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PROOF

Oral Administration of *Basella alba* Leaf Methanol Extract and Genistein Enhances the Growth and Non-Specific Immune Responses of *Oreochromis niloticus*

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Abstract

The effects of the isoflavone genistein and methanol extract of *Basella alba* leaves were evaluated in Nile tilapia, *Oreochromis niloticus* on growth and immunostimulation. Adult tilapia (mean weight 39.55 g) was fed diets containing genistein (1 gm/kg) and methanol extract of *B. alba* (1 g/kg) for 35 days. *Basella alba* extract treated tilapia showed significantly higher ($P<0.05$) weight gain, respiratory burst, phagocytic activity, plasma protein content and plasma lysozyme activity compared to fish fed control diet. The *B. alba* extract treated fish showed the highest final individual mean body weight, final individual length, specific growth rate, hepatosomatic index and total immunoglobulin content. The phagocytic and respiratory burst activities of genistein treated fish were significantly higher ($P <0.05$) compared to the control group. The weight gain, lysozyme activity and total protein content of genistein treated fish did not differ significantly ($P>0.05$) from both control and *B. alba* treated fish. There was no significant difference ($P > 0.05$) in the food conversion ratio as well among the different treatment groups. The study indicates that *B. alba* methanol extract might positively influence the growth and protect the health status of tilapia.

Keywords: *Basella alba*, Genistein, Growth, Immunostimulation, Nile tilapia

Introduction

In the last three decades (1980–2010), world food fish production of aquaculture has expanded by almost 12 times, at an average annual rate of 8.8% (FAO, 2012). With increasing consumer demand, aquaculture is expanding in almost all regions of the world. Improving fish growth by treatment with different hormones such as thyroid hormones, androgens and growth hormones has been studied in several teleost species (Turan and Akyurt, 2005). Several chemical agents are used as immunostimulators to enhance innate or non-specific immune response in fish to augment fish growth (Citarasu, 2010). However, recent consumer demand for farm fish has increasingly stressed quality and safety, and the absence of concomitant pollutants, antibiotics and carcinogens. Traditional use of antibiotics and other chemotherapeutics in fish culture has been criticized because of the potential development of antibiotic-resistant bacteria, environmental pollution and accumulation of residues in fish. Since the European Union ratified a ban in 2006 for the use of all sub-therapeutic antibiotics intensified efforts to identify and develop safe dietary supplements and additives that enhance the life activity, health and immune system of farm fish (Chakraborty and Hancz, 2011; Chakraborty *et al*., 2014).

Plants are considered storehouse and sources of many safer and cheaper chemicals. Plant-derived compounds such as alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids and essential oils have been reported to promote various activities like antistress, growth promotion, appetite stimulation, tonic and immunostimulation, and antimicrobial properties in fish culture (Citarasu, 2010). Phytochemicals, in the form of herbal biomedicine has a long history, mainly in Asian countries (Ji *et al*., 2009). These might provide a useful source of new medicines, pharmaceutical entities and bioactive compounds for effective treatment of infectious fish diseases; enhancing fish health; and food safety and quality, while conserving aquatic environment. Herbal preparations are promising to be an important source of therapeutics in fish culture due to their antioxidant and antimicrobiological activities.

Genistein, a well-characterized isoflavone, is an important secondary plant metabolite that has been

