide dismutase activity; SR, survival rate; SSI, spleno/somatic index; TGF-β, transforming growth factor-β; TL, total lipids; TNF-α, tumour necrosis factor α; TRB, trombocytes; TRIG, triglycerides; TP, total proteins; VSI, viscerosomatic index; WBC, white blood cell count; WG, weight gain; wk, weeks.

Variation in treated fish compared to controls: ↑, significant increase; ↓, significant decrease; ↔, no significant changes.

n.a., not available.

*Evaluated in terms of cumulative mortality reduction after challenge with a pathogen.

†Evaluation performed on samples collected after the challenge.

**Table 2** Impact of plant-derived immunostimulants used by injection on growth, survival, haematological profile, immune response and disease resistance of cultured fish

| Immunostimulants – intraperitoneal administration<br>Single plant/plant mixture | Parts/products | Concentrations | Exposure | Fish species | Weight (g) | Growth performance and survival | Haematological and immunological parameters | Disease resistance* | References |
|---|---|---|---|---|---|---|---|---|---|
| *Ocimum sanctum* | Leaves extract (water) | 0.0001%, 0.01%, 1% | 200 μL fish$^{-1}$ | *O. mossambicus* | 25 | n.a. | RB (↑), specific Ig (↑) | *A. hydrophila* | Logambal *et al.* (2000) |
| *Solanum trilobatum* | Leaves extract (water, hexane) | 4, 40, 400 mg kg$^{-1}$ BW | 200 μL fish$^{-1}$ | *O. mossambicus* | 25 ± 5 | n.a. | LYZ (↑), RB (↑), RNI (↑) | *A. hydrophila* | Divyagnaneswari *et al.* (2007) |
| | Leaves extract (water, hexane) | 4, 6.4, 32, 40, 160, 400, 800 mg kg$^{-1}$ BW | 200 μL fish$^{-1}$ | *O. mossambicus* | 25 ± 5 | n.a. | LYZ (↑), specific Ig (↑) | *A. hydrophila* | Divyagnaneswari *et al.* (2008) |
| *Tinospora cordifolia* | Leaves extract (water) | 6, 60, 600 mg kg$^{-1}$ BW | 200 μL fish$^{-1}$ | *O. mossambicus* | 25 ± 5 | n.a. | LYZ (↑), COMP (↑), AP (↑), RB (↑), RNI (↑), MPO (↑) | *A. hydrophila* | Alexander *et al.* (2010) |
| | Leaves extract (ethanol, petroleum ether) | 0.8, 8, 80 mg kg$^{-1}$ BW | 200 μL fish$^{-1}$ | *O. mossambicus* | 25 – 30 | n.a. | RB (↑), specific Ig (↑) | *A. hydrophila* | Sudhakaran *et al.* (2006) |
| *Toona sinensis* | Leaves extract (water) | 0.2%, 0.4% | 10 μL fish$^{-1}$ | *O. mossambicus* | 10.7 ± 2.5 | n.a. | LYZ (↔), PHAG (↑), RB (↑), Ig (↔) | *A. hydrophila* | Wu *et al.* (2010) |
| *Azadirachta indica* + *Curcuma longa* + *Ocimum sanctum* | Leaves extracts (water, ethanol, methanol) | 5, 50, 100 mg kg$^{-1}$ BW | 50 μL fish$^{-1}$ | *C. aurata* | 23 ± 2 | n.a. | LYZ (↑), COMP (↑), PHAG (↑), RB (↑) | *A. hydrophila* | Harikrishnan, Balasundaram, Kim *et al.* (2009b) |
| *Chrysanthemum cinerariaefolium* + *Punica granatum* + *Zanthoxylum schinifolium* | Leaves extracts (water, ethanol, methanol) | 5, 50, 100 mg kg$^{-1}$ BW | 50 μL fish$^{-1}$ | *P. olivaceus* | 63.2 ± 2.4 | n.a. | LYZ (↑), COMP (↑), PHAG (↑), RB (↑) | *U. marinum* | Harikrishnan, Heo, Balasundaram *et al.* (2010f) |

AP, antiprotease activity; COMP, complement activity; Ig, immunoglobulins; LYZ, lysozyme activity; MPO, myeloperoxidase of leucocytes; PHAG, phagocytosis; RB, respiratory burst activity; RNI, nitrogen reactive intermediates.

Variation in treated fish compared to controls: ↑, significant increase; ↓, significant decrease; ↔, no significant changes.

n.a., not available.

*Evaluated in terms of cumulative mortality reduction after challenge with a pathogen.



 

**Table 3** Effect of plant-derived medicines used *per os* in the therapy of fish infectious diseases

**Medicines – oral administration**

| Single plant/plant mixture | Parts/ products | Concentrations | Exposure | Fish species | Weight (g) | Pathogen | Growth performance and survival | Haematological and immunological parameters | Disease resistance | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Allium sativum* | Plant extract (solvent n.a.) | 0.1%, 0.4%, 0.8% | 45 d | *O. niloticus* | 100 ± 20 | *Trichodina* sp. *A. hydrophila* | n.a. | n.a. | Reduction of parasite number and fish mortality | Omima (2010) |
| *Azadirachta indica* | Leaves extract (ethanol) | 0.2% | 3% BW day$^{-1}$ 4 weeks | *C. mrigala* | 63 ± 2 | *A. invadans* | n.a. | *RBC (↔), Hct (↔), Hb (↔), WBC (↔), LYM (↔), MON (↔), NEU (↔), EOS (↔), TP (↔), GLU (↔), CHO (↔), Ca (↔) | n.a. | Harikrishnan, Balasundaram & Heo (2010c) |
| *Origanum minutiflorum* | Essential oil | 8 mL 5 kg$^{-1}$ | 30 d | *D. puntazzo* | 1.5 | *Myxobolus* sp. | WG (↔) | LYMP (↑), LYZ (↓), PHAG (↔), RNI (↔) | Reduction of infection prevalence and intensity | Karagouni *et al.* (2005) |
| *Rosmarinus officinalis* | Leaves powder, leaves extract (ethyl acetate) | 4%, 6% | 2% BW day$^{-1}$ 15 d | *Oreochromis* sp. | 7.5 ± 1.0 | *S. inieae* | n.a. | n.a. | Reduction of fish mortality | Abutbul *et al.* (2004) |
| | Leaves | 4%, 8%, 16% | 14–20 d | *Oreochromis* sp. | 5.5 ± 0.5 7.0 ± 1.0 44.0 ± 19.0 | *S. agalactiae S. inieae* | n.a. | n.a. | Reduction of fish mortality | Zilberg *et al.* (2010) |
| *Azadirachta indica* + *Curcuma longa* + *Ocimum sanctum* | Leaves extract (water) | 0.01%, 0.02%, 0.04%, 0.08% | to satiety 4 weeks | *C. aurata* | 47 ± 3 | *A. hydrophila* | n.a. | *RBC (↓), Hct (↓), Hb (↔), MCH (↓), WBC (↑), TP (↓), GLU (↓), CHO (↓), TRIG (↓), LYZ (↑), PHAG (↑), RB (↑) | n.a. | Harikrishn *et al.* (2010a) |
| | Leaves extract (ethanol) | 0.25% | 3% BW day$^{-1}$ 4 weeks | *C. aurata* | 23 ± 2 | *A. hydrophila* | n.a. | *LYZ (↑), COMP (↑), PHAG (↑), RB (↑) | Reduction of fish mortality | Harikrishnan *et al.* (2009a) |
| | Leaves extract (water) | 0.1% | 2% BW day$^{-1}$ 4 weeks | *C. carpio* | 52 ± 2 | *A. hydrophila* | n.a. | *RBC (↓), Hct (↓), Hb (↓), MCV (↑), MCH (↑), MCHC (↓), WBC (↑), TP (↓), GLU (↓), CHO (↓), COMP (↔), RB (↑) | Reduction of fish mortality | Harikrishnan *et al.* (2010b) |

Ca, calcium; CHO, cholesterol; COMP, complement activity; d, days; EOS, eosinophils; GLU, glucose; Hb, haemoglobin concentration; Hct, haematocrit value; LYM, lymphocytes; LYZ, lysozyme activity; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MON, monocytes; NEU, neutrophils; PHAG, phagocytosis; RBC, red blood cell count; RB, respiratory burst activity; RNI, nitrogen reactive intermediates; TRIG, triglycerides; TP, total proteins; WBC, white blood cell count; WG, weight gain; wk, weeks.
Variation in infected and treated fish compared to infected untreated controls: ↑, significant increase; ↓, significant decrease; ↔, no significant changes.
*Statistical differences between infected and treated fish and uninfected untreated controls.
n.a., not available.

**Table 4** Effect of plant-derived medicines used by injection in the therapy of fish infectious diseases

| | | | Medicines – intraperitoneal administration | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Single plant/plant mixture | Parts/ products | Concentrations | Exposure | Fish species | Weight (g) | Pathogen | Growth performance and survival | Haematological and immunological parameters | Disease resistance | References |
| *Allium sativum* | Plant extract (water) | 1% | 500 μL fish$^{-1}$ biweekly 3 months | *D. labrax* | 156.9 ± 37.4 | *M. marinum* | n.a. | specific Ig (↑) | No effect | Colorni *et al.* (1998) |
| *Punica granatum* | Leaves extract, (water, ethanol, methanol) | 5, 50, 100 mg kg$^{-1}$ BW | 50 μL fish$^{-1}$ | *P. olivaceus* | 547 ± 14.5 | lymphocystis virus | n.a. | LYZ (↑), COMP (↑), PHAG (↑), RB (↑) | Reduction of fish mortality | Harikrishnan, Heo, Balasundaram *et al.* (2010e) |

COMP, complement activity; Ig, immunoglobulins; LYZ, lysozyme activity; PHAG, phagocytosis; RB, respiratory burst activity.
Variation in infected and treated fish compared to uninfected untreated controls: ↑, significant increase; ↓, significant decrease; ↔, no significant changes.
n.a., not available.

