

Effect of Methanolic Extract of *Andrographis paniculata* (Nees) on Growth and Haematology of *Oreochromis mossambicus* (Peters)

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Abstract: A 45 day study was undertaken to evaluate the effect of dietary aqueous extract of *Andrographis paniculata* in Tilapia fish, *Oreochromis mossambicus* on growth performance and basic haematological parameters. Fish were randomly distributed into glass aquaria at 15 fish /tank in triplicate. Five diets (45% crude protein) containing varying levels of aqueous extracts of *A. paniculata* at 0, 500, 1000, 2000 and 3000 mg/kg were prepared and fed to the fish twice daily at 2% of their body weight. The results for each test group were compared statistically with those for the control. Analysis of the results showed a significant increase in RBCs, WBCs, thrombocytes, Hb, PCV, and MCHC, and significant decrease in weight gain. The increase in cellular immunological indicators such as RBCs, WBCs and thrombocytes in the experimental fish may be due to the increase in the levels of immunity which in turn could be due to the action of the extract present in the diets. The critical examination of the parameters of the various treatment groups shows that fish treated with an extract of 2000 mg/kg of feed exhibited more optimum features in most of the haematological and growth factors. The observations made in the present investigation show that *A. paniculata* can act as an immunostimulant even at low concentrations and, hence, *A. paniculata* can be considered as a potential as well as a natural additive to fish feed in commercial aquaculture applications to augment the cellular immunity to control the common diseases of fish.

Key words: Haematology • Erythropoiesis • Leucopoeisis • Immunostimulant • *Andrographis paniculata*
• *Oreochromis mossambicus*

INTRODUCTION

Fish culture is an age-old practice in India, which is the second largest culture fish producer in the world. With the emergence of large-scale commercial fish culture, diseases of varied etiology are being increasingly recognized as a major hurdle to successful and sustainable farming [1]. The use of chemotherapeutics for controlling diseases has been criticized for their negative impacts, including accumulation of tissue residues, environmental pollution, development of drug resistance in pathogens and immunosuppression [2]. There is a need to look for ecofriendly disease preventive measures to promote sustainable fish culture in India. In order to reduce the risk of disease at different stages of growth, the level of resistance to infection in the cultured organisms should be increased by the use of better feed, vaccines, immunostimulants or by selective breeding for

disease resistance [3]. An immunostimulant is a substance that elevates non-specific defense mechanisms and specific immune response if the treatment is followed by vaccination or infection [5]. They increase resistance to disease by enhancing the non-specific immune system and their use has been given considerable attention in aquaculture. Immunostimulants, used in vaccines to amplify the specific immune response or administered as feed additives to modulate non-specific immunity, have been demonstrated to play role in protection against diseases in fish [4] and enhancing growth [5]. The modulation of immune response by using medicinal plant products as a possible therapeutic measure has become a subject of active scientific investigations [6].

India has a rich resource of traditional herbal medicines to treat human and animal diseases. These have no or little side effects during treatment. Commonly used herbal extracts are from *Ocimum sanctum* (Tulsi),

Withania somnifera (Ashwagandha) *Tinospora cordifolia* (Guduchi) and *Emblia officinalis* (Amlaki) for the treatment of immunosuppressive conditions for humans and animals [7]. Though many synthetic and natural substances have been tested for their immunostimulating abilities, traditional medicinal herbs seem to have the potential to be a rich source of active substances for immunomodulation. Indian medicinal plants are a rich source of substances that are claimed to induce paraimmunity, the nonspecific immunomodulation of granulocytes, macrophages, natural killer cells and complement functions in mammalian models.