important secondary plant metabolite that has been reported to be present in soybean meal and pulp mill effluents and exert estrogenic and/or antiestrogenic effects (Green and Kelly, 2009). Besides, attempts have been made to use the therapeutic potential of this isoflavone for growth induction and health promotion in fish (Chakraborty and Hancz, 2011; Chakraborty *et al*., 2014). Malabar spinach, *Basella alba* (family *Basellaceae*) is a fast growing vegetable, native to tropical Asia, probably originating from India or Indonesia and extremely heat tolerant (Bamidele *et al*., 2010). Methanol extract from the dry leaves of *B. alba* has been reported to possess active components that increase testosterone production in adult male rat testes during an *in vitro* study (Moundipa *et al*., 2005). This edible plant has also been described to possess nutritional values in traditional medicines of several countries (Siriwatanametanon *et al*, 2010). However, studies related to its *in vivo* effect on growth and immunostimulation of fish is limited. The Nile tilapia, *Oreochromis niloticus* (Linnaeus) is a well-studied, fast-growing and widely cultured fish species throughout the world. Plant-derived immunostimulants could improve the immune status of fish by raising the number of phagocytes and enhancing lysozyme and complement activities and the immunoglobulin level leading to increase in fish production (Chakraborty and Hancz, 2011; Chakraborty *et al*., 2014). Considering these aspects, the present study was focused to evaluate the efficacy of genistein and methanol extract of *B. alba* for promotion of general health and growth in Nile tilapia.

Materials and Methods

Chemicals and Reagents

All chemicals and reagents including genistein were purchased from Sigma Chemical Company (St. Louis, MO).

Extraction Procedure for *B. alba*

Fresh leaves of *B. alba* were collected from farmland in West Bengal, India, washed with distilled water and dried under shade at about 35-40ºC for several days. Air-dried leaves of *B. alba* (0.2 kg) were then grounded using an electric grinder and the powder was subjected to an organic solvent extraction by maceration under gentle agitation in a glass vessel for 48 h at room temperature using successively hexane (200 ml for 5 h, 3 times), methylene chloride (200 ml for 5 h, 3 times) and methanol (200 ml for 5 h, 3 times). The methanol extract was evaporated to dryness under pressure at 45ºC using a rotary evaporator and stored under nitrogen at -20ºC in amber glass bottle until it was used.

dry weight basis was calculated from the following equation:

$$\text{Yield (\%)} = (W_1 \times 100) / W_2$$

where W_1 was the weight of extract after evaporation of methanol and W_2 was the dry weight of the fresh plant sample.

Qualitative Phytochemical Analysis of Methanol Extract of *B. alba*

Qualitative phytochemical analysis of methanol extract of *B. alba* leaves was carried out using standard procedures to identify the presence of flavonoids, carbohydrates, steroids, tannins and saponins in the extract (Malpani *et al*., 2011; Kumar and Bhardwaj, 2012; Ray *et al*., 2013).

Determination of Antioxidant Activity

The free radical-scavenging activity of *B. alba* methanol extract was evaluated using the stable radical DPPH, according to the method of Masuda *et al*. (1999) with modifications (Maisuthisakul *et al*., 2007). A series of extract concentrations with different ratios of the extract to methanol, i.e. 1:10, $1:10^2$, $1:10^3$, $1:10^4$, $1:10^5$, $1:10^6$, $1:10^7$, were prepared. Then, 4.9 ml of each diluted plant extract was mixed with 100 µl of 5 mM DPPH in methanol. The mixtures of different extract concentrations and DPPH were placed in the dark at 37ºC for 30 min. The absorbance of each sample of plant extract containing DPPH (A_1) was read at 517 nm using a spectrophotometer (UV–vis model 1601, Shimadzu, Kyoto, Japan). The absorbance of each sample of plant extract dilution without DPPH (A_s), and only DPPH solution without plant extract (A_o, called control) were also recorded. All determinations were performed in triplicate. The percentage of DPPH radical-scavenging activity of the plant extract determined at these seven concentrations within the range of dose response (at least 10–90% reduction in absorbance) was calculated as shown:

$$\text{DPPH radical scavenging activity (\%)} = [\{A_0 - (A_1 - A_s)\} / A_0] \times 10$$

The percentage of DPPH radical-scavenging activity was plotted against the plant extract concentration (µg/ml) to determine the amount of extract necessary to decrease DPPH radical concentration by 50% (called EC_{50}). The EC_{50} value of each extract was estimated by sigmoid non-linear regression using SigmaPlot 2000 Demo (SPSS Inc., Chicago, IL, USA). The unit of EC_{50} was later converted to µg/µg DPPH. These values were changed to antiradical activity (A_{AR}) defined as $1/EC_{50}$.