*sinensis*, *Artemisia capillaries*, *Astragalus membranaceus*, *Azadirachta indica*, *Cnidium officinale*, *Crataegi fructus*, *Cynodon dactylon*, *Echinacea purpurea*, *Eclipta alba*, *Lonicera japonica*, *Massa medicata*, *Nyctanthes arbortristis*, *Punica granatum*, *Scutellaria baicalensis*, *Solanum nigrum*, *Solanum trilobatum*, *Tinospora cordifolia*, *Toona sinensis*, *Whitania somnifera* and *Zataria multiflora*. Nevertheless, other plants which are used all over the world for both curative and culinary purposes such as garlic (*Allium sativum*), garlic chives (*Allium tuberosum*), green tea (*Camellia sinensis*), cinnamom (*Cinnamomum verum* or *zeylanicum*), turmeric (*Curcuma longa*), Sundial lupine (*Lupinus perennis*), mango (*Mangifera indica*), peppermint (*Mentha piperita*), nutmeg (*Myristica fragrans*), basil (*Ocimum basilicum* and *sanctum*), oregano (*Origanum vulgare*), rhubarb (*Rheum officinale*), rosemary (*Rosmarinus officinalis*) and ginger (*Zingiber officinale*) have been also screened. The herbal remedies have been generally administered as plant materials (seeds, bulbs, leaves) or plant-derived products, including extracts obtained using a range of extraction procedures and different aqueous or organic solvents (ethanol, methanol, ethyl acetate, hexane, butane, acetone, benzene, petroleum ether, etc.), or other preparations such as essential oils, concoctions and decoctions.

## Fish species under investigation

The fish species widely reared in Asian aquaculture have been the most studied ones. In fact, 29.9% of the research has been conducted in tilapias (*Oreochromis mossambicus* and *niloticus*), 25.2% in carps (*Cyprinus carpio, Labeo rohita* and *Catla catla*), 7.5% in the goldfish (*Carassius auratus*), 7.5% in groupers (*Epinephelus tauvina* and *bruneus*), 5.6% in the olive flounder (*Paralichthys olivaceus*). The remaining studies dealt with other Asian fish species such as yellow croaker (*Pseudosciaena crocea*), rockfish (*Sebastes schlegeli*), Japanese eel (*Anguilla japonica*), rock bream (*Oplegnathus fasciatus*), spotted snakehead (*Channa punctatus*). Among fish species that are cultured in western countries, the rainbow trout (*Oncorhynchus mykiss*) has been the most investigated one with 10.3% of references. Other studies have been performed on the channel catfish (*Ictalurus punctatus*), sharpsnout sea bream (*Diplodus puntazzo*), red sea bream (*Pagrus major*), sea bass (*Dicentrarchus labrax*) have been little studied, while no data exist on gilthead sea bream (*Sparus aurata*), turbot (*Scophthalmus*


 

**Table 5** Effect of plant-derived medicines used by immersion in the therapy of fish infectious diseases

| Medicines – immersion administration | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Single plant/plant mixture** | **Parts/products** | **Concentrations** | **Exposure** | **Fish species** | **Weight (g)** | **Pathogen** | **Growth performance and survival** | **Haematological and immunological parameters** | **Disease resistance** | **References** |
| *Allium sativum* | Plant extract (solvent n.a.) | 800 ppm | 96 h | *O. niloticus* | 3.62 ± 0.06 | *Trichodina* sp. | n.a. | n.a. | Reduction of parasite number | Chitmanat *et al.* (2005) |
| *Artemisia annua* | Leaves extract (ethanol) | 0.005%, 0.01%, 0.015%, 0.02% | 30–180 min | *H. longifilis* | n.a. | Monogenean parasites | n.a. | n.a. | Reduction of parasite number | Ekanem and Brisibe (2010) |
| *Azadirachta indica* | Leaves extract (water) | 1% | 5 min day$^{-1}$ 24 d | *C. carpio* | 40 ± 10 | *A. invadans* | n.a. | *RBC (↔), Hct (↓), Hb (↓), WBC (↑), LYM (↓), MON (↔), NEU (↓), EOS (↔) | Lesions recovery | Harikrishnan *et al.* (2005) |
| *Camellia sinensis* | Leaves extract (solvent n.a.) | 0.03% 0.3%, 0.6%, 0.9% | 30, 60 min 1–10 min | *O. keta* | 0.5–0.6 | *I. necator* | n.a. | n.a. | Reduction of parasite number | Sutzuki, Misaka and Sakai (2006) |
| | | 0.03% 0.6%, 0.9% | 30, 60 min 1–5 min | *O. masou* | 0.2 | *I. necator* | n.a. | n.a. | Reduction of parasite number | Sutzuki *et al.* (2006) |
| | | 0.9% | 5 min | *O. niloticus* | 0.2–0.9 | *Trichodina* sp. | n.a. | n.a. | Reduction of parasite number and fish mortality | Noor El-Deen (2010) |
| | | 0.05% | 15 min | | | | | | | |
| *Carica papaya* | Seeds extract (petroleum ether) | 0.02%, 0.025% | 96 h | *C. auratus* | n.a. | *I. multifilis* | n.a. | n.a. | Reduction of parasite number and fish mortality | Ekanem *et al.* (2004a) |
| *Centella asiatica* | Plant extract (water) | 0.02%, 0.04%, 0.06%, 0.08% | n.a. | *O. niloticus* | 10 ± 1 | *F. columnare* | n.a. | n.a. | Reduction of fish mortality | Rattanachaikunsopon and Phumkhachorn (2010b) |

**Table 5** Continued

**Medicines – immersion administration**

| Single plant/plant mixture | Parts/products | Concentrations | Exposure | Fish species | Weight (g) | Pathogen | Growth performance and survival | Haematological and immunological parameters | Disease resistance | References |
|---|---|---|---|---|---|---|---|---|---|---|
| *Macuna pruriens* | Leaves extract (methanol) | 0.01%, 0.015%, 0.02% | 72 h | *C. auratus* | n.a. | *I. multifilis* | n.a. | n.a. | Reduction of parasite number and fish mortality | Ekanem *et al.* (2004a) |
| *Piper guineense* | Seeds extract (methanol) | 0.00005%, 0.0001%, 0.00015%, 0.0002% | 96 h | *C. auratus* | n.a. | *G. elegans* *D. extensus* | n.a. | n.a. | Reduction of parasite number and fish mortality | Ekanem *et al.* (2004b) |
| *Solanum nigrum* | Leaves extract (ethyl acetate) | 1% | 10 min day$^{-1}$ | *C. punctatus* | 25 ± 3 | *A. hydrophila* | n.a. | RBC (↑), Hb (↑), MCV (↑), WBC (↓) | Lesions recovery | Rajendiran *et al.* (2008) |
| *Terminalia catappa* | Plant extract (solvent n.a.) | 800 ppm | 96 h | *O. niloticus* | 3.62 ± 0.06 | *Trichodina* sp. | n.a. | n.a. | Reduction of parasite number | Chitmanat *et al.* (2005) |
| *Azadirachta indica* + *Curcuma longa* + *Ocimum sanctum* | Leaves extract (water) | 1% | 5 min day$^{-1}$, two times | *C. aurata* | 10 ± 2 | *A. hydrophila* | n.a. | *RBC (↔), Hct (↑), Hb (↑), MCV (↑), MCH (↑), MCHC (↑), WBC (↓) | n.a. | Harikrishnan and Balasundaram (2008) |
| | Leaves extract (water) | 1% | 5 min day$^{-1}$, two times | *C. aurata* | 15 ± 2 | *A. hydrophila* | n.a. | n.a. | Lesions recovery | Harikrishnan, Balasundaram, Moon *et al.* (2009c), Harikrishnan, Moon, Kim *et al.* (2010g) |

EOS, eosinophils; Hb, haemoglobin concentration; Hct, haematocrit value; LYM, lymphocytes; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MON, monocytes; NEU, neutrophils; RBC, red blood cell count; WBC, white blood cell count.

n.a., not available.

Variation in infected and treated fish compared to uninfected untreated controls: ↑, significant increase; ↓, significant decrease; ↔, no significant changes.

*Statistical differences between infected and treated fish and uninfected untreated controls.

*maximus*), solea (*Solea* spp.) and Atlantic salmon (*Salmo salar*) (Fig. 2).

## Route of plant administration and dosage

Medicinal plants have been applied almost exclusively via oral administration as immunostimulants and less frequently via injection or immersion for preventive purposes or disease treatment.

Oral delivery, although it is the least effective method for immunostimulants administration, as the product is slowly absorbed by the fish (Harikrishnan, Balasundaram, Kim, Kim, Han & Heo 2009b), is regarded as the most suitable method for fish farming. It is non-stressful for fish and allows a large number of subjects to be treated with the minimum cost and effort (Sakai 1999). Different strategies have been used for the oral administration of plants and plant-derived products in fish (Tables 1 and 3). Very frequently plant extracts/plant parts have been included as ingredients of the diet (pellet/extruded feed) at doses ranging from 0.1 to 2% and fish were fed for a period ranging from 2 to 5 weeks, according to a dietary regime (% BW day$^{-1}$) calibrated for fish species and size (Dügenci, Arda & Candan 2003; Vasudeva, Romesh, Singh & Chakrabarti 2004; Vasudeva & Chakrabarti 2004, 2005a,b; Vasudeva, Das, Jyotyrmayee & Chakrabarti 2006; Christybapita, Divyagnaneswari & Michael 2007; Choi, Park, Yoon, Kim, Jang & Choe 2008; Awad & Austin 2010; Harikrishnan, Balasundaram & Heo 2010b; Kirubakaran, Alexander & Michael 2010; Rattanachaikunsopon & Phumkhachorn 2010a,c; Awad, Mitchell & &Austin 2011 Harikrishnan, Kim, Kim, Balasundaram & Heo 2011d,f,g). In other experiments similar percentages of herbs have been included in the diet but fish were fed until satiation or for a longer time period (6–12 weeks) (Kim, Hwang & Bai 1999; Jian & Wu 2003, 2004; Shalaby, Khattab & Abdel Rahman 2006; Yin, Jeney, Racz, Xu, Jun & Jeney 2006; Ji, Takaoka, Jeong, Lee, Ishimaru, Seoka & Takii 2007b; Ji, Jeong *et al.* 2007a; Sahu, Das, Mishra, Pradhan & Sarangi 2007a; Sahu, Das, Pradhan, Mohapatra, Mishra & Sarangi 2007b; Xie, Liu, Zhou, Su, He, Pan, Ge & Xu 2008; Immanuel, Uma, Iyapparaj, Citarasu, Punitha, Michael & Palavesam 2009; Nya & Austin 2009a,b, 2011; Yin, Ardò, Thompson, Adams, Jeney & Jeney 2009; Aly & Mohamed 2010; Bilen & Bulut 2010; Mohamad & Abasali 2010; Sharma, Deo, Riteshkumar, Chanu & Das 2010; Bilen, Bulut & Bilen 2011; Harikrishnan, Balasundaram & Heo 2011b). Otherwise fish were fed diets supplemented with smaller percentages (0.002–0.08%) of plants (Sivaram, Babu, Immanuel, Murugadass, Citarasu & Marian 2004; Aly, Atti & Mohamed 2008a; Aly, Mohamed & John 2008b; Goda 2008; Rattanachaikunsopon & Phumkhachorn 2009a; Zheng, Tan, Liu, Zhou, Xiang & Wang 2009; Harikrishnan, Balasundaram & Heo 2010a; Sheikhzadeh, Nofouzi, Delazar & Oushani 2011). Occasionally diets supplemented with higher doses (between 4% and 16%) of plants have been used (Abutbul, Golan-Goldhirsh, Barazani & Zilberg 2004; Shalaby *et al.* 2006; Xie *et al.* 2008; Dorucu, Ozesen, Ispir, Altinterim & Celayir 2009; Sharma *et al.* 2010; Zilberg, Tal, Froyman, Abutbul, Dudai & Golan-Goldhirsh 2010).

