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Growth performance and immune response of silver striped catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) fed with Lapsi *Choerospondias axillaris* (Roxburgh, 1832) during intensive aquaculture

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Abstract
Background: Lapsi (*Choerospondias axillaris)* is a medicinal plant native to Nepal; its fruit is widely used for the treatment of various diseases in various countries like Mongolia, China, Vietnam, etc.
Hypothesis: The lapsi fruit is rich in high level of antioxidant compounds which may be useful for fish health.
Objectives: Four hundred fingerlings @ 7.27 ± 03 g were procured, acclimatized and 360 fingerlings from the stock were distributed randomly at the rate of 15 fishes per tank into 24 plastic tanks (30 inch x 24 inch x 18 inch) equally. Eight (T1, T2, T3, T4, T5, T6, T7 and T8) purified experimental diets containing 40% crude protein were prepared using different doses of lapsi extracts (LE) and lapsi powder (LP) as 0, 100, 200, 400, 800, 1600 mg kg^{-1} LE and then 100 and 200 mg kg^{-1} LP in T7 and T8 respectively along with other usual ingredients. Proximate analysis, weight gain%, specific growth rate (SGR) and feed conversion ratio (FCR) was studied. Various enzymes like SGOT, SGPT, ALP, NBT, SOD and Catalase were studied from the blood, liver and gills of experimental fishes to understand the immune response.
Results: It has been concluded that T4 diet fed fish i.e. 400 mg kg^{-1} LE will be better for fish farmers for better growth and better fish immunity.
Discussion: Medicinal herbs have strong antibacterial effects of active compounds known to and play an important role in preventing bacterial infections.
Conclusions: Lapsi *Choerospondias axillaris* may be useful for the aqua-farmers for better aquaculture productions and it may enhance the socioeconomic status of the country.
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Keywords: *Choerospondias axillaris*, lapsi, antioxidant, fish growth, immune response, protein profile

1. Introduction
Aquaculture means "growing aquatic organism in confined waters and harvesting the production for human benefits [1]. Aquaculture remains a growing, vibrant and important production sector for high protein food. India is the second largest producer in the world, a long way behind to China. Inland fish production in India has increased at a higher rate since 1980 [2]. In India, the inland water resources of the country are in terms of 29,000 km of rivers, 0.3 million ha of estuaries, 0.19 million ha of backwaters and lagoons, 3.15 million ha of reservoirs, 0.2 million ha of floodplain wetlands and 0.72 million ha of upland lakes, which contributes about 1.05 million tonnes of fish annually. Fisheries in India were recognized as an important allied sector of Indian agriculture only after independence [3]. The vibrancy of the sector can be visualized by 11-folds increase in fish production in just six decades, i.e. from 0.75 million tonnes in 1950-51 to 8.3 million tonnes at present (2011-12). The unparallel average annual growth rate of over 4.5% over the years has placed the country on the forefront of global fish production, only after China [4]. Besides meeting the domestic needs, the dependence of over 14.5 million people on fisheries activities for their livelihood and foreign

exchange earnings to the tune of Rs 129 billion (2010-11) from the fisheries produce, amply justifies the importance of the sector on the country's economy and also livelihood security. On the other hand, Nepal lies between India and China. The country touches with India at its southern, western and eastern borders, while the northern boundar is with China. In the south the altitude is about 50 metres above sea level, while at northern end the elevation goes up to the highest peak 8848 m (Mt. Everest) of the world [5]. Being landlocked, the country is deprived of any oceanic resources and overwhelmed by mountains, which comprise about 83% of the total area of 147,181 sq. km. and approximately 5% of the total area of the country is known to be occupied by different freshwater aquatic habitats where some 232 fish species are reported to thrive [6]. In general, the aquatic habitats and fish species can be viewed as prospects for fisheries and aquaculture development in the country. This also implies that aquatic resources located at different altitudes and climatic zones can offer potential for different fisheries and aquaculture activities in Nepal. Aquaculture is the main source of livelihood for many poor people in the developing countries including Nepal [7]. The main purpose of fish farming is the production of white meat at large scale for human consumption [8]. The aqua culturist faces the challenges of disease outbreaks like bacterial, fungal, viral protozoans and so on [9]. Diseases and pathogens are part of every intensive aquaculture production systems [10]. Farmers are in great loss due to farmed species are increasing exposed to pathogens where prophylactic treatment with antibiotics are no longer acceptable and ineffective for many pathogens [11]. Due to this reason, net production is low and is unable to fulfill the demands in the market.