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Preparation of Experimental Diets

Genistein was added to finely ground diets (crude protein 35.4%) using 10 ml of a 1:1 mixture of dimethylsulfoxide (DMSO) and ethanol for 1 kg feed (Green and Kelly, 2009) while methanol extract of *B. alba* was added after dissolving in DMSO only (Moundipa *et al*., 2005). Control diet was treated with a 1:1 mixture of DMSO and ethanol only. All the feed were then wetted with deionized water, mixed thoroughly, formed into pellets with a pelleter (diameter 2 mm), and dried at room temperature.

Experimental Design

The experiment was conducted on a group of adult tilapia (39.55 ± 0.8 g, 13.1 ± 0.09 cm) collected from research Institute for Fisheries, Aquaculture and Irrigation (HAKI, Szarvas, Hungary). Fish were transported to the laboratory, acclimatized to the laboratory condition and randomly distributed into glass aquaria (50 l) at a constant temperature of 20±2°C, and a density of 5 fish per tank with 4 replicates per treatment. Experimental diets were formulated and designated as follows: Control (CON), genistein (GEN) 1 g/kg and methanol extract of *B. alba* (BAS) 1 g/kg. Fish were fed ad libitum twice daily for 35 days with the experimental diets.

Analysis of Growth Parameters

After 35 days, all fish were measured individually for weight and length. Growth parameters such as specific growth rate (SGR) and weight gain (WG) were calculated as follows (Pechsiri and Yakupitiyage, 2005):

SGR (%body weight gain/day) = [(ln final weight–ln initial weight) / time (days)] × 100

WG (g) = mean final weight (g) – mean initial weight (g)

The total weight of liver was taken to measure the hepatosomatic index (HSI) according to the following formula (Sadekarpawar and Parikh, 2013):

HSI = [Liver weight (g) / Fish weight (g)] X 100

Blood Sampling and Separation of Leukocytes

Blood samples (4 fish/group) were collected from the caudal vein 35 days after start of feeding. Heparin was used as an anticoagulant. Leukocytes for assay were separated from each blood sample by density gradient centrifugation. One ml of histopaque 1.119 (Sigma) containing 100 µl of Bacto haemagglutination buffer, pH 7.3 (Difco, USA) was dispensed into siliconised tubes. One ml of a mixture of histopaque 1.077 and haemagglutination buffer was layered on the top. One ml of blood sample was then layered carefully on the top of the gradient. Sample preparations were centrifuged at 700 x g for 30 min at 4ºC. After centrifugation, plasma was collected and stored at −20°C for future analysis. Separated leukocytes were gently removed and dispensed into siliconised tubes, containing phenol red free Hank's balanced salt solution (HBSS). Cells were then washed in HBSS and adjusted to 107 viable cells/ml.

Respiratory Burst Activity

Respiratory burst activity of isolated leukocytes was quantified by the nitroblue tetrazolium (NBT) assay (Secombes, 1990), which measures the quantity of intracellular oxidative free radicals. This method was slightly changed, the concentration of NBT solution was 2 mg/ml.

Phagocytosis Assay

Phagocytosis activity of blood leukocytes was determined spectrophotometrically by the method of Seeley *et al*. (1990).

Lysozyme Assay

Plasma lysozyme activity was measured spectrophotometrically by the method of Sankaran and Gurnani (1972).

Plasma Total Protein Assay

The total protein concentration of the plasma was determined by a colorimetric assay based on the Biuret reaction, using a protein diagnostic reagent kit (Reanal, Hungary). The assay was performed in 96-well microtiter plates. 10 µl plasma, reagent blank or standard solution and 300 µl diluted Biuret solution was added to the wells in triplicates. After 20 min of incubation at room temperature, absorbance was measured with a multiscan spectrophotometer at 550 nm. The protein concentrations of the samples were calculated by the following formula: $y=A_m/A_{st} \times x$, where y is the protein concentration of the sample, A_m is the absorbance of the sample, A_{st} is the absorbance of the standard and x is the standard's known protein concentration.