An extensive review on the use of immunostimulating herbs in fish was made by Jeney *et al.* [8] and they opined that the herbal extracts can be used in fish culture as alternatives to vaccines, antibiotics or chemotherapeutic agents. Harikrishnan *et al.* [9] have studied innate immune response and disease resistance in *Carassius auratus* by triherbal solvent extracts. The results showed that ethanol triherbal solvent extract from *Azadirachta indica*, *Ocimum sanctum* and *Curcuma longa* enhanced the phagocytic activity of leucocytes after fourth week. Citarasu [10] had made a review on herbal biomedicines and advocated that the herbal biomedicines are a promising opportunity for aquaculture industry. The review shows that the herbal based drugs act as growth promoter, immunostimulants, antibacterial agents etc. in cultured fishes [11]. India is a large depository of medicinal plants but studies of the effects of a number of medicinal plant extracts on immunostimulation and growth promotion of cultured fishes are fragmentary. The efficacy of *A. paniculata* on growth and immunostimulating mechanism in fishes has not yet been reported from India or elsewhere. Hence, the present experiment has been formulated to understand the efficiency of methanolic extract of *A. paniculata* (Nees) on growth and immunostimulation in a cichlid, *Oreochromis mossambicus* (Peters). The outcome of this study may help to get a wider acceptance for the usage of medicinal plants in disease prevention and improved health management in aquaculture practices.

MATERIALS AND METHODS

Experimental Design: *A. paniculata* was collected from the medicinal garden of Kerala University campus and authenticated by Department of Botany, University of Kerala. The extraction was done in a Soxhlet apparatus for 72 hours using methanol as solvent. The sun dried leaves and shoots of the *A. paniculata* was used for this purpose. The extract was concentrated using a rotary vacuum evaporator and after complete evaporation of the solvent, residue of the extract was stored in refrigerator. This extract was used in different concentrations for the preparation of experimental diets. A basal diet (D1) containing 40% crude protein which has been proved to be the optimum protein for the growth of this fish [12] has been prepared. Graded level of methanol extract of *A. paniculata* were added to the basal diet at 500, 1000, 2000, and 3000 mg/kg and designated as treatments D2, D3, D4, and D5 respectively. The feed stuffs were thoroughly mixed and hot water was added at intervals to gelatinize starch and then steam cooked in a pressure cooker. After cooking, vitamin mineral mixture, fish oil and plant extract were added. Then the diets were pelletized using a hand pelletiser and sun dried to a moisture level of below 10%. These pellets are broken into small pieces and stored in air tight containers and labeled. The proximate composition of the experimental diets is given in Table 1.

The fish, *O. mossambicus* was collected from a freshwater pond near Thiruvananthapuram city and the experiment was conducted in glass aquaria with well water. After measuring the individual weights, male fish with body weight ranging from 20.00 to 40.00 g were randomly distributed into glass aquaria, at fifteen fish/tank in triplicate treatments. These fish were acclimatized for five days in the respective aquaria and fed with a balanced diet prepared in the laboratory. Tanks were aerated throughout the period of experiments. The water quality in glass aquaria was maintained by removal of faecal matter and the water was partially (a quarter) replaced every day with the same quantity of matured well

Table 1: Proximate composition of major ingredients used for diet preparation

Ingredients	Crude Protein (CP)	Ether extract (lipid)	Nitrogen free extract (NFE)	Crude fibre (CF)	Ash
Fish meal	45	12	8.2	2.9	21.3
Soyabean flour	50	6.4	34.6	5.1	6.4
GNOC flour	38	15.2	28.5	11.8	6.2
Wheat flour	13.9	8.3	60.1	13.1	4.6
Tapioca flour	2	0.6	92.5	27	2.2

Table 2: Ingredients and proximate composition of the Experimental diets

Ingredients	% level	Control			Experiments	
		D1	D2	D3	D4	D5
Fish meal	25	250	250	250	250	250
Groundnut oil cake flour	30	300	300	300	300	300
Soyabean flour	35	350	350	350	350	350
Tapioca flour	3	30	30	30	30	30
Wheat flour	3	30	30	30	30	30
Vitamin & mineral mixture	3	30	30	30	30	30
Fish oil	1	10	10	10	10	10
Methanolic extract of <i>A.paniculata</i> Nees (mg/kg)			500	1000	2000	3000