When plant administration has been performed by intraperitoneal injection (Tables 2 and 4), fish were treated with low doses of extracts, between

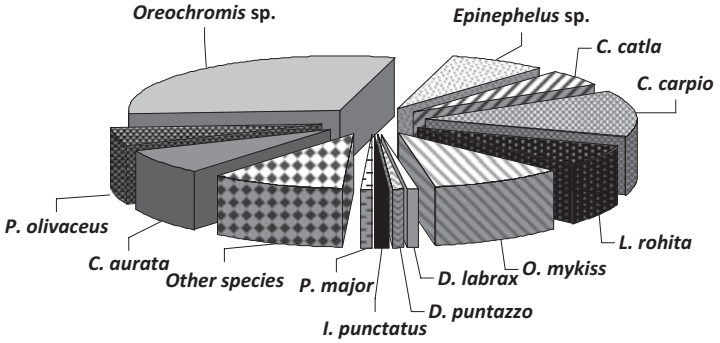


**Figure 2** Fish species treated with plant-derived products. Reference publications = 105.

0.8 and 800 mg $kg^{-1}$ of BW (Sudhakaran, Sirekha, Devasree, Premsingh & Michael 2006; Divyagnaneswari, Christybapita & Michael 2007, 2008; Harikrishnan, Balasundaram, Kim *et al.* 2009b; Alexander, Kirubakaran & Michael 2010; Harikrishnan, Heo, Balasundaram, Kim, Kim, Han & Heo 2010e; Harikrishnan, Heo, Balasundaram, Kim, Kim, Han & Heo 2010f). Intraperitoneal injection has been proved to be the most effective way of administration because enables the immunostimulant to be quickly absorbed and functional (Harikrishnan, Balasundaram, Kim *et al.* 2009b), but it is stressful, labour intensive, relatively time-consuming and becomes impractical when the fish weigh is less than 10–15 g (Sakai 1999; Galindo-Villegas & Hosokawa 2004).

The administration of plant extracts to fish by immersion (Table 5) has been tested in the treatment of diseases as alternative to traditional drugs and the protocols adopted included single or repeated treatments (Harikrishnan, Rani & Balasundaram 2003; Harikrishnan, Balasundaram & Bhuvaneswari 2005; Harikrishnan & Balasundaram 2008; Rajendiran, Natarjan & Subramanian 2008; Harikrishnan, Balasundaram, Moon, Kim, Kim & Heo 2009c; Harikrishnan, Moon, Kim, Kim & Heo 2010g; Rattanachaikunsopon & Phumkhachorn 2010b). Dip treatment is logistically more practical for a large number of small fish (weighing less than 5 g) but dilution, exposure time and levels of efficacy are usually not well defined for the majority of immunostimulants (Sakai 1999; Galindo-Villegas & Hosokawa 2004).

## Effects on fish growth and survival

In aquaculture, various growth-promoting additives are commonly added to the diets to improve the nutrient utilization, growth performance and survival of cultured fish. Dietary supplements include probiotics, yeast, amino acids, antioxidants, carnitine, colourants, enzymes, lipid derivatives, nutraceutics, vitamins, hormones, aromatic compounds, plant extracts and certain organic acids/salts (Goda 2008). The beneficial effects of the inclusion of plants or their derived products in fish feed are well documented (Table 1). Diets enriched with *A. sativum* have been reported to significantly increase survival (Aly & Mohamed 2010), weight gain (WG), specific growth rate (SGR), feed efficiency ratio (FER) and decrease the feed conversion ratio (FCR) in Nile tilapia (Shalaby *et al.* 2006; Aly *et al.* 2008a; Aly & Mohamed 2010). Similar effects have been observed in this species fed diets including extract of ginseng (Goda 2008) or *E. purpurea* (Aly *et al.* 2008b; Aly & Mohamed 2010). Tilapia *O. mossambicus* fed a diet supplemented with acetone extract of *A. marmelos, C. dactylon, W. somnifera* or *Z. officinale* showed increased SGR (Immanuel *et al.* 2009). Ji, Takaoka *et al.* (2007b) found increased FBW and survival rates in red sea bream fed diet supplemented with *A. capillaries, C. officinale* or *C. fructus*. An improvement of the growth parameters WG, SGR and FCR was observed in rainbow trout fed diets containing *A. sativum* (Nya & Austin 2009a) or *Z. officinale* (Nya & Austin 2009b). Catfish fed a diet including *O. vulgare* essential oil showed reduced FCR and elevated WG, SGR and survival (Zheng *et al.* 2009). The oral administration of *A. aspera* (Vasudeva *et al.* 2006) increased the SGR value and decreased the FCR value in *L. rohita* and similar results have been reported in *C. carpio* fed *Rheum officinale* enriched diets (Xie *et al.* 2008). Moreover, the survival in *L. rohita* was improved by feeding diets with *C. longa* (Sahu, Das, Mishra, Pradhan, Samal & Sarangi 2008). A mixture of *A. sinensis* and *A. membranaceus* in the diet increased the FBW value in *C. carpio* var. *Jian* (Jian & Wu 2004). In greasy groupers, the dietary incorporation of *O. sanctum* or *W. somnifera* extract induced a significant increase in both WG and SGR values (Sivaram *et al.* 2004). In the same fish species, increased SGR value was also observed after feeding a mixture of *C. dactylon, Piper longum, Phyllanthus niruri, Tridax procumbens* and *Z. officinale* extracts (Punitha, Babu, Sivaram, Shankar, Dhas, Mahesh, Immanuel & Citarasu 2008). Diets containing *A. capillaries, C. officinale, C. fructus* or *M. medicata* increased survival rates, FBW, WG and SGR in olive flounder juveniles (Ji, Jeong *et al.* 2007a).

Some authors suggest that a dietary supplementation with plants could improve lipid metabolism and modulate the activities of trypsin-like enzymes during digestive processes, resulting in an efficient protein deposition and growth performance. Furthermore, it has been reported that alcohol extracts of herbs might inhibit the colonization and proliferation of pathogenic bacteria in fish digestive tract and preferentially maintain the intestinal bacterial flora, improving consequently the digestibility of feeds and subsequent nutrient absorption (Ji, Jeong *et al.* 2007a; Aly *et al.*

  

2008a; Goda 2008; Xie *et al.* 2008; Aly & Mohamed 2010). In support of these hypotheses there is some evidence of an increase in the protein efficiency ratio (PER), protein productive value (PPV) or energy retention (ER) in different fish species fed herbs supplemented diets (Shalaby *et al.* 2006; Goda 2008; Nya & Austin 2009a,b).

## Effects on fish haematological profile

Although the reference values for haematological and biochemical blood parameters of fish species have not been fully defined and the correlation between changes in these parameters and the occurrence of specific diseases or metabolic disorders has not been well characterized, haematological analysis may prove to be an important tool to diagnose diseases and to monitor the fish physiological status in response to therapeutic and dietary treatments, environmental changes and stress (Shalaby *et al.* 2006).