Plasma Total Immunoglobulin Assay

This assay was similar to the previous one. 50 µl plasma and 50 µl polyethylene glycol (PEG) was added to each well of a 96-well microtiter plate. After 2 h of incubation at room temperature, plates were centrifuged at 1000 G for 15 min. The protein content of the supernatant was determined by the assay described above. This value was subtracted from the total protein level, and the result was equal to the total

immunoglobulin concentration of the plasma.

Statistical Analysis

All data are expressed in terms of mean ± standard error (SE). Different parameters were analyzed by one-way analysis of variance (ANOVA), where significant differences were found, a Tukey test was performed. All statistical analysis was performed using the SPSS version 13.0 for Windows.

Results

The yield of evaporated dried extracts based on dry weight basis with methanol as organic solvent was 6.5%. The methanol extract was found to contain steroids, alkaloids, soluble starch, tannins, saponins and flavonoids. The methanolic extract showed a free radical-scavenging activity (A_{AR}) of 0.64 ± 0.009.

The weight gain of tilapia fed diets supplemented with *Basella* extract (9.8±1.2 g) was significantly higher (P<0.05) compared to fish fed control diet (5.7±1.1 g), while tilapia fed genistein supplemented diet showed no significant difference (P>0.05) in weight gain (9.1±0.9 g) from both the treatment groups (Table 1). The BAS group showed the highest final individual mean body weight (49.9±1.9 g), final individual length (14.3±0.2 cm) and SGR (0.62±0.1). However, no significant difference (P>0.05) was observed for these growth parameters and HSI as well, among the three groups of fish (Table 1).

Both GEN and BAS groups showed significantly higher (P<0.05) respiratory burst and phagocytic activities compared to the CON group, while there was no significant difference (P>0.05) in immunoglobulin content between the three groups of fish (Table 2). The phagocytic activity of the BAS group is significantly higher (P<0.05) compared to the GEN group as well. The total plasma protein content of and the plasma lysozyme activity in BAS group was significantly higher (P<0.05) compared to the CON group, while the GEN group showed no significant difference (P>0.05) in protein content and plasma lysozyme activity from the other two treatment groups (Table 2).

Discussion

The growth enhancing and immunostimulating effect of an important dietary vegetable *B. alba* and the flavonid genistein have been investigated in this study. In order to eliminate the influence of the moisture content of the plant, the yield of the methanolic extract from *B. alba* leaves was calculated depending on a dry weight basis and the yield was found to be moderately high. Similar high yield from *B. alba* leaves has been reported with methanol in successive extraction with petroleum ether, ethyl acetate and methanol (Siriwatanametanon *et al*., 2010). In general, compounds such as flavonoids have been associated with the antioxidant activity of the plant extract. Other phytochemicals found to be present in the methanol extract of *B. alba* leaves are

Table 1. Comparative growth parameters in tilapia fed control, genistein and *B. alba* methanol extract supplemented diets after 35 days of culture*

Treatment category	Growth parameters				
	Final Weight (g)	Final Length (cm)	Weight Gain (g)	SGR (%)	Hepatosomatic index
Control	45.85 ± 1.9^{a}	14.0 ± 0.2^{a}	5.7 ± 1.1^{a}	0.37 ± 0.13^{a}	2.12 ± 0.3^{a}
Basella	49.9 ± 1.9^{a}	14.3 ± 0.2^{a}	9.8 ± 1.2^{b}	0.62 ± 0.13^{a}	2.57 ± 0.2^{a}
Genistein	47.55 ± 2.1^{a}	14.2 ± 0.2^{a}	9.1 ± 0.9^{ab}	0.6 ± 0.09^{a}	2.46 ± 0.2^{a}

*Notations a and b are to compare among the means of different treatment categories within the same column. Values with different superscripts are significantly different (P<0.05). Initial mean weight and length of fish for all treatment were 39.55 ± 0.8 g and 13.1 ± 0.09 cm, respectively.