Vitamin /mineral mixture : Vitamin A- 700000 IU, Vitamin D3 70000 IU, Vitamin E- 250mg, Nicotinamide- 1000mg, Cobalt- 150 mg, Copper- 1200mg, Iodine- 325mg, Iron- 1500mg, Magnesium-6000mg, Manganese-1500mg, Potassium-100mg, Selenium-10mg, Sodium-5mg, Sulphur-0.72%, Zinc-9600mg, Calcium 25.5%, Phosphorus-12.75.

water of the same source. The water quality parameters like temperature and pH were monitored throughout the study. The water temperature ranged from 28°C to 31°C and the pH ranges between 6.9 and 7.1. Animals are fed daily at *ad libitum* at morning and evening. There was no mortality observed throughout the experiment. The final weights of the fish were taken after 45 days of rearing.

Blood Sample Collection: At the end of the experiment, feeding was suspended for 24 hours before blood samples were collected. From randomly picked fish, after anaesthetizing with MS-222, blood was collected from the caudal vein with a 1 ml plastic syringe with heparin. Individual fish were sampled only once to avoid the influence on the assays due to multiple bleeding and handling stress on fish.

Determination of Haematological Parameters: Haematological analyses were carried out by standard methods suggested by [13]. Haemoglobin estimation was done by acid-haematin method using Sahli's haemoglobinometer and the value is expressed in g%. The packed cell volume (PCV) was determined by microhaematocrit tube method [14] and has been calculated using the following formula:

$$\text{PCV} = \frac{\text{Height of RBC column after centrifugation}}{\text{Total height of the blood column}} \times 100$$

Total erythrocyte count, total leucocyte count and total thrombocytes were determined by using a Neubauer's haemocytometer. Hendricks solution [15] is used as the diluting fluid for RBCs, WBCs and

thrombocytes. These data were used to calculate the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and the mean corpuscular haemoglobin concentration (MCHC) suggested by Dacie and Lewis [16].

Statistical Analysis: Experimental data are presented as mean±SD and were analyzed with one way Analysis Of Variance (ANOVA) followed by Tukey's test to compare the means between individual treatments in SPSS at a significance level of P<0.05.

RESULTS

The results on growth of the fishes are given in table 3. Generally there is an increase in the body weight of the fishes in control and all experimental groups. The increase in the weight gain has showed a highly significant between the control and experiment one (D2) and no statistically significant difference between the other experimental groups. Body weight gain in D2 is significantly higher than those of groups D4 at 1% level (P<0.01) and D5 at 5% level (P<0.05). SGR also showed similar pattern of increase.

The results of the haematological parameters have been given in table 4. RBCs count has been showed an increasing trend in all experimental groups when compared to control and except in group 1 (D2) this increasing trend is statistically significant. The RBCs values ranged from 1.55-4.92. The highest mean value of 4.65±0.14 was observed in D4 and the lowest one in control. The RBCs count shows significant increase in D4 and D5 (P<0.01) when compared to D3 and there is significant increase between the treatments of D3 and D4.

Table 3: The Growth Performance of *O. mossambicus*

Parameters	Control				Experiments					
	D1		D2		D3		D4		D5	
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
Initial weight (g)	20.50-40.00	30.10±5.34	25.00-40.00	32.41±6.35	20.00-31.00	24.47±3.76	20.00-32.00	26.24±3.23	20.00-34.00	25.07±4.12
Final weight (g)	26.00-45.00	36.60±7.45	32.00-53.00	41.31±5.42	23.00-42.00	32.04±6.54	24.50-41.00	32.70±4.64	26.00-40.50	31.98±3.65
Weight gain (g)	5.00- 9.50	6.50±2.46	6.00- 12.50	8.92±1.05**	3.50- 13.00	7.58±0.73	4.00- 11.00	6.47±0.32***	4.50- 10.50	6.92±0.33**
SGR (%)	11.11-21.11	14.44±2.54	11.11-27.11	19.81±2.35**	7.29- 27.77	16.83±1.62	7.77- 24.44	14.36±0.71***	8.88- 24.44	15.36±0.74

*** indicates significant difference with control and * and ** indicates the significant difference within the treatments D2 to D4. ** and * indicates significance at 1% and 5% level respectively.