### Haematological indices

The red blood cell count (RBC), haemoglobin concentration (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) are haematological indices, which indicate the erythrocyte status and oxygen-carrying capability in fish (Houston 1997). The amount of oxygen received by tissues depends on the maturity of the erythrocytes and their haemoglobin content. Therefore, these parameters can be helpful in detecting any abnormal changes in fish health conditions, for example during the use of immunostimulants (Goda 2008). The leucocrit (Lct) and white blood cell count (WBC) are indicators of the leucocyte percentages in the blood. A sharp increase in the total number of leucocytes is known to be correlated with the activation of a protective response against infectious agents and chemicals (Harikrishnan *et al.* 2010a). Several authors have observed positive changes in these cell parameters following the administration of plants to fish (Tables 1, 3 and 5), probably as a result of the stimulation of erythropoiesis and leucopoiesis by plant active compounds. Nya and Austin (2009a, 2011) reported that the dietary administration of garlic in rainbow trout enhanced the maturation and haemoglobin content of erythrocytes as well as the number of total leucocytes and lymphocytes, leading to an improvement in fish health status and immune competence. Similarly, diets with *L. perennis*, *M. indica* or *Urtica dioica* increased the RBC, Hct and WBC values (Awad & Austin 2010), with *Z. officinale* elevated RBC, Hct, WBC, lymphocytes, monocytes and neutrophils (Nya & Austin 2009b), with *Nigella sativa* increased Lct and Hct (Dorucu *et al.* 2009). Diets supplemented with garlic (Sahu, Das, Mishra *et al.* 2007a), mango (Sahu, Das, Pradhan *et al.* 2007b) or *C. dactylon* extract (Kaleeswaran, Ilavenil & Ravikumar 2010) significantly increased the blood indices RBC, Hb and WBC in Indian major carp. Similar increases in RBC and WBC values in this fish species were described by Sahu *et al.* (2008) after the administration of turmeric. Moreover, common carps fed diets containing a mixture of *Brussica nigra*, *Chelidonium majus*, *E. purpurea*, *Inula helenium* and *Tussilago farfara* extracts (Mohamad & Abasali 2010), a mixture of *C. zeylanicum*, *Juglans regia*, *M. piperita* and *O. basilicum* extracts (Abasali & Mohamad 2010), *A. marmelos* extract (Pratheepa, Ramesh & Sukumaran 2010) or *Euphorbia hirta* extract (Pratheepa & Sukumaran 2011) had significantly higher haemoglobin content, RBC and WBC indices. Common carps fed diets with *Z. multiflora* essential oil (Soltani, Sheikhzadeh, Ebrahimzadeh-Mousavi & Zargar 2010) showed higher WBC. The dietary administration of garlic (Shalaby *et al.* 2006; Aly *et al.* 2008a; Aly & Mohamed 2010) and ginseng root extract (Goda 2008) in Nile tilapia induced significant increases in RBC, Hb and Hct, although these plants did not seem to have an evident positive effect on WBC. On the other hand, the extract of *E. purpurea* increased Hct as well as WBC, lymphocytes and eosinophils counts (Aly *et al.* 2008b; Aly & Mohamed 2010). Tilapia *O. mossambicus* fed diets enriched with acetone extract of *A. marmelos*, *C. dactylon*, *W. somnifera* or *Z. officinale* had higher Hct and Lct values (Immanuel *et al.* 2009). Higher Hb concentration was observed in greasy grouper fed diets containing *O. sanctum* extract or *W. somnifera* extract (Sivaram *et al.* 2004), kelp grouper fed diets including *E. japonica* extract (Kim, Harikrishnan, Kim, Jang, Kim, Hong, Balasundaram & Heo 2011) or *Kalopanax pictus* extract (Harikrishnan, Kim, Kim, Balasundaram & Heo 2011f), red sea bream fed a diet supplemented with *A. capillaries*, *C. officinale*, *C. fructus* or *M. medicata* (Ji, Takaoka *et al.* 2007b). Interestingly, the use of

traditional herbal medicines has been reported to restore the altered haematological parameters to nearly normal values and to heal lesions caused by *Aeromonas hydrophila* and *Aphanomyces invadans* in *C. carpio* and *C. auratus* (Harikrishnan *et al.* 2003, 2005; Harikrishnan *et al.* 2010a; Harikrishnan & Balasundaram 2008; Harikrishnan *et al.* 2010b).

### Biochemical parameters

Serum/plasma proteins include albumin, globulins and various humoral elements of the non-specific immune system, such as transferrin, precipitins, agglutinins, antimicrobial peptides, complement factors, lysozyme and antiproteases (Ellis 1999; Magnadottir 2006). Albumin is essential for maintaining the osmotic pressure needed for the proper distribution of body fluids and acts as a plasma carrier or non-specific ligand with many binding domains (Nya & Austin 2009a). Serum globulins, such as gamma globulins, are the source of immunoglobulins, so their level in blood reflects the concentration of antibodies and consequently the immune status of fish (Goda 2008). Certain herbal medicines have been reported to increase serum total proteins, albumin and globulin (Table 1), suggesting a stimulation of humoral immune response and the improvement in health status. In Indian major carp fed diets enriched with *A. sativum* (Sahu, Das, Mishra *et al.* 2007a), *C. longa* (Sahu *et al.* 2008), *C. dactylon* extract (Kaleeswaran *et al.* 2010) or *M. indica* (Sahu, Das, Pradhan *et al.* 2007b) significant increases in serum total proteins, albumin and globulins were noted. Higher levels of albumin (Vasudeva *et al.* 2006) and globulins (Vasudeva & Chakrabarti 2004, 2005a,b; Vasudeva *et al.* 2004) were found in carps fed diets containing *A. aspera*. Positive variations in total proteins, albumin and globulins were observed in common carp fed diets supplemented with a mixture of ethanolic extracts from *B. nigra*, *C. majus*, *E. purpurea*, *I. helenium* and *T. farfara* (Mohamad & Abasali 2010) or *C. zeylanicum*, *J. regia*, *M. piperita* and *O. basilicum* (Abasali & Mohamad 2010). Goda (2008) reported an increase in these parameters in *O. niloticus* fed diets including ginseng root extract while Shalaby *et al.* (2006) described only an increase in proteins after feeding diets with *A. sativum*. Total proteins, albumin and globulins were elevated in *O. mossambicus* after the dietary administration of *C. dactylon*, *W. somnifera* or *Z. officinale* acetone extract (Immanuel *et al.* 2009). Higher serum proteins level was reported in rainbow trout fed diets supplemented with *Cotinus coggyria* (Bilen *et al.* 2011), *M. indica* (Awad & Austin 2010) or *N. sativa* (Dorucu *et al.* 2009) as well as with aqueous extract of *U. dioica* or *Viscum album* (Dügenci *et al.* 2003). Furthermore, in the same fish species a significant increase in both protein and globulin concentrations was detected after feeding diets enriched with *A. sativum* (Nya & Austin 2009a) or *Z. officinale* (Dügenci *et al.* 2003; Nya & Austin 2009b). Kim *et al.* (2011) reported an increase in total proteins, albumin and globulins in kelp grouper fed diets containing *Eriobotrya japonica* ethanolic extract, similarly other authors observed an increase in proteins using diets enriched with ethanolic extract of *K. pictus* (Harikrishnan *et al.* 2011f) or *Styrax japonica* (Harikrishnan, Kim, Kim, Balasundaram & Heo 2011g). The dietary administration of *O. sanctum* extract or *W. somnifera* extract increased globulins in greasy grouper (Sivaram *et al.* 2004).

Blood glucose concentration is often used as an indicator of non-specific stress in fish rather than raised cortisol and adrenaline levels. In fact, during stressful situations there is an abrupt increase in blood cortisol which causes a breakdown of glycogen from the liver through glycogenolysis and, consequently, a rise in blood glucose levels (Shalaby *et al.* 2006; Xie *et al.* 2008; Kaleeswaran *et al.* 2010). Several studies (Tables 1 and 4) indicated that the administration of medicinal plants in fish can significantly reduce blood glucose and cortisol concentrations, limiting the effects of environmental stressors or infections, which normally cause their increase. Blood glucose significantly decreased in *O. niloticus* (Shalaby *et al.* 2006) and *L. rohita* (Sahu, Das, Mishra *et al.* 2007a) after the dietary administration of garlic. In *L. rohita*, this parameter was also reduced after feeding diets containing *M. indica* (Sahu, Das, Pradhan *et al.* 2007b). In common carp, both blood cortisol and glucose decreased after feeding diets including rhubarb anthraquinone extract (Xie *et al.* 2008), while lower levels of glucose were induced by diets enriched with *B. nigra*, *C. majus*, *E. purpurea*, *I. helenium*, *T. farfara* (Mohamad & Abasali 2010), *C. zeylanicum*, *J. regia*, *M. piperita* and *O. basilicum* (Abasali & Mohamad 2010) extracts. Diets with extract of *A. marmelos*, *C. dactylon*, *W. somnifera* or *Z. officinale* significantly reduced plasma glucose in

  

*O. mossambicus* (Immanuel *et al.* 2009). Harikrishnan *et al.* (2010a) reported an effective resistance to stress caused by *A. hydrophila* infections in *C. auratus* treated with diets containing different herbal extracts. Similarly, *E. bruneus* infected with *Vibrio carchariae* and fed diets supplemented with *E. japonica* ethanolic extract had lower glucose level in comparison with control untreated group (Kim *et al.* 2011).

It is suggested that several medicinal herbs promote lipid metabolism that catabolizes body fatty acids as a main energy expenditure, resulting in an efficient protein accumulation and growth performance (Ji, Jeong *et al.* 2007a). However, information concerning the effects of the administration of plants on blood lipids is limited (Tables 1 and 3). Diets containing *A. sativum* significantly decreased total lipids in Nile tilapia (Shalaby *et al.* 2006). Triglycerides and cholesterol have been reported as being reduced in tilapia *O. mossambicus* fed a diet with acetone extract of *A. marmelos*, *C. dactylon*, *W. somnifera* or *Z. officinale* (Immanuel *et al.* 2009). Moreover, lower plasma triglycerides and higher plasma HDL-cholesterol were observed in red sea bream fed a fishmeal diet supplemented with *C. officinale* (Ji, Takaoka *et al.* 2007b) and in juvenile flounder fed a diet containing a mixture of *A. capillaries*, *C. fructus*, *C. officinale* and *M. medicata* (Ji, Jeong *et al.* 2007a).

Alkaline phosphatase (ALP) and acid phosphatase (ACP) are important enzymes that regulate a number of essential functions in organisms. An increase in serum ALP activity indicates higher breakdown of the energy reserves which are then used for the growth and survival of fish (Sahu *et al.* 2008). ACP is widely considered to be a valuable parameter of macrophage activation (Pratheepa *et al.* 2010). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are ubiquitous aminotransferases that represent indices for the diagnosis of hepatopancreas injury. Liver is rich in ALT and AST and hepatic damage (induced by chemical, infectious and physiological factors) or a disturbance in the Kreb's cycle may result in their increase (Shalaby *et al.* 2006; Ji, Takaoka *et al.* 2007b). A range of evidences suggests that the administration of medicinal herbs in fish can modulate the activities of these enzymes (Table 1). A diet including leaf extract of *A. marmelos* significantly enhanced ACP and ALP activity in *C. carpio*, probably as a consequence of the macrophage activation (Pratheepa *et al.* 2010). Shalaby *et al.* (2006) found lower serum ALT and AST activities in Nile tilapia fed diets with *A. sativum*. Similarly, a diet enriched with *A. capillaries*, *C. officinale*, *C. fructus* or *M. medicata* has been reported to reduce the activity of these enzymes in red sea bream (Ji, Takaoka *et al.* 2007b) and olive flounder (Ji, Jeong *et al.* 2007a). Diets supplemented with *A. aspera* (Vasudeva *et al.* 2006; Vasudeva & Sunil 2009) or *C. longa* (Sahu *et al.* 2008) increased ALP activity and reduced both ALT and AST concentrations in the serum of Indian major carp.