Lapsi, *Choerospondias axillaris* (Roxb.) Burtt & Hill is indigenous fruit tree of Nepal found growing within 900–2000 m above sea level in many parts of the country [5]. It is grown in 301 village Development committees of 29 hill districts of Nepal for some socio-economic purpose [5]. Lapsi trees are commonly found in places like Pharping, Machhaya gaon (Kirtipur), Phulbari, Panchkhal, Namobuddha, Kavre, Panauti and Dhulikhel of Kavrepalanchowk district as well as in Jiri, Charikot of Dholkha district and Chautara of Sindupalchowk district (Fig. 1). Lapsi (*Choerospondias axillaris)* is one of the known medicinal plants rich in vitamin C content [12] and used as a medicinal plant to enhance the immune system of the body [13]. The constituents of lapsi fruits have been investigated chemically and shown to include phenolic compounds and flavonoid content [14]. The ability of phenolic compounds to serve as antioxidants has been recognized, leading to speculation about the potential benefits of ingesting phenolic rich foods [15]. It is assumed that the antioxidant activity of fruits of lapsi *Choerospondias axillaris* (Roxb.) may enhance the blood parameters of carp by improving immunity in the body so that carp can survive in adverse conditions and have capacity to fight against the diseases.

Fig 1: Distribution of Lapsi *Choerospondias axillaris* (Roxb.) in various districts of Nepal.

Catfishes are the second major group of freshwater fishes. India, being a mega-diverse country, harbors 197 catfish species from 52 genera. The Indian families include Amblycipitidae (Torrent catfishes), Akysidae (Stream catfishes), Bagridae (Bagrid catfishes), Chacidae (Square head or angler catfish), Clariidae (Air breathing catfishes), Heteropneustidae (Air-sac catfishes), Olyridae (Long-tail catfishes), Pangasiidae (Shark catfishes), Plotosidae (Eel-tail catfishes), Schilbeidae (Schilbid catfishes), Siluridae (Sheat fishes), Sisoridae (Sisorid catfishes). Striped catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) is popularly known as pangas or pyaasi in India, belonging to the family pangasiidae, under the order Siluriformes. The origin of *P. hypophthalmus* is reported from the Mekong River of Vietnam to the Chao Phraya River to Thailand [16]. Later the fish was introduced to several countries such as Malaysia,

Indonesia, Philippines, Bangladesh and China, where it spread and was adopted under aquaculture. In India, the fish was introduced from Bangladesh [17]. In India, the *Pangasius hypophthalmus* is produced in Andhra Pradesh and West Bengal on a commercial scale. However, its production moved into the states like Uttar Pradesh, Punjab, Rajasthan, Bihar, Chhattisgarh and Haryana [18]. Thus, after the study of all these information, an experiment was conducted to understand the effects of lapsi *C. axillaris* on growth and immune response in striped catfish *P. hypophthalmus*.

2. Materials and Methods

2.1 Selection of Sites and Study Area

This experiment was conducted over a period of 90 days from July to October 2015 at the wet lab of Central Institute of Fisheries Education (CIFE), Old Campus, Versova, Mumbai and laboratory work was carried out in Fish Nutrition, Physiology and Biochemistry laboratories of ICAR-CIFE, Mumbai, New Campus.

2.2 Collection and Identification of Lapsi Fruits

Bhaktpur is the main center for lapsi production in Nepal. Lapsi *Choerospondias axillaris* (Roxb.) fruits collected from Bhaktapur were identified by National Herbarium and Plant laboratories, Department of Plant Resources, Ministry of Forests and Soil Conservation, Government of Nepal, located at Godavari, Nepal.

Fig 2: Lapsi tree cultivated at Champadevi Hill of Kathmandu, Nepal

2.3 Preparation of Crude Extract of Lapsi Fruit

Lapsi fruits were taken to laboratory soon after their collection. The crude extract of the pulp of lapsi fruits were prepared by using ethanol (70%) as described by Labh *et al* 2015. The known quantity (10 g) of lapsi powder was taken in a 500 ml conical flask and added 500 ml of 70% ethanol. The ethanol lapsi powder mixture was kept for 48 hours on orbital shaker. After 48 hours, the mixture was filtered using Whatman filter paper No.1. All the residues were removed and the filtrate was centrifuged at 10,000 rmp for 5 minutes to collect the supernatant. The supernatant was concentrated using the water bath at the temperature of 70 °C. A greasy substance, the crude extract of the lapsi, was obtained and transferred to screw-cap bottle, labeled and stored at 4 °C until use.