Table 4: Haematological Characteristics of *O. mossambicus*

Blood Parameters	Control				Experiments					
	D1		D2		D3		D4		D5	
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
RBC x 10 ⁶ /mm ³	1.62-2.48	2.04±0.43	1.56- 2.65	2.32±0.21	2.99- 4.22	3.65±0.22***	3.04- 5.19	4.65±0.14***	3.43- 4.92	4.29±0.02***
WBC x 10 ³ /mm ³	1.65-3.50	2.18±0.84	1.75- 5.88	2.77±0.79	4.65- 6.75	5.63±0.09***	5.24- 6.84	6.23±0.29***	5.03- 7.02	6.08±0.09***
Thrombocytes x 10 ³ /mm ³	3.81-6.15	4.54±4.65	4.03- 29.20	11.70±5.28*	20.70- 30.05	27.05±1.51***	26.90- 40.50	37.17±0.49***	20.00-38.65	34.02±0.74***
Hb (g%)	5.40-7.20	6.40±0.26	7.80- 10.00	8.38±0.16**	8.40- 10.00	8.92±0.21***	8.40-10.00	9.18±0.08***	8.00- 10.40	9.55±0.20***
PCV (%)	16.67-30.18	23.78±3.80	24.48- 48.97	35.4±2.91**	31.62- 45.00	33.62±2.38**	25.00-50.00	37.91±2.93**	22.90- 34.78	27.45±1.65***
MCV (μm ³)	85.45-154.08	116.78±18.58	91.21- 222.48	155.27±20.86	67.10- 116.21	75.00±37.94***	70.45-118.34	81.66±5.48**	51.55- 101.39	65.10±4.29***
MCH (pg)	27.08-34.61	31.47±3.12	31.77- 44.66	37.09±2.92*	19.72- 30.02	24.70±2.25***	17.68- 26.97	20.11±0.43***	19.23- 28.65	22.51±0.60***
MCHC (%)	2.12-3.95	2.81±0.42	1.63- 4.45	2.44±0.29	1.91- 3.63	2.69±0.15	2.00- 2.75	2.48±0.16	2.70- 4.13	3.43±0.18***

* indicates significant difference with control and ** and *** indicates the significant difference within the treatments D2 to D4. ** and * indicates significance at 1% and 5% level respectively.

The D5 group has showed significant increase when compared to D3 and D4. The WBCs count in D3, D4, D5, were significantly higher than those of control group (D1) and experiment one (D2) ($P<0.01$). The values for WBC ranged from 1.65-7.02 and the highest mean value was observed in D4 (6.23 ± 0.29) and the lowest one in control. The thrombocytes count showed all-time higher values than the control and the increase is statistically significant in all experimental groups when compared to control. In addition, the thrombocytes count in D3, D4 and D5 were significantly higher than that of experiment D2 ($p<0.01$). Haemoglobin values in all experimental groups also showed an upward trend when compared to control. This increase is statistically significant. These values are significant when compare between the experiments D2-D4. The Hb in D3, D4 and D5 have significant increase with reference to experiment D2, and the Hb in D5 is significantly higher than that of experiment D3 ($p<0.01$). The haemoglobin value is ranged between 5.40 and 10.40 and the highest mean value is found in D5 (9.55 ± 0.20) and the lowest is in D1 (6.40 ± 0.26). The PCV in all experimental groups reported higher values than the control. The PCV value of the group D5 has not varied much from the control but showed highly significant downward trend between other experimental groups ($P<0.01$). The MCV registered a higher value than that of control group for the experiment D2 and for all other groups a drastic decline is observed. The decline in MCV between control and D5 is

statistically significant. D3, D4, D5 have significant reduction than that of D2 ($P<0.01$). The MCH values also exhibited the similar pattern of trends as like MCV and the values ranges between 1.63 - 4.13 and the highest mean value is reported in D5 (3.43 ± 0.18) and the lowest one in D2 (2.44 ± 0.29).