## Effects on fish immune response

The immunomodulatory effects of herbal medicines have been well reported in various fish species. The products derived from plants improved mainly the innate/non-specific immune response, being able to stimulate both humoral and cellular defence mechanisms, as reported by Galindo-Villegas and Hosokawa (2004) also for other immunostimulant substances. Immunostimulants probably interact with specific receptors on cells surface and promote the expression of intracellular genes encoding for antimicrobial molecules (Raa 1996; Bricknell & Dalmo 2005). The research findings demonstrate also positive effects of plants on fish specific immune response.

### Non-specific immune response

Plant products enhance various components of the innate immunity such as serum lysozyme, complement, antiproteases, phagocytes microbicidal activities (Tables 1–4).

Lysozyme is a bactericidal enzyme that hydrolyses the β-1,4 glycosidic linkage between N-acetyl glucosamine and N-acetyl muramic acid of bacterial cell wall peptidoglycan, thereby causing bacteriolysis and preventing the growth of bacteria. Lysozyme is also known to activate the complement system and phagocytes by acting as an opsonin, as well as to display anti-viral and anti-inflammatory properties (Magnadottir 2006; Saurabh & Sahoo 2008). The serum lysozyme activity was reported to be enhanced in rainbow trout fed diets with *A. sativum* bulbs (Nya & Austin 2009a, 2011), *C. sinensis* extract (Sheikhzadeh *et al.* 2011), *C. coggyria* (Bilen *et al.* 2011), *L. perennis* seeds, *M. indica* fruits, *U. dioica* leaves (Awad & Austin 2010) or *Z. officinalis* root (Nya & Austin

2009b). A similar immunostimulatory effect was observed in tilapia *O. niloticus* fed diets supplemented with extract of *A. membranaceus* (Yin *et al.* 2006; Ardò, Yin, Xu, Váradi, Szigeti, Jeney & Jeney 2008), *Cratoxylum formosum* (Rattanachaikunsopon & Phumkhachorn 2010c), *E. purpurea* (Aly *et al.* 2008b) or *L. japonica* (Ardò *et al.* 2008) as well as in tilapia *O. mossambicus* fed diets enriched with extract of *C. dactylon*, *W. somnifera*, *Z. officinale* (Immanuel *et al.* 2009), *E. alba* (Christybapita *et al.* 2007) or *N. arbortristis* (Kirubakaran *et al.* 2010). The lysozyme activity significantly increased in *O. mossambicus* also after intraperitoneal injection of water and hexane soluble fractions of *S. trilobatum* leaves (Divyagnaneswari *et al.* 2007, 2008) and water soluble fraction of *T. cordifolia* leaves (Alexander *et al.* 2010). A significant increase in serum lysozyme activity was reported in Indian major carp after feeding diets with *A. aspera* seeds (Vasudeva *et al.* 2006; Vasudeva & Sunil 2009), *A. sativum* bulbs (Sahu, Das, Mishra *et al.* 2007a), *C. dactylon* extract (Kaleeswaran, Ilavenil & Ravikumar 2011a), *C. longa* (Sahu *et al.* 2008), *M. indica* kernels (Sahu, Das, Pradhan *et al.* 2007b) or *W. somnifera* roots (Sharma *et al.* 2010). Besides, the medicinal herbs *A. aspera* (Vasudeva & Chakrabarti 2005b), *A. membranaceus* (Yin *et al.* 2009), *Rheum officinale* (Xie *et al.* 2008), *B. nigra, C. majus, E. purpurea, I. helenium, T. farfara* (Mohamad & Abasali 2010), *C. zeylanicum*, *J. regia*, *M. piperita*, *O. basilicum* (Abasali & Mohamad 2010), *A. marmelos* (Pratheepa *et al.* 2010) and *E. hirta* (Pratheepa & Sukumaran 2011) enhanced lysozyme activity in common carps. This immune parameter was higher also in channel catfish fed a diet with *O. vulgare* essential oil (Zheng *et al.* 2009), in red sea bream fed a diet with *C. officinale* root (Ji, Takaoka *et al.* 2007b), in goldfish fed a diet with triherbal extracts of *A. indica, C. longa* and *O. sanctum* (Harikrishnan, Balasundaram, Kim *et al.* 2009b), in kelp grouper fed diets with extract from green tea (Harikrishnan *et al.* 2011b), *K. pictus* (Harikrishnan *et al.* 2011f), *S. japonica* (Harikrishnan *et al.* 2011g), *V. album* (Harikrishnan, Balasundaram & Heo 2011c) or *E. japonica* (Kim *et al.* 2011), in yellow croaker fed diets with *A. sinensis* and *A. membranaceus* (Jian & Wu 2003), in rock bream fed diets with *S. baicalensis* extract (Harikrishnan, Kim, Kim, Balasundaram & Heo 2011h), in Japanese eel fed diets with *V. album* extract (Choi *et al.* 2008), in olive flounder fed diets with *P. vulgaris* extract (Harikrishnan, Kim, Kim, Balasundaram & Heo 2011e). The lysozyme activity was promoted in juvenile flounders also by dietary/intraperitoneal administration of aqueous, methanolic, ethanolic thriherbal extracts of the traditional Korean medicinal plants *Crysanthemum cinerariaefolium*, *P. granatum*, *Zanthoxylum schinifolium* (Harikrishnan, Balasundaram, Kim, Kim, Han & Heo 2010d; Harikrishnan, Heo, Balasundaram *et al.* 2010f).

The alternative complement pathway is known to be one of the powerful non-specific defence mechanisms which protects fish from a wide range of bacteria (Ellis 1999, 2001). The serum natural haemolytic complement activity was improved by oral administration of *A. sinensis* and *A. membranaceus* mixture in yellow croaker (Jian & Wu 2003) and Jian carp (Jian & Wu 2004), *E. alba* (Christybapita *et al.* 2007) or *N. arbortristis* (Kirubakaran *et al.* 2010) extract in tilapia, *C. sinenis* (Harikrishnan *et al.* 2011b), *K. pictus* (Harikrishnan *et al.* 2011f), *S. japonica* (Harikrishnan *et al.* 2011g) or *E. japonica* (Kim *et al.* 2011) extract in kelp grouper, *S. baicalensis* extract in rock bream (Harikrishnan *et al.* 2011h), *C. dactylon* extract in *C. catla* (Kaleeswaran *et al.* 2011a), *P. vulgaris* extract (Harikrishnan *et al.* 2011e) or mixed *C. cinerariafolium*, *P. granatum*, *Z. schinifolium* extracts (Harikrishnan, Balasundaram, Kim *et al.* 2010d) in olive flounder. Similarly, the alternative complement pathway was enhanced by intraperitoneal injection of aqueous, methanolic, ethanolic extracts of *A. indica*, *C. longa*, *O. sanctum* in goldfish (Harikrishnan, Balasundaram, Kim *et al.* 2009b), aqueous extract of *T. cordifolia* in tilapia (Alexander *et al.* 2010), aqueous, methanolic, ethanolic extracts of *C. cinerariafolium*, *P. granatum*, *Z. schinifolium* (Harikrishnan, Heo, Balasundaram *et al.* 2010f) in olive flounder.

Antiproteases, principally α2-macroglobulin, α1-antiprotease and α2-antiplasmin, are protease inhibitors that restrict the ability of bacteria to invade and grow in fish, by inhibiting their extracellular enzymes (Ellis 2001). Serum antiprotease activity was elevated in different species of carp treated with *A. aspera* (Vasudeva & Chakrabarti 2004, 2005a,b; Vasudeva *et al.* 2004; Vasudeva & Sunil 2009) or *C. dactylon* extract (Kaleeswaran *et al.* 2010, 2011a; Kaleeswaran, Ilavenil & Ravikumar 2011b). Positive effects on these enzymes have been also exhibited by *E. alba* (Christybapita *et al.* 2007) or *T. cordifolia* (Alexander *et al.* 2010) extract in tilapia, *C. sinensis* extract (Sheikhzadeh

  

*et al.* 2011) and *Z. officinale* (Nya & Austin 2009b) in rainbow trout, *C. sinensis* (Harikrishnan *et al.* 2011b), *K. pictus* (Harikrishnan, Kim, Kim *et al.* 2011f) or *S. japonica* (Harikrishnan *et al.* 2011g) extract in kelp grouper, *S. baicalensis* extract in rock bream (Harikrishnan *et al.* 2011h).