2.4 Formulation and Preparation of Purified Diets

Altogether eight purified diets (40% protein) were prepared for this experiment. Diet T1 was control diet (0.0 mg kg^{-1}) without the extract of lapsi fruits where as dietsT2, T3, T4, T5 and T6 were supplemented as 100 mg kg^{-1}, 200 mg kg^{-1}, 400 mg kg^{-1}, 800 mg kg^{-1} and 1600 mg kg^{-1} extract of lapsi fruits (LE) respectively. Besides these two diets T7 and T8 were supplemented by 100 and 200 mg dry grinded powder of lapsi fruits (LP) to compare it with ethanol extracts. The other ingredients were as per the standard norm of Nutrition Lab, CIFE (Table 1). Purified ingredients such as casein (technical grade), gelatin, dextrin, starch, cellulose, Carboxy-Methyl Cellulose (CMC), choline chloride, cod liver oil, sunflower oil and vitamin & mineral premixture were procured from Hi-Media Company and used for feed formulation. Except the control diet (T1), Five (T2–T6) diets with identical composition were prepared with different concentrations of lapsi fruits extracts (LE) and two (T7 & T8) with lapsi powder (LP). All the ingredients were weighed properly as per the requirement and kept in plastic containers separately for each treatment. Gelatin crystals were weighed and first dissolved in hot water to form a jelly, which mixes easily with other ingredients. By adding sufficient water these were made into dough. The dough was then subjected to heat treatment for gelatinization. To the gelatinized dough micro-ingredients (vitamin-mineral premixture and choline chloride), oils, LE and LP were added, as a measure to prevent their loss during heat application. After incorporation of these ingredients, the dough was mixed properly and passed through the pelletizer to get uniform sized pellets, which were spread out on a paper for proper air drying. After drying these feeds were packed in containers, closed airtight and labeled according to the treatments.

Table 1: Preparation of Eight (1 control, 5 lapsi extracts and 2 lapsi powder treatments) purified diets used during the experiment

Ingredients	T1 (0% LE)	T2 (0.1% LE)	T3 (0.1% LE)	T4 (0.1% LE)	T5 (0.1% LE)	T6 (0.1% LE)	T7 (0.1% LP)	T8 (0.2% LP)
Casein††	33	33	33	33	33	33	33	33
Gelatin††	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
Dextrin††	16.8	16.8	16.8	16.8	16.8	16.8	16.8	16.8
Starch††	19	19	19	19	19	19	19	19
Cellulose††	11	11	11	11	11	11	11	11
Cod Liver Oil†	4	4	4	4	4	4	4	4
Sun Flower Oil†	4	4	4	4	4	4	4	4
Vitamin Premix§	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mineral Premix§	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
CMC††	2.8	2.7	2.6	2.4	2	1.2	1.8	0.8
Choline Chloride††	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Lapsi extracts†	0	0.1	0.2	0.4	0.8	1.6	1	2

§Composition of vitamin mineral mix (EMIX PLUS) (quantity 2.5kg $^{-1}$)

Vitamin A 55,00,000 IU; Vitamin D_3 11,00,000 IU; Vitamin B_2 2,000 mg; Vitamin E 750 mg; Vitamin K 1,000 mg; Vitamin B_6 1,000 mg;

Vitamin B_{12} 6 µg; Calcium Pantothenate 2,500 mg; Nicotinamide 10 g; Choline Chloride 150 g; Mn 27,000 mg; I 1,000 mg; Fe 7,500 mg; Zn 5,000 mg; Cu 2,000 mg; Co 450 mg; Ca 500 g; P 300g; L- lysine 10 g; DL-Methionine 10 g; Selenium 50 mgl^{-1}; Selenium 50 mgl^{-1}; Satwari 250 mgl^{-1}; (Lactobacillus 120 million units and Yeast Culture 3000 crore units).

†Fruits of *C. Axillaris* were collected from Bhaktapur, brought to laboratory and then extracts were prepared from the pulp of lapsi fruits.

††Himedia Laboratories, Mumbai, India.

†Ingredients like sunflower oil and Cod Liver Oil wereprocured from local market.

LE=Ethanol extract of Lapsi fruits

LP=Lapsi Powder

2.5 Fish Species: Sources and Maintenance

Silver stripped catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) has been selected for the present experiment. Four hundred healthy farm-raised fingerlings were procured from the local hatchery form in Mumbai and fish were packed in 10 l polyethylene bags filled with oxygenated water and were transported to Wet Laboratory of ICAR-CIFE, Mumbai. After arrival, these fingerlings were kept in the four stocking plastic containers (300 liters capacity each) at the rate of 100 fish per container and acclimatized to experimental conditions for 30 days prior to the start of the experiment.