DISCUSSION

To develop alternative practices for growth promotion and disease management in aquaculture, attention has also been focused in identifying novel drugs, especially from plant sources. These drugs may be delivered to the cultivable organisms either through feed supplementation or oral delivery through predator larvae or any other microparticulate diets mode. Several herbs have been tested for their growth promoting activity in aquatic animals [17, 18]. The present study demonstrated that diets supplemented with methanolic extracts from *A. paniculata* enhanced growth and immunity in *O. mossambicus* fed with 45 days. The growth of the fish has been increased in all experimental groups when compared to control except in D4 which reported a similar value as control. Among the tested diets, D2 showed highest rate of growth and the enhanced growth rate could be due to the growth promoting effect of *A. paniculata* extract as reported by Mathivanan *et al.* [19] in broilers.

There is all time high value for RBCs in all experimental groups when compared with control. This result is in conformity with that of Sahu *et al.* [20] who reported that RBCs counts were higher in *Labe rohita* fingerlings fed *Magnifera indica* kernel and they postulates that this increase is an indication of enhanced cellular immunity. This increase in RBCs values in the treated group may be because of the possible mechanism by which the *A. paniculata* extract may trigger erythropoiesis and also decrease in the rate of oxidant induced haemolysis due to the presence of the antioxidants present in the plant extract as reported by Sheeja *et al.* [21].

It is generally accepted that WBCs such as monocytes, granulocytes, neutrophils and macrophages are main components of the non-specific immune system [22]. Within aquaculture there are studies reporting herbal medicine extracts can be used as immunostimulants to enhance non-specific immune system of cultured fish species [22]. In the present study also the extract supplemented diets induced significant increase in total WBCs count. Similar results are obtained by Sahu *et al.* [20] when *L. rohita* has been fed with *Magnifera indica* kernel and by Dada and Ikurowo [23] when studied the effect of ethanolic extract of *G. cambogia* in catfish, *Clarias gariepinus* brood stock. Some others such as Gopalakannan and Arul [24] and Mohamad and Abasali [25] are also reported similar observations in fishes. The proliferation of WBCs in a dose dependent manner in experimental groups may be because of the leucopoiesis, particularly lymphopoiesis as a response to enhanced immunity.

Fish blood contains nucleated cells termed thrombocytes which are thought to be functionally analogous to mammalian platelets. There are reports suggests that fish thrombocytes have phagocytic ability, and participate in defense mechanisms [26, 27]. Piscine thrombocytes represent a link between innate and adaptive immunity [28] and express surface and intracellular molecules that are involved in the immune function [29]. It is already agreed that the fish thrombocytes are blood phagocytes that form one of the protective barriers [11, 30, 31]. In the present study, the thrombocytes count is increased in all experiments and the same is significant in D2 to D5 groups. The major reason for this enhanced concentration of thrombocytes in the experimental groups may be their participatory role in immune functions as observed by Kollner *et al.* [29], since the antibiotic properties such as antiviral, anti fungal and antimicrobial properties of the *A. paniculata*.

The blood indices such as MCV, MCH and MCHC are particularly important for the diagnosis of anemia in most animals [32]. The MCV and MCH values were decreased in most of the plant extract treated groups. MCHC has been maintained a steady state in experimental groups when compared to control except in D5 where the value showed an increase. The maintenance of constant level or increase of MCHC of tilapia fed on *A. paniculata* extract may be attributed to improvement of fish health as suggested by Suresh and Amolkumar [33].