Phagocytosis is the most important cellular mechanism of the non-specific immune system of teleosts, and together with humoral components it constitutes the first line of defence against invading pathogens. Phagocytes (monocytes/macrophages and neutrophils) engulf the microorganisms and kill them by degranulation, metabolic activation and release of microbicidal oxygen and nitrogen reactive species (ROS and NOS) (Neumann, Stafford, Barreda, Ainsworth & Belosevic 2001). In rainbow trout, phagocytosis and respiratory bust activity of leucocytes have been significantly promoted by *A. sativum* (Nya & Austin 2009a, 2011), *C. coggyria* (Bilen *et al.* 2011), *Laurus nobilis* (Bilen & Bulut 2010) or *Z. officinale* (Dügenci *et al.* 2003; Nya & Austin 2009b) enriched diets, while *L. perennis*, *M. indica* or *U. dioica* enriched diets enhanced only the ROS production (Awad & Austin 2010). Diets including extract form *A. membranaceus* (Yin *et al.* 2006; Ardò *et al.* 2008), *L. japonica* (Ardò *et al.* 2008), *C. dactylon*, *W. somnifera*, *Z. officinalis* (Immanuel *et al.* 2009), *C. formosum* (Rattanachaikunsopon & Phumkhachorn 2010c) or *T. sinensis* (Wu, Liu, Chang & Hsieh 2010) effectively enhanced the phagocytic efficiency of white blood cells in tilapia. Furthermore, the killing capability of tilapia leucocytes, evaluated by measuring ROS, NOS and myeloperoxidases, has been improved after feeding diets containing *A. sativum* (Aly *et al.* 2008a) or extracts from *A. membranaceus*, *L. japonica* (Ardò *et al.* 2008), *C. formosum* (Rattanachaikunsopon & Phumkhachorn 2010c), *E. alba* (Christybapita *et al.* 2007), *N. arbortristis* (Kirubakaran *et al.* 2010) as well as after intraperitoneal injection of extracts from *O. sanctum* (Logambal, Venkatalakshmi & Michael 2000), *S. trilobatum* (Divyagnaneswari *et al.* 2007), *T. cordifolia* (Sudhakaran *et al.* 2006; Alexander *et al.* 2010) or *T. sinensis* (Wu *et al.* 2010). In Indian major carp, diets with *A. aspera* (Vasudeva *et al.* 2006; Vasudeva & Sunil 2009), *A. sativum* (Sahu, Das, Mishra *et al.* 2007a), *C. longa* (Sahu *et al.* 2008), *M. indica* (Sahu, Das, Pradhan *et al.* 2007b) or *W. somnifera* (Sharma *et al.* 2010) induced a significant stimulation of blood phagocytes respiratory burst, diets with *A. marmelos* enhanced the phagocytosis activity (Pratheepa, Madasamy & Sukumaran 2011), diets with *C. dactylon* promoted the oxidative burst, NOS production and myeloperoxidase activity (Kaleeswaran *et al.* 2011a). Common carps showed an improvement of phagocytosis after feeding diets with *A. membranaceus* and *A. sinensis* mixture (Jian & Wu 2004), an increase in both phagocytosis and respiratory burst activity after feeding diets with extract of *A. membranaceus* (Yin *et al.* 2009), *A. marmelos* (Pratheepa *et al.* 2010) or *E. hirta* (Pratheepa & Sukumaran 2011), an increasing trend in the respiratory burst activity after feeding diets with *B. nigra*, *C. majus*, *E. purpurea*, *I. helenium*, *T. farfara* ethanolic extracts (Mohamad & Abasali 2010) or *C. zeylanicum*, *J. regia*, *M. piperita* and *O. basilicum* ethanolic extracts (Abasali & Mohamad 2010). Oral, immersion and intraperitoneal treatments with *A. indica*, *C. longa* and *O. sanctum* extracts enhanced phagocytosis and ROS generation of goldfish leucocytes (Harikrishnan, Balasundaram, Kim *et al.* 2009b). The antimicrobial mechanisms of phagocytes were improved also in greasy grouper fed diets enriched with *O. sanctum* extract, *W. somnifera* extract (Sivaram *et al.* 2004) or *C. dactylon*, *P. longum*, *P. niruri*, *T. procumbens*, *Z.officinale* extracts mixture (Punitha *et al.* 2008), in kelp grouper fed diets enriched with extract from *C. sinensis* (Harikrishnan *et al.* 2011b), *E. japonica* (Kim *et al.* 2011), *K. pictus* (Harikrishnan *et al.* 2011f), *Lactuca indica* (Harikrishnan *et al.* 2011d), *S. japonica* (Harikrishnan *et al.* 2011g) or *V. album* (Harikrishnan, Balasundaram & Heo 2011c), in yellow croaker fed diets supplemented with *A. membranaceus* and *A. sinensis* (Jian & Wu 2003), in Japanese eel fed diets with *V. album* extract (Choi *et al.* 2008), in rock bream fed diets with *S. baicalensis* leaves extract (Harikrishnan *et al.* 2011h). The function of phagocytic cells was promoted in olive flounder by diets with *Prunella vulgaris* ethanolic extract (Harikrishnan *et al.* 2011e) or mixed *C. cinerariafolium*, *P. granatum*, *Z. schinifolium* extracts (Harikrishnan, Balasundaram, Kim *et al.* 2010d). The release of myeloperoxide enzymes by azurophilic granules of neutrophils is measured also through the serum peroxidase activity. A significant increase in this parameter was detected in *C. carpio* fed diets including *A. marmelos* leaf extract (Pratheepa *et al.* 2010), in *O. mykiss* fed diets containing decaffeinated green tea extract (Sheikhzadeh *et al.* 2011), in *E. bruneus* fed diets with extract of

*K. pictus* (Harikrishnan *et al.* 2011f) or *S. japonica* (Harikrishnan *et al.* 2011g).

### Specific immune response

The administration of herbal medicines in fish in association with immunization trials or infections has been reported to enhance the adaptive/specific immune response by improving the synthesis of specific antibodies (Tables 1, 2 and 4). Colorni, Avtalion, Knibb, Berger, Colorni and Timan (1998) observed an enhancing effect on antibody level in *D. labrax* infected with *M. marinum* and i.p. treated with *A. sativum* extract. Moreover, it has been demonstrated that the ethanolic extract of the medicinal herb *C. dactylon* induces a significant increase in serum specific antibody titre and an aggregation of spleen melanomacrophage centres in *C. catla* vaccinated against *A. hydrophila* (Kaleeswaran *et al.* 2010). Similarly, extract from astragalus (Yin *et al.* 2009) or *A. vera* (Alishahi, Ranjbar, Ghorbanpour, Peyghan, Mesbah & Razi-jalali 2010) and the essential oil of *Z. multiflora* (Soltani *et al.* 2010) promoted the production of anti-*A. hydrophila* immunoglobulins in vaccinated *C. carpio*. Moreover, *O. mossambicus* intraperitoneally/orally treated with *O. sanctum* (Logambal *et al.* 2000) or *S. trilobatum* (Divyagnaneswari *et al.* 2008) and immunized against *A. hydrophila* showed a higher serum antibody response compared to controls. Similar immunostimulatory effects were observed also in tilapia injected with *T. cordifolia* ethanol or petroleum ether extract (Sudhakaran *et al.* 2006). Feeds with aqueous extract from *A. marmelos* or *E. hirta* strongly enhanced the primary antibody response of *C. carpio* experimentally infected with *A. hydrophila* (Pratheepa *et al.* 2010) and *Pseudomonas fluorescens* (Pratheepa & Sukumaran 2011) respectively.

## Effects on fish resistance to infections

### Bacterial infections

It has been widely established that certain herbs can improve the resistance of fish to bacterial diseases, as a overall consequence of their immunostimulatory effects (Tables 1–2). Diets supplemented with *A. sativum* (Shalaby *et al.* 2006; Aly *et al.* 2008a; Aly & Mohamed 2010), *A. membranaceus* extract (Ardò *et al.* 2008), *E. purpurea* extract (Aly & Mohamed 2010), *L. japonica* extract (Ardò *et al.* 2008) or *Psidium guajava* (Pachanawan, Phumkhachorn & Rattanachaikunsopon 2008) have been reported to significantly ameliorate the immune competence and the survival of Nile tilapia submitted to a challenge with a virulent strain of *A. hydrophila*. Similarly, diets with extract of *A. paniculata* (Rattanachaikunsopon & Phumkhachorn 2009b), *C. verum* (Rattanachaikunsopon & Phumkhachorn 2010a) or *C. formosum* (Rattanachaikunsopon & Phumkhachorn 2010c) enhanced the disease resistance of this fish species to S*treptococcus* sp. while *A. tuberosum* essential oil (Rattanachaikunsopon & Phumkhachorn 2009a) or *Centella asiatica* extract (Rattanachaikunsopon & Phumkhachorn 2010b) improved its resistance to *Flavobacterium columnare.* Oral administration of *E. alba* aqueous extract (Christybapita *et al.* 2007) or *N. arbortristis* chloroform extract (Kirubakaran *et al.* 2010) significantly increased the resistance of tilapia *O. mossambicus* to haemorrhagic septicaemia by enhancing lysozyme, complement, antiproteases activity and phagocyte microbicidal capability. Similarly, the intraperitoneal injection of *O. sanctum* aqueous extract (Logambal *et al.* 2000), *S. trilobatum* water and hexane soluble fractions (Divyagnaneswari *et al.* 2007, 2008), *T. cordifolia* water, ethanol and petroleum ether fractions (Sudhakaran *et al.* 2006; Alexander *et al.* 2010) or *T. sinensis* hot water extract (Wu *et al.* 2010) reduced the susceptibility of *O. mossambicus* to this disease due to the improvement of the non-specific/specific humoral and cellular immune response. In other researches, diets enriched with acetone extract from *A. marmelos*, *C. dactylon*, *W. somnifera* or *Z. officinale* have been reported to reduce mortality in *O. mossambicus* challenged with *Vibrio vulnificus*, and were able to enhance blood leucocrit, phagocytic and lysozyme activity (Immanuel *et al.* 2009). Long-term dietary administration of *A. aspera* (Vasudeva *et al.* 2006; Vasudeva & Sunil 2009), *A. sativum* (Sahu, Das, Mishra *et al.* 2007a), *C. dactylon* extract (Kaleeswaran *et al.* 2011a), *C. longa* (Sahu *et al.* 2008), *M. indica* (Sahu, Das, Pradhan *et al.* 2007b) or *W. somnifera* (Sharma *et al.* 2010) largely prevented *A. hydrophila* infections in Indian major carp, enhancing serum and phagocyte bactericidal activities. Similarly, diet supplementation with *Rheum officinale* (Xie *et al.* 2008), *A. membranaceus* (Yin *et al.* 2009), *A. marmelos* extract (Pratheepa *et al.* 2010), *B. nigra*, *C. majus*, *E. purpurea*, *I. helenium*, *T. farfara* extracts (Mohamad & Abasali 2010),