Table 2. Comparative immunological parameters in tilapia fed control, genistein and *B. alba* methanol extract supplemented diets after 35 days of culture*

Treatment Category	Immunological parameters				
	Phagocytic Activity (OD 510 nm)	Respiratory burst activity (OD 620 nm)	Lysozyme (µg/ml)	Total protein (mg/ml)	Total Immunoglobulin (mg/ml)
Control	0.34 ± 0.03^{a}	0.13 ± 0.007^{a}	12.1 ± 0.5^{a}	41.46 ± 1.5^{a}	14.3 ± 0.9^{a}
Basella	1.09 ± 0.07^{c}	0.2 ± 0.01^{b}	13.25 ± 0.4^{b}	47.5 ± 1.5^{b}	20.1 ± 1.8^{a}
Genistein	0.8 ± 0.06^{b}	0.19 ± 0.009^{b}	13.16 ± 0.4^{ab}	42.1 ± 1.3^{ab}	18.6 ± 2.4^{a}

*Notations a, b and c are to compare among the means of different treatment categories within the same column. Values with different superscripts are significantly different (P<0.05).

reported to have many beneficial health effects as well (Chakraborty and Hancz, 2011; Chakraborty *et al*., 2014). However, quantitative estimation of flavonoids and phenols needs to be done with the extract to determine the correlation between the available phytochemicals and the antiradical activity of the plant.

B. alba leaves are found to be high in proteins, fat, vitamin A, vitamin C, vitamin E, vitamin K, vitamin B9 (folic acid), riboflavin, niacin, thiamine and minerals such as calcium, magnesium and iron. (Grubben and Denton, 2004). *B. alba* was shown to significantly increase red blood cell count, white blood cell count, packed cell volume, hemoglobin concentration and platelet count, thereby reducing anaemia and maintaining good health in Wister albino rats (Bamidele *et al*., 2010). Hepatosomatic Index (HSI) provides an indication on status of energy reserve in an animal. In a poor environment, fish usually have a smaller liver with less energy reserved in the liver (Sadekarpawar and Parikh, 2013). Although not significantly higher than the other groups, fish fed *B. alba* supplemented diet showed the highest HSI that indicated comparatively better general health of this group. The significant growth increase in fish fed diets supplemented with *B. alba* extract might be attributed to the general good health of the fish. Moreover, methanol extract of *B. alba* leaves has been reported to stimulate testosterone production in purified leydig cells of Sparague Dawley rats (Nantia *et al*., 2011). Dietary treatment with such extract might also stimulate androgen production and subsequent growth in tilapia. However, further study and analysis of androgen concentration is required to establish the exact mechanism of augmented growth in tilapia fed *B. alba* extract supplemented diet.

Genistein can have both estrogenic and antiestrogenic effects, either of which, in turn, can have stimulatory or suppressive actions on growth and reproduction in fish (Green and Kelly, 2009). In the present study, fish fed genistein supplemented diet showed no significant change in growth ($P>0.05$) compared to the control fish. Similarly, growth performance of rainbow trout *Oncorhynchus mykiss* was not affected by dietary treatments with genistein-enriched diets (Bennetau-Pelissero *et al*., 2001). Exposure to genistein showed no significant reduction in survival and growth in fathead minnows, *Pimephales promelas*. However, chronic exposure to high concentration of this phytoestrogen may result in reproductive impairment in fish (Ingham *et al*., 2004). Although isoflavone content within diets, soybean meal, and the beans themselves is highly variable, the increased replacement of fish meal with soybean products has increased the amount of total isoflavones in fish diets. Soy protein concentrate and diets with high proportions of soybean meal replacing fish meal contain a high amount of genistein. Under this context, further studies are required to determine the ideal treatment regime with this phytochemical during aquaculture.