The overall results of the present study proved that the extract of *A. paniculata* induced the innate immunity of the fish in all treated groups. A critical examination of the parameters of the various experimental groups shows that fishes treated with extract of 1000mg/kg feed (D3) exhibited higher growth rate and at the same time this dose seems to be sufficient to swell the innate immunity by way of enhancing the major haematological parameters. Hence, from the present results it is recommended a dose of 1000mg/kg feed of plant extract for this species of fish culture conditions.

Our results suggest the protective ability of *A. paniculata* mediated through cellular and may be non cellular immune mechanisms, as evident from the enhanced haematological parameters such as RBCs, WBCs and thrombocytes. The exact mechanism of action of the leaf extract on the immune system of fish is not known. But it has been observed that the immunostimulant might act directly on the immunopoietic cells [34]. The leaf of *A. paniculata* has been shown to contain water soluble phenolic compounds, and various other constituents such as alkaloids, glycosides, saponins, etc. [35] that might act as a potential immunostimulant. However, the active principle responsible for the immunostimulatory property observed in the present study has to be identified. Similar immunostimulatory effect has been observed in *O. mossambicus* administered with azadirachtin, a triterpenoid derived from the seed kernel of *Azadirachta indica* [36] and with other plant extracts in mice [37] rats [38] and broiler chicken [39]. The prospects of using natural products including plant extracts in the treatment of Epizootic Ulcerative Syndrome [40] and some parasitic diseases like myxobolosis, trichodinosis, gyrodactylosis, argulosis, etc., in farmed tropical freshwater fish has been reported [41]. The results of the present investigation show that *A. paniculata* can act as an immunostimulant even at low concentrations. Even though the exact modulation of immune response elicited

by the natural compound in fish is not fully understood, it has been observed that this plant extract will function as an immunostimulant and might act directly on the immunopoietic cells. The critical examination of the parameters of the various treatment groups shows that a dosage of 1000mg/kg of feed is sufficient to maintain the growth rate and enhance innate immunity.