*C. zeylanicum*, *J. regia*, *M. piperita* and *O. basilicum* extracts (Abasali & Mohamad 2010) or *A. vera* extract (Alishahi *et al.* 2010) improved the resistance of *C. carpio* to *A. hydrophila*. Nya and Austin (2009a,b, 2011) demonstrated the potential value of garlic and ginger in terms of conferring protection against *A. hydrophila* in rainbow trout while Harikrishnan, Balasundaram and Heo (2009a) found equivalent results in goldfish preventively fed *A. indica*, *C. longa* and *O. sanctum*. Resistance to *A. hydrophila* was also increased in channel catfish fed a diet supplemented with oregano essential oil (Zheng *et al.* 2009) and in Japanese eel fed diets enriched with Korean mistletoe extract (Choi *et al.* 2008). Ji, Takaoka *et al.* (2007b) reported that red sea bream juveniles fed a diet with *A. capillaries*, *C. fructus*, *C. officinale* or *M. medicata* showed reduced mortality when infected with *Vibrio anguillarum*. Moreover, diets supplemented with *O. sanctum* or *W. somnifera* methanolic extract increased the survival rates in *E. tauvina* juveniles during *Vibrio harveyi* infections (Sivaram *et al.* 2004) and similar results were obtained using a mixture of *C. dactylon*, *P. longum*, *P. niruri*, *T. procumbens* and *Z. officinale* petroleum ether extracts (Punitha *et al.* 2008). Diet with *Alnus firma* ethanol extract protected olive flounder against *Tenacibaculum maritimum* (Harikrishnan, Kim, Kim, Kim, Hong & Heo 2011i) while a dietary mixture containing *A. sinensis* and *A. membranaceus* significantly reduced the cumulative mortality of yellow croaker infected with *Vibrio alginolyticus* (Jian & Wu 2003). *S. baicalensis* extract showed a protective effect against *Edwardsiella tarda* in rock bream, improving the haematological and immune status (Harikrishnan *et al.* 2011h). Green tea (Harikrishnan *et al.* 2011b) and *E. japonica* (Kim *et al.* 2011) extracts incorporated into the diet yielded high survival rate of *E. bruneus* against *V. carchariae*, *L. indica* extract enhanced its resistance against *Streptococcus iniae* (Harikrishnan, Balasundaram & Heo 2011c) while *S. japonica* extract enhanced its resistance against *V. harveyi* (Harikrishnan *et al.* 2011g). However, further studies are required to elucidate how long the plant-based treatments must be provided to fish to get a long-term protection. Aly *et al.* (2008a), Aly and Mohamad (2010) suggested that the longest lasting protection can be obtained by increasing the period of plant application. In fact, *O. niloticus* fed diets supplemented with garlic or echinacea extract for 1 month were protected against an immediate challenge with *A. hydrophila*, while a protection lasting 8 months was ensured by giving the same diets for 2 or 3 months.

On the other hand, Harikrishnan *et al.* demonstrated that different extracts of *A. indica*, *C. longa* and *O. sanctum* can be used as therapeutic agents to control *A. hydrophila* infections in goldfish (Harikrishnan *et al.* 2009a; Harikrishnan, Balasundaram, Moon *et al.* 2009c, Harikrishnan *et al.* 2010a; Harikrishnan, Moon, Kim *et al.* 2010g) and common carp (Harikrishnan *et al.* 2010b), being able to induce gradual reduction in the clinical signs, complete recovery of health status and increased survival (Tables 3 and 5). Similar results were described by Rajendiran *et al.* (2008) in spotted snakehead dip-treated with *S. nigrum* ethyl acetate leaf extract (Table 5). In tilapia spp., the dietary application of rosemary provided good results in the treatment of streptococcal infections (Abutbul *et al.* 2004; Zilberg *et al.* 2010) whereas bath treatment with *C. asiatica* aqueous extract demonstrated therapeutic effects against *F. columnare* (Rattanachaikunsopon & Phumkhachorn 2010b) (Tables 3 and 5).

### Parasitic infections

Herbal-based immunostimulants are also capable of reducing parasitic infections (Tables 1 and 2). Oral administration of *P. vulgaris* extract (Harikrishnan *et al.* 2011e) and intraperitoneal administration of traditional Korean medicinal (TKM) triherbal extracts (Harikrishnan, Heo, Balasundaram *et al.* 2010f) clearly enhanced the resistance of olive flounder *P. olivaceus* against the parasite *Uronema marinum*. Moreover, a diet with mixed *C. cinerariafolium*, *P. granatum* and *Z. schinifolium* extracts protected olive flounder against *Philasterides dicentrarchi* (Harikrishnan, Balasundaram, Kim *et al.* 2010d). These results are in agreement with the significantly increased survival rates observed in kelp grouper *E. bruneus* fed diets including *S. japonica* extract (Harikrishnan *et al.* 2011g) or *V. album* extract (Harikrishnan, Balasundaram & Heo 2011c). Furthermore, in this species the dietary supplementation with *K. pictus* extract conferred protection from a mixed infection by *V. alginolyticus* and *P. dicentrarchi* (Harikrishnan *et al.* 2011f).

Other plant extracts showed potential therapeutic effects also on parasitic infections in cultured fish (Tables 3 and 5). Methanolic extract of

*Mucuna pruriens* leaves and petroleum ether extract of *Carica papaya* seeds demonstrated their potential in the control of *Ichthyophthirius multifiliis* infections in goldfish, having produced a significant reduction in the parasite burden and the fish recovery (Ekanem, Obiekezie, Kloas & Knopf 2004a). Similarly, methanolic extracts of *Piper guineense* seeds were active in the treatment of monogenean diseases (Ekanem, Wang, Simon, Obiekezie & Morah 2004b). Baths with green tea (Noor El-Deen 2010), garlic or Indian almond (Chitmanat, Tongdonmuan & Nunsong 2005; Omima 2010) extract have produced good results in *O.niloticus* against *Trichodina* sp. infestations. Furthermore, oral administration of *O. minutiflorum* essential oil reduced the prevalence of infection caused by *Myxobolus* sp. in sharpsnout sea bream (Karagouni, Athanassopoulou, Lytra, Komis & Dotsika 2005).

### Fungal and viral infections

The information concerning the plant-based control of fungal and viral infections is limited. Immersion treatment with *A. indica* extract demonstrated a restorative effect in common carp experimentally infected with the fungus *A. invadans* (Harikrishnan *et al.* 2005) (Table 5). Intraperitoneal administration of *P. granatum* extracts reduced the mortality of olive flounder infected with the lymphocystis disease virus (LDV) (Harikrishnan, Heo, Balasundaram *et al.* 2010e) (Table 4).

## Adverse effects

The application of medicinal herbs in aquaculture has received attention for its promoting effects on growth and immune functions but also because, unlike chemotherapeutic agents, their administration in several animal models including humans is usually associated with few or no side effects (Briskin 2000). There are few reports with regard to this aspect in fish, still they underline similar evidences. Abutbul *et al.* (2004) found that the oral administration of *Rosmarinus officinalis* in tilapia spp. had no negative effects on fish survival, appearance and behaviour. No apparent toxic effects and mortality were observed in Nile tilapia (*O. niloticus*) fed with *A. paniculata* extract (Rattanachaikunsopon & Phumkhachorn 2009b), *C. verum* essential oil (Rattanachaikunsopon & Phumkhachorn 2010a), *C. formosum* extract (Rattanachaikunsopon & Phumkhachorn 2010c) or *P. guajava* extract (Pachanawan *et al.* 2008). Further insights and appropriate trials are necessary to evaluate whether plant extracts are toxic to fish at high levels or if administered for long periods, as well as to study how the herbal bioactive compounds are metabolized and/or stored in fish tissues.

## Factors influencing the effectiveness of plants and plant products

### Plant/plant products concentration and duration of administration

Among factors that may influence the effectiveness of herbal products in fish, their dosage and the duration of administration are crucial. In fact, only an appropriate dosage can significantly induce a stimulation of immune responses increasing disease resistance, without being toxic to animals (Sakai 1999).

It has been observed that the response of fish after the administration of plants and plant products was usually dose-dependent and therefore more evident at higher concentrations. However, sometimes high doses did not yield the enhanced effects observed at lower concentrations and were occasionally found to be less effective, as has already been demonstrated for many other immunostimulants (Sakai 1999). For example, Jian and Wu have shown that an increase in traditional Chinese medicine (a product composed of several herbs) concentration in the diet did not improve the immune response of yellow croakers (2003) and Jian carps (2004), as they observed that 1.0% and 1.5% dietary levels resulted in similar lysozyme, complement and phagocyte activities. Similarly, Sivaram *et al.* (2004) reported that diets supplemented with 100 and 200 mg $kg^{-1}$ of ethanol extract from *O. sanctum* or *W. somnifera* significantly improved growth, immune response and resistance to *V. harveyi* infections of greasy groupers but higher levels (400 and 800 mg $kg^{-1}$) did not provide the same positive results. Yin *et al.* (2006) observed that the roots of *Astragalus* effectively stimulated phagocytosis and lysozyme activity of Nile tilapia when added to the diet at concentrations of 0.1% and 0.5%, but a dose of 1% was not equally effective. Choi *et al.* (2008) found no significant differences in ROS production

  

of leucocytes when eels were fed 0.5% and 1.0% dietary mistletoe, suggesting that the lower dose is sufficient to induce the maximum response. Sharma *et al.* (2010) reported that carps fed a diet enriched with 2 g $kg^{-1}$ of *W. somnifera* root showed higher immune response and relative percentage of survival when compared to carps fed doses of 1 g $kg^{-1}$ or 3 g $kg^{-1}$. The absence of a linear relationship between dose and effect has been described by other authors for other plants and other species of fish (Xie *et al.* 2008; Harikrishnan, Balasundaram, Kim *et al.* 2009b; Kirubakaran *et al.* 2010; Mohamad & Abasali 2010; Soltani *et al.* 2010). Pratheepa *et al.* (2011) observed a progressive increase in the immune capability of *C. catla* by increasing the level of *A. marmelos* extract in the diet from 5 g $kg^{-1}$ to 30 g $kg^{-1}$, whereas a higher concentration (50 g $kg^{-1}$) induced immunosuppression.

Different effects on fish health status, humoral and cellular immune mechanisms and disease resistance have also been detected following different periods of stimulation. Kirubakaran *et al.* (2010) reported that the administration of 0.1% *N. arbortristis* chloroform extract supplemented diet in *O. mossambicus* for 3 weeks induced a more effective immune response then a feeding lasting 1 or 2 weeks. Harikrishnan *et al.* (2003) observed that an immersion treatment repeated for 30 days with *A. indica* aqueous extract was necessary to improve the haematological parameters in *C. carpio*. However, other authors measured a maximum immune response after just a few days of administration and a decrease in immune competence during the following weeks of treatment. For example, humoral and cellular non-specific immune responses in tilapia (*O. mossambicus*) fed diets including *E. alba* aqueous extract significantly increased after 1 week while no modulation was observed after 3 weeks feeding (Christybapita *et al.* 2007).

## Bioactive compounds contained in plants and plant extracts

Medicinal plants synthesize and accumulate natural bioactive substances responsible for their pharmacological properties in humans and animals. Based on their chemical structure, plant active compounds can principally be categorized into alkaloids, terpenoids (triterpenes and steroid saponins), phenolic compounds, glycosides, flavonoids, tannins and polysaccharides (Lovkova *et al.* 2001).