The plant extract as well as the individual phytochemical were able to enhance the nonspecific immune response of Nile tilapia. The phagocytic activity of leukocytes is a primitive defence mechanism and an important characteristic of the non-specific innate immune system in fish (Jeney *et al*, 2009). Herbal medicine extracts have been reported to enhance phagocytosis in various fish species (Chakraborty and Hancz, 2011). In the present study, the phytochemicals significantly enhanced the phagocytic activity of leukocytes isolated from Nile tilapia, 35 days after the start of feeding. Phagocytes also produce toxic oxygen forms during a process called respiratory burst. This activity can be measured photometrically by detecting the amount of superoxide (O_2^-) anion. Feeding of genistein and *B. alba* extract were observed to increase the intracellular respiratory burst activity of fish phagocytic cells. Several herbs containing phytochemicals including flavonoids have been reported to enhance intracellular respiratory burst activity in leukocytes of different fishes including tilapia (Chakraborty and Hancz, 2011). The innate immune system also consists of different humoral elements like the complement system, lysozyme, transferrin, agglutinins and precipitins (Magnadóttir, 2006). Immunostimulants, vaccines and probiotics were observed to enhance the plasma lysozyme activity (Jeney *et al.*, 2009; Kim and Austin, 2006). In our experiment, a significantly ($P<0.05$) elevated lysozyme activity was measured at the end of the culture period in fish fed diets containing *B. alba* extract, while fish fed genistein supplemented diet showed marginal increase in lysozyme activity compared to the control group (Table 2). Though dietary administration of *B. alba* extract significantly increased ($P<0.05$) total protein level, the total immunoglobulin level of plasma was not significantly affected ($P>0.05$) by either the extract or genistein. Variable results have been observed regarding the effect of immunostimulating herbs on total protein and immunoglobulin levels in fish (Jeney *et al*, 2009).

Phytochemicals are natural bioactive compounds from plants with wide-ranging benefits to human health. Suggestions have been made that plant constituents might directly activate innate defense mechanisms by acting on receptors and trigger gene activation, which might result in production of anti-microbial molecules (Bricknell and Dalmo, 2005). Therefore it seems very likely that at least part of the stimulatory capacities of *B.alba* methanol extract might be associated with one or more components present in it. *B. alba* showed presence of tannins, flavonoids, saponins, steroids and phenolic compounds that are reported to have antioxidant, analgestic, anti-inflammatory, anti-hypertensive, cytotoxic and anti-microbial activity (Siriwatanametanon *et al*, 2010; Sivasankar *et al*.,

2011). The isoflavone genistein has been found to manifest potent antioxidative activity in the LPO assay in Japanese medaka, *Oryzias latipes* (Zhang *et al*., 2003).

The enhancement of non-specific immune responses might imply the immunostimulatory activity of the methanol extract of *B. alba* leaves and genistein. But, the *B. alba* extract seems to be a better growth promoting and immunostimulating agent, and can have a promising role in development of sustainable eco-friendly aquaculture. However, further investigation regarding the dietary treatment regime and mechanism of growth induction and immunostimulation with *B. alba* extract in fish is warranted. The principal bioactive compounds responsible for such functions must also be identified for comprehensive understanding of the plant's potential application in aquaculture.

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References

Bamidele, O., Akinnuga, A.M., Olorunfemi, J.O., Odetola, O.A., Oparaji, C.K. and Ezeigbo, N. 2010. Effects of aqueous extract of *Basella alba* leaves on haematological and biochemical parameters in albino rats. African Journal of Biotechnology, 9(41): 6952-6955. doi: 10.5897/AJB10.957.