REFERENCES

1. Rao, K.G., C.V. Mohan and D. Seenappa, 1992. The use of chemotherapeutic agents in fish culture in India, in *Diseases in asian aquaculture I* edited by Shariff I.M., Subasinghe R.P., Arthur J.R., (Fish Health Section, Asian Fisheries Society, Manila, Philippines), 505.
2. Ellis, A.E., 1988. General principles of fish vaccination, in *Fish vaccination*, edited by Ellis A E: (Academic Press, London), pp: 20.
3. Raa, J., G. Roerstad, R. Ingested and B. Robertson, 1922. The use of immunostimulants to increase resistance of aquatic organisms to microbial infections, in *Diseases in asian aquaculture I* edited by Shariff, I.M., R.P. Subasinghe and J.R. Arthur, (Fish Health Section, Asian Fisheries Society, Manila, Philippines), pp: 39.
4. Jaya, K., T. Swain and P.K. Sahoo, 2003. Dietary bovine lactoferrin induces changes in immunity level and disease resistance in Asian catfish *Clarias batrachus*, Vet. Immunol Immunopathol., 94: I.
5. Siwicki, A.K. and M. Korwin-Kossakowski, 1988. The influence of levamisole on the growth of carp (*Cyprinus carpio* L.) larvae, J. Appl. Ichthyol., 4: 178.
6. Upadhyay, S.N., *Immunomodulation* (Narosa Publishing House New Delhi).
7. Devasagayam, T.P.A. and S.K. Sainis, 2002. Immune system and antioxidants, especially those derived from Indian medicinal plants, Indian J. Expt. Biol., 40: 639.
8. Jeney, G., L.. Yin and Z.J. Ardo, 2009. The use of immunostimulation herbs in fish. An overview of research. Fish physiol Biochem. 35: 669-676.
9. Harikrishnan, R., C. Balasundaram M.C. Kim J.S. Kim Y.J. Han and M.S. Heo, 2009. Innate immune response and disease resistance in *Carassius auratus* by triherbal solvent extracts. Fish Shellfish Immunol., 27: 508-515.
10. Citarasu, T., 2010. Herbal biomedicines a new opportunity for aquaculture industry Aquacult Int., 18: 403-414.
11. Prasad, G. and G. L. Priyanka, 2011. Effect of fruit rind extract of *Garcinia gummi-gutta* on haematology and plasma biochemistry of catfish *Pangasianodon hypophthalmus*. Asian J. Biochem., 6(3): 240-251.
12. Zhao, M., S. Xie, X. Zhu, Y. Yang, N. Gan and L. Song, 2006. Effect of dietary Cyanobacteria on growth and accumulation of microcystins in Nile tilapia (*Oreochromis mossambicus*). Aquaculture, 261: 960-966.
13. Blaxhall, P.C. and K.E. Daisley, 1973. Routine haematological methods for use with fish blood. J. Fish Biol., 5: 771-781.
14. Bull, B.S., J.A. Koepke, E. Simson and O.W. Assendelft, 2000. Procedure for determining packed cell volume by the microhematocrit method approved standard, 3rd edn, NCCLC document. Clinical and Laboratory standards Institute, Wayne PA. H7-A3 20(18).
15. Hendricks, L.J., 1952. Erythrocytes counts and haemoglobin determinations for two species of sucker, genus *Catostomus* from Colorado. Copeia, 4: 265-266.
16. Dacie, J.V. and S.M. Lewis, 1984. Practical Haematology 6. Ed. New York, Churchill, pp: 22.
17. Rao, Y.V., B.K. Das, J. Pradhan and R. Chakrabarthi, 2006. Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. Fish Shellfish Immunol., 20: 263-273.
18. Immanuel, G., T. Citarasu, V. Sivaram, B.M. Michael and A. Palavesam, 2007. Delivery of HUFA, probionts and biomedicine through biocapsulated Artemia as a means to enhance the growth and survival and reduce the pathogenicity in shrimp *Penaeus monodon* post larvae. Aquacult Internat, 15: 137-152.
19. Mathivanan, R., C.S. Edwin and K. Viswanathan, 2008. Effect of *A. paniculata* supplementation on growth and feed conversion efficiency of broilers. Indian J. Poultry Sci. 43: ISSN :0019-5529.
20. Sahu, S., B.K. Das, J. Pradhan, B.C. Mohapatra, B.K. Mishra and N.N. Sarangi, 2007. Effect of *Magnifera indica* kernel as a feed additive in immunity and resistance to *Aeromonas hydrophila* in *Labeo rohita* fingerlings. Fish Shellfish Immunol., 23: 109-118.
21. Sheeja, K., P.K. Shihab and G. Kuttan, 2006. Antioxidant and anti-inflammatory activities of the plant *Andrographis paniculata* Nees. J. Immunopharmacology and Immunotoxicol., 28(1): 129-140.