The studies regarding the use of plants in aquaculture usually do not provide any data about the chemical composition of the tested plant products (extracts, oils…), hovewer it is thought that the antimicrobial/immunomodulatory properties could be ascribed to secondary metabolites of the abovementioned classes of compounds. For instance, Zheng *et al.* (2009) considered carvacrol, a phenolic compound, as the main active substance in oregano essential oil administered in *I. punctatus*. Similarly, Dada and Ikuerowo (2009) attributed the effects of *G. cola* to its content of different phenols, including biflavonoids, xanthones and benzophanones while Logambal *et al.* (2000) suggested that the water extract of *O. sanctum* leaves is rich in eugenol, methyl eugenol and caryophyllene. Wu *et al.* (2010) reported that water extract of the Chinese herb *T. sinensis* consists of tritrpenes and phenolic substances including methyl gallate, gallic acid, kaempferol, quercetin, quercitrin, rutin, kaempferol-D-glucoside, catechin, epicatechin, β-sitosterol, stigmasterol, β-sitosteryl-glucoside, stigmasterolglicoside, phytol and toosendanin. *Rosmarinus officinalis* contains terpenes (1,8-cineol, o-pinene, α-pinene, limonene, terpineol-4-ol, α-terpineol), camphor and polyphenols (carnosic acid, rosmarinic acid) (Abutbul *et al.* 2004). Harikrishnan *et al.* (2003, 2005) and Harikrishnan *et al.* (2009a) consider the terpenes azadirachtin, nimbin, nimbinin, nimbinidin, nimbolide and nimbidic acid responsible for antimicrobial and immunostimulant roles of neem (*A. indica*) extracts. Polysaccharides, organic acids, alkaloids, glucosides and volatile oil are the major active components of *A. membranaceous* and *S. baicalensis* extracts that have been found to enhance immune function in fish (Yin *et al.* 2009; Harikrishnan *et al.* 2011h). The Korean herb *P. vulgaris* includes flavonoids, triterpenes, phenolic acids, triterpenoids, tannins and polysaccharides (Harikrishnan *et al.* 2011e), *E. alba* contains eclalbatin, α-amyrin, urosilic acid, oleanolic acid, acliptasaponin, daucosterol, stigmasterol-3-O-glucoside (Christybapita *et al.* 2007), *T. cordifolia* contains alkaloids, diterpenoid lactones, glycosides, steroids and sesuiterpenoids (Alexander *et al.* 2010), *C. dactylon*, *A. marmelos*, *W. somnifera* and *Z. officinale* contain different alkaloids, coumarins, triterpenoids, β-sitosterol, steroidal lactones and volatile oils (Immanuel *et al.* 2009).

On the other hand, Kaleeswaran *et al.* (2011a, b) performed a preliminary phytochemical screening of *C. dactylon* ethanolic extract, revealing tannins, quinines and phenols as responsible for immunostimulatory activity in *C. catla*. A similar experimental approach, which includes both chemical analysis and biological activities determination, should be strongly requested.

Accumulation of plant secondary metabolites varies according to season, temperature, water availability and geographical source (Croteau, Kutchan & Lewis 2000). The current literature does not provide information on eventual differences in the activity of extracts obtained from various batches of plants, therefore further research is needed to explore this aspect.

### Solvent used for plant extraction

The solvent used for the extraction (water, methanol, ethanol, ethyl acetate, hexane, butane, acetone, benzene, petroleum ether, etc.) is another factor which can influence the spectrum of antibacterial and immunomodulatory properties of plant extracts in fish.

Some studies concerning the effectiveness of extraction methods highlighted that alcoholic or organic solvents always provide a higher efficiency in extracting secondary bioactive metabolites (polar or non-polar) with antimicrobial and immunostimulant activity, compared to water-based methods. Divyagnaneswari *et al.* (2007) demonstrated that the hexane soluble fraction of *S. trilobatum* was more protective than the water soluble fraction when administered intraperitoneally to tilapia. Harikrishnan, Balasundaram, Kim *et al.* (2009b) reported that the injection of triherbal aqueous, ethanol or methanol solvent leaf extracts from *A. indica, O. sanctum* and *C. longa* enhanced non-specific immune parameters and disease resistance against *A. hydrophila* in goldfish, but the ethanol solvent extract appeared more effective as an immunostimulant. On the other hand, the use of aqueous extracts, which contain soluble and particulate components, is more suitable when the plants are being administered by immersion (Rajendiran *et al.* 2008). Similar results were also obtained in studies performed *in vitro*. For example, Borisutpeth, Kanbutra, Weerakhun, Sarachoo and Porntrakulpipat (2005) demonstrated that methanol extracts from *Cassia fistula* and *Hibiscus sabdariffa* exhibited slightly higher efficacy against *A. hydrophila* and *S. agalactiae* compared to water extracts. Moreover, Rattanachaikunsopon and Phumkhachorn (2009b) observed that ethanol extracts from *A. sativum*, *Cassia alata*, *Gracinia mangostana* and *P. guajava* had a greater inhibitory activity *in vitro* against *S. agalactiae* when compared to aqueous extracts of the same plant species. Ponnusamy, Ebenezer, Marimuthu, Selvakumar and Nelson (2010) reported that *Clitoria ternatea* extracted using ethyl acetate, ethanol, acetone and petroleum ether showed higher antibacterial effects against a range of fish pathogens than that extracted using water.

Other reports show that there may be also differences in the capability of the alcoholic and organic solvents to recover the antimicrobial compounds that could lead to different susceptibilities of the target bacterial strains. Abutbul *et al.* (2004) observed that rosemary was more active against *S. iniae* growth when extracted using ethyl acetate compared to other organic solvents (ethanol, methanol, methanol/ethyl acetate). Punitha *et al.* (2008) tested different solvent extracts from *C. dactylon*, *P. longum*, *P. niruri*, *T. procumbens* and *Z. officinalis* against *V. harveyi* and reported that those based on petroleum ether and benzene effectively limited the pathogen viability. Dhayanithi, Ajith Kumar and Kathiresan (2010) established that ethanol and methanol extracts obtained from neem (*A. indica*) were highly inhibitory towards several bacterial fish pathogens when compared to extracts produced using other solvents (chloroform or acetone).

### Sinergism or antagonism between plants

Further investigations are needed to elucidate possible synergistic or antagonistic effects between the plants included in the mixtures. However, some studies demonstrated that the combined use of medicinal herbs brings a synergistic action on fish physiology, as compared with the use of individual herbs. Ji, Takaoka *et al.* (2007b) reported that a diet supplemented with a mixture of *M. medicata* (fruits), *C. fructus* (fruits), *A. capillaries* (leaves) and *C. officinale* (roots) enhanced growth performance, serum constituents and resistance to *V. anguillarum* in red sea bream to a greater extent than a diet containing a single medicinal herb. Similarly, the results of Harikrishnan & Balasundaram (2008) revealed that a triherbal extract of *A. indica, C. longa* and *O. sanctum* is effective *in vitro*

  

against *A. hydrophila* at lower concentrations than the extracts obtained from each single plant.

## Legislation on herbal products in aquaculture

Currently several herbal products are included in a list of feed additives (based on the EC REGULATION No 1831/2003) continuously updated by the EU. Within this list, herbal products are classified in category 2 – sensory additives, functional group b – flavouring compounds, subclass – natural products botanically defined. The list also provides information concerning the animal species (sometimes including fish) to which each plant can be administered the dosages.

As regards the use of plants for curative purposes in fish, the Commission Regulation (EC) No 710/2009 covering 'organic aquaculture' allows the use of substances of plant origin at a homeopathic dilution as well as plants and plant extracts with no anaesthetic effects as veterinary treatments for fish. The use of allopathic medicines must be limited to a maximum of two treatments per year or to a single treatment when the production cycle takes less than 1 year. If these limits are exceeded for allopathic treatment, fish can not be marketed as organic products. In addition, plants may be employed for cleaning and disinfection in fish farms addressed to organic production. In the United States, the use of plant-derived products in the aquaculture industry is under the control of the US FDA and the US EPA, which also approve drugs and chemicals to be used in fish farming. Among plants, only garlic and onion have undergone review by the FDA as new animal drugs of low regulatory priority. Their administration is permitted in salmonids at all stages of life for the treatment of helminth and lice infestations (PPM number 1240.4200 2011).

## Conclusions and future perspectives

Infectious diseases represent the main problem for the development and sustainability of the aquaculture industry as they cause significant economic losses inasmuch as they restrict productivity and require the use of control measures that are often very expensive. However, the indiscriminate administration of antibiotics or other drugs in fish leads to the selection of antibiotic-resistant bacterial strains as well as to the accumulation of chemical residues in water and fish tissues, which may prove damaging to the environment and be potentially dangerous for consumers. On the other hand, the production of effective vaccine formulations for a number of fish pathogens is usually difficult and not cost-effective for pharmaceutical companies.

In this context, plant-derived products seem to represent a promising tool, complementary to vaccination and traditional drugs, being able to improve growth, survival, health status, innate (lysozyme, complement, antiproteases phagocytosis and microbicidal capacity of phagocytic cells) and adaptive (specific Ig production) immune responses as well as disease resistance in various marine and freshwater fish species. In addition, unlike chemotherapeutics, their administration to fish does not seem to be associated with side effects. Medicinal plants are also easily commercially available, inexpensive and biocompatible.

Further investigations are still strongly recommended to define the optimal doses and timings of administration as well as to isolate, characterize and quantify the bioactive compounds contained in plants and phytoextracts, to identify the most effective substances/metabolites that could be included in new natural formulations to be use in fish. Moreover, research on mode of action, stability of plant materials in aquatic environment and digestability in fish as well as *in vitro* and *in vivo* toxicological tests are prerequisites for their safe application.

Nowadays, only few commercial herbal products are available at a global level for large-scale use in aquaculture. In many countries a review of the current legislation should be undertaken to allow a greater flexibility in their use taking into consideration the benefits that they might have in intensive farming conditions, in terms of fish welfare and public health. Plants and plant bioactives might be proposed in acquaculture primarily as feed additives or immunostimulants, rather that therapeutics, as the registration of herbal remedies to be used in this field is a time-consuming process and implies higher economic costs.

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