Bennetau-Pelissero, C., Breton, B.B., Bennetau, B., Corraze, G., Le Menn, F., Davail-Cuisset, B., Helou, C. and Kaushik, S.J. 2001. Effect of genistein-enriched diets on the endocrine process of gametogenesis and on reproduction efficiency of the rainbow trout *Oncorhynchus mykiss*. General and Comparative Endocrinology, 121(2): 173-187. doi:10.1006/gcen.2000.7585

Bricknell, I. and Dalmo, R.A. 2005. The use of immunostimulants in fish larval aquaculture. Fish and Shellfish Immunology, 19(5): 457-472. doi: 10.1016/j.fsi.2005.03.008

Chakraborty, S.B. and Hancz, C. 2011. Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture. Reviews in Aquaculture, 3: 103-119. doi: 10.1111/j.1753-5131.2011.01048.x

Chakraborty, S.B., Horn, P. and Hancz, C. 2014. Application of phytochemicals as growth-promoters and endocrine modulators in fish culture. Reviews in Aquaculture, 6: 1-19. doi: 10.1111/raq.12021

Citarasu, T. 2010. Herbal biomedicines: a new opportunity for aquaculture industry. Aquaculture International, 18: 403–414. doi: 10.1007/s10499-009-9253-7

Food and Agriculture Organization of the United Nations (FAO). 2012. The State of World Fisheries and Aquaculture, Rome.

Green, C.C. and Kelly, A.M. 2009. Effects of the estrogen mimic genistein as a dietary component on sex differentiation and ethoxyresorufin-O-deethylase (EROD) activity in channel catfish (*Ictalurus punctatus*). Fish Physiology and Biochemistry, 35: 377–384. doi: 10.1007/s10695-008-9260-z

Grubben, G.J.H. and Denton, O.A. 2004. Plant Resources of Tropical Africa 2. Vegetable. PROTA Foundation, Wageningen; Backhuys, Leiden; CTA, Wageningen, 4: 103-111.

Ingham, R.R., Gesualdi, D.A., Toth, C.R. and Clotfelter, E.D. 2004. Effects of genistein on growth and development of aquatic vertebrates. Bulletin of Environmental Contamination and Toxicology, 72: 625-631. doi: 10.1007/s00128-004-0289-0.

Jeney, G., Yin, G., Ardó, L. and Jeney, Z. 2009. The use of immunostimulating herbs in fish. An overview of research. Fish Physiology and Biochemistry, 35: 669–676. doi: 10.1007/s10695-009-9304-z

Ji, S-C., Takaoka, O., Lee, S-W., Hwang, J-H., Kim, Y-S., Ishimaru, K., Seoka, M., Jeong, G-S. and Takii, K. 2009. Effect of dietary medicinal herbs on lipid metabolism and stress recovery in red sea bream *Pagrus major*. Fisheries Science, 75: 665–672. doi: 10.1007/s12562-009-0058-4

Kim, D. and Austin, B. 2006. Innate immune responses in rainbow trout (*Oncorhynchus mykiss*, Walbaum) induced by probiotics. Fish and Shellfish Immunology, 21: 513–524. doi: 10.1016/j.fsi.2006.02.007

Kumar, A. and Bhardwaj, A. 2012. Comparative, qualitative and quantitative chemotypic characterization among north Indian *Tribulus terrestris*. International Research Journal of Pharmacy, 3(6): 212-218.

Magnadóttir, B. 2006. Innate immunity of fish (overview). Fish and Shellfish Immunology, 20: 137–151. doi: 10.1016/j.fsi.2004.09.006

Maisuthisakul, P., Pongsawatmanit, R. and Gordon, M.H. 2007. Assessment of phenolic content and free-radical scavenging capacity of some Thai indigenous plants. Food Chemistry, 100(4): 1409–1418. doi: 10.1016/j.foodchem.2005.11.032

Malpani, M.O., Rajput, P.R., Mane, V.D. and Dhabe, P.S. 2011. Phytochemical screening, anifungal activity and curative impact on *Channa punctatus* fish of *Butea monosperma* (Lam): flower, leaves and gum. Der Pharmacia Lettre, 3(5): 271-280.