22. Ardo, L., G. Yin, P. Xu, L. Varadi, G. Szigeti, Z. Jeney, and G. Jeney, 2008. Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of Nile tilapia (*Oreochromis mossambicus*) and resistance against *Aeromonas hydrophila*. Aquaculture, 275: 26-33.
23. Dada, A.A. and M. Ikuero, 2009. Effects of ethanolic extracts of *Garcinia kola* seeds on growth and haematology of catfish (*Clarias gariepinus*) broodstock. African J. Agri. Res., 4: 344-347.
24. Gopalakannan, A. and V. Arul, 2006. Immunostimulatory effects of dietary intake of chitin, Chitosan and levamisole on the immune system of *Cyprinus carpio* and control of *Aeromonas hydrophila* infection in ponds. Aquaculture, 255: 179-187.
25. Mohamad, S. and H. Abasali, 2010. Effect of Plant Extract Supplemented Diets on Immunity and Resistance to *Aeromonas hydrophila* in common Carp (*Cyprinus carpio*). Agri. J., 5(2): 119-127.
26. Stosik, M., 1993. Morphology and phagocytic activity of common carp, *Cyprinus carpio* L. thrombocytes. Med. Wet., 49: 184-185. Stosik, M. and W. Deptula, 1992) Thrombocytes in fish. Med. Wet. 48: 556-558.
27. Stosik, M. and W. Deptula, 1992. Thrombocytes in fish. Med. Wet., 48: 556-558.
28. Passantino, L., A. Cianciotta, R. Patruno, M.R. Ribaud, E. Jirillo and G.F. Passantino, 2005. Do fish thrombocytes play an immunological role? Their cytoenzymatic profiles and function during an accidental piscine candidiasis in aquarium. Immunopharmacol Immunotoxicol., 27: 345-356.
29. Kollner, B.U. Fischer J.H.W.M. Rombout, J.J. Taverne-Thiele and J.D. Hansen, 2004. Potential involvement of Rainbow trout thrombocytes in immune functions: a study using a panel of monoclonal antibodies and RT-PCR. Dev Comp Immunol., 28: 1049-1062.
30. Tavares-Dias, M. and F.R. Moraes, 2004. Haematology in Teleost Fish. Sao Paulo: Ribeirao Preto.
31. Prasad, G. and S. Charles, 2010. Haematology and leucocyte enzyme cytochemistry of a threatened yellow catfish *Horabagrus brachysoma* (Günther 1864) Fish Physiol. Biochem., 36: 435-443.
32. Colese, 1986. Veterinary clinical pathology Philadelphia, Saunders, pp: 615.
33. Suresh, R.N. and H. Amolkumar, 2009. Evaluation of immunomodulatory activity of an extract of Andrographolides from *Andrographis paniculata*. Thiemee J. Planta Med., 75(8): 785-791.
34. Jeney, G. and D.P. Anderson, 1993. Enhanced immune response and protection in rainbow trout to *Aeromonas salmonicida* bacteria following prior immersion in immunostimulants. Fish Shell. Immunol., 33: 51-58.
35. Jayalakshmi, T., A. Sankaravadiyoo and S. Savitha, 2010. Comparative study of phytochemical studies and antibacterial activity of *Andrographis Paniculata*, *Ocimum Sanctum* and *Phyllanthus niruri*. J. Ecotoxicol. Environ. Monit., 20(2): 169-173.
36. Logambal, S.M. and R.D. Michael, 1997. Azadirachtin-An immunostimulant in *Oreochromis mossambicus* (Peters) Abstract, National Workshop on fish and shellfish health management, CIFA, Bhubaneswar, India.
37. Ray, A., B.D. Banerjee and P. Sen, 1996. Modulation of humoral and cell mediated immune responses by *Azadirachta indica* (Neem) in mice. Indian J. Exp. Biol., 34: 698-701.
38. Wali, N., S. Dhawan, S. Garg and S.N. Upadhyay, 1993. Antiinflammatory effect of extract of neem leaf. In Chary, MS, Singh RP, Krus and Saxena RC (eds). Abstract. World Neem Conference, Bangalore, India.
39. Sadekar, R.D., A.Y. Kolle, B.S. Barmase and V.F. Desai, 1998. Immunopotentiating effects of *Azadirachta indica* (Neem) dry leaves powder in broilers, naturally infected with IBD virus. Indian J. Exp. Biol., 36: 1151-1153.
40. Campbell, R.E., J.H. Lilley and R.H. Richards, 1998. The use of natural products in the treatment of EUS (Epizootic Ulcerative Syndrome). In Kane A.S. and S.L. Poynton (eds), Proceedings of the International Symposium on Aquatic Animal Health. Baltimore, U.S.A, pp: 114.
41. Dey, R.K., 1997. On the use of herbal materials for managing diseases and health conditions of fish during sustainable aquaculture practices. Abstract, National workshop on fish and shellfish health management, CIFA, Bhubaneswar, India.