Masuda, T., Yonemori, S., Oyama, Y., Takeda, Y., Tanaka, T. and Andoh, T. 1999. Evaluation of the antioxidant activity of environmental plants: activity of the leaf extracts from seashore plants. Journal of Agricultural and Food Chemistry, 47(4): 1749–1754. doi: 10.1021/jf980864s

Moundipa, P.F., Beboy, N.S.E., Zelefack, F., Ngoula, S., Tsamo, E., Schill, W-B. and Monsees, T.K. 2005. Effects of *Basella alba* and *Hibiscus macranthus* extracts on testosterone production of adult rat and bull Leydig cells. Asian Journal of Andrology, 7(4): 411-417. doi: 10.1111/j.1745-7262.2005.00056.x

Nantia, E.A., Travert, C., Manfo, F-P.T., Carreau, S., Monsees, T.K. and Moundipa, P.F. 2011. Effects of the Methanol Extract of *Basella alba* L (Basellaceae) on Steroid Production in Leydig Cells. International Journal of Molecular Sciences, 12(1): 376-384. doi:10.3390/ijms12010376

Pechsiri, J. and Yakupitiyage, A. 2005. A comparative study of growth and feed utilization efficiency of sex-reversed diploid and triploid Nile tilapia, *Oreochromis*

niloticus L. Aquaculture Research, 36(1): 45-51. doi: 10.1111/j.1365-2109.2004.01182.x.

Ray, S., Chatterjee, S. and Chakrabarti, C.S. 2013. Antiprolifertive activity of allelochemicals present in aqueous extract of *Synedrella nodiflora* (L.) Gaertn. in apical meristems and Wistar rat bone marrow cells. IOSR Journal of Pharmacy, 3(2): 1-10. doi: 10.9790/3013-3220110

Sadekarpawar, S. and Parikh, P. 2013. Gonadosomatic and hepatosomatic indices of freshwater fish *Oreochromis mossambicus* in response to a plant nutrient. World Journal of Zoology, 8(1): 110-118. doi: 10.5829/idosi.wjz.2013.8.1.7268

Sankaran, K. and Gurnani, S. 1972. On the variation in the catalytic activity of lysozyme in fishes. Indian Journal of Biochemistry & Biophysics, 9(2): 62–165.

Secombes, C.J. 1990. Isolation of salmonid macrophages and analysis of their killing activity. In: J.S. Stolen, D.P. Anderson, B.S. Robertson, W.B. van Miuswinkell (Eds.), Techniques in Fish Immunology, SOS Publications, Fair Haven, New Jersey, USA: 137–154.

Seeley, K.R., Gillespie, P.D. and Weeks, B.A. 1990. A simple technique for the rapid spectrophotometric determination of phagocytosis by fish macrophages. Marine Environmental Research, 30(1): 37-41. doi: 10.1016/0141-1136(90)90009-D

Siriwatanametanon, N., Fiebich, B.L., Efferth, T., Prietoa, J.M. and Heinrich, M. 2010. Traditionally used Thai medicinal plants: In vitro anti-inflammatory, anticancer and antioxidant activities. Journal of Ethnopharmacology, 130(2): 196–207. doi: 10.1016/j.jep.2010.04.036

Sivasankar, V., Moorthi, A., Sarathi Kannan, D. and Suganya devi, P. 2011. Anthocyanin, and its antioxidant properties in *Basella alba*. Journal of Pharmacy Research; 4(3): 800-806.

Turan, F. and Akyurt, I. 2005. Effects of androstenedione, a phytoandrogen, on growth and body composition in the African catfish *Clarias gariepinus*. The Israeli Journal of Aquaculture – Bamidgeh, 57(1): 62-66.

Zhang, L., Khan, I.A., Willett, K.L. and Foran, C.M. 2003. In vivo effects of black cohosh and genistein on estrogenic activity and lipid peroxidation in Japanese Medaka (*Oryzias latipes*). Journal of Herbal Pharmacotherapy, 3(3): 33-50. doi: 10.1080/J157v03n03_04