2.6 Experiment design and set-up

The experimental set-up consisted of 24 plastic rectangular tubs (120 L capacity) covered with perforated lids to prevent the escaping of fish. The 24 experimental tubs were washed properly using acid followed by fresh water and sundried for a day. Later it was filled with water; aeration was provided and was kept for overnight. On the next day, the tubs were again washed using $KMnO_4$ solution (4ppm) followed by fresh water rinsing. Then the tubs were thoroughly washed with clean water. Three hundred and sixty fishes with average initial weight of 7.5 to 8 g were randomly distributed to the eight experimental groups including control. Each group was having three replicates. Completely randomized design was followed during the experimental trial. Fifteen fishes were stocked to each tub, which had well aerated water. Round the clock aeration was provided to each tub during the experimental period. Initially the total weight of fishes in each tanks were measured and recorded. The body weights were measured at intervals of 15 days to assess the growth. The fishes were starved overnight before taking the body weight.

2.7 Culture Period, Conditions and Maintenance

De-chlorinated tap water was used during experiment and replaced 2-3rd on every alternate day. Fingerlings were acclimated for 10 days and were fed with control diet without lapsi during acclimatized period. After that, fingerlings were fed with test and control diet at the rate of 3% of their body weight twice daily at 11 a.m. and 4 p.m. Temperature ranged from 25 °C to 29 °C and pH ranged from 7.53 to 7.92 throughout the study period. Dissolved oxygen was maintained above 5 mg/l with the help of aerators. The experimental tubs were cleaned manually and siphoning was done every day morning to remove the faecal wastes and uneaten feeds in the tubs. An equal volume of clean water is again filled in the tubs to replace the siphoned water. Feeding was done @ 3% of the body weight initially and the feeding rate was adjusted accordingly. The daily ration was divided into two equal parts and was fed twice daily once in the morning and next in the afternoon. Initial weight were recorded in the beginning of experiment, then to balance the feeding system on regular check basis, weight of whole body mass were taken on every fifteen days. At the end of experiment sampling were done by measuring individual weight basis. Mortality was checked to understand the survival rate during the entire experimental periods.

2.8 Proximate Analysis

Proximate analysis of the diets and carcass tissues in the beginning and at the end of the experiment was done by standard methods (AOAC, 1995) at Fish Nutrition Laboratory, CIFE. Moisture, crude protein (CP), ether extract (EE), total ash (TA) organic matter and total carbohydrate (TC) were measured by following formulae

$$\text{Moisture (\%)} = \frac{\text{(Wet weight of sample – Dried weight of sample)}}{\text{Wet weight of sample}} \times 100$$

$$\text{Crude Protein (\%)} = \text{N2 (\%)} \times 6.25$$

$$\text{Ether Extract (\%)} = \frac{\text{(Initial weight of sample – final weight of sample)}}{\text{Initial weight of sample}} \times 100$$

$$\text{Total Ash (\%)} = \frac{\text{Weight of ash} \times 100}{\text{Weight of sample}}$$

Organic matter of the experimental diets and carcass tissue was calculated by subtracting the ash (%) from 100

$$\text{TC (\%)} = 100 - (\text{Crude Protein \%} + \text{Ether Extract \%} + \text{Total Ash \%})$$

2.9 Growth Performance

Percentage weight gain, specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency ratio (FER), protein efficiency ratio (PER) and fish su rvival were measured by using following formulae:

$$\text{Weight Gain (\%)} = \frac{\text{(Final weight – Initial weight)}}{\text{Initial weight}} \times 100$$

$$\text{SGR} = \frac{\text{Loge Final weight – Loge Initial weight}}{\text{Number of days}} \times 100$$

$$\text{FCR} = \frac{\text{Feed given (Dry weight)}}{\text{Body weight gain (Wet weight)}}$$

$$\text{FER} = \frac{\text{Net weight gain (Wet weight)}}{\text{Feed given (Dry weight)}}$$

$$\text{PER} = \frac{\text{Net weight gain (Wet weight)}}{\text{Protein fed}}$$

- Fish survival: Difference in number of fish between the time at stocking and at harvest was determined for the estimation of survival. This was expressed in percent of the initial number of fish.

2.10 Immunological Parameters

After completion of the feeding trial, sampling was done for the analysis of immune-haematological parameters. Carps from each replicate from each group were rapidly netted and

anaesthetized with 50 mg/L of tricaine methane sulfonate (MS222, Sigma Chemical Co. St. Louis, MO, USA). 1-2 ml of Blood was drawn from the *vena caudalis* with disposable hypodermic needle (26 gauge). From the collected sample a drop of blood was used for smear on a grease free clean glass slide from each fish. Half the blood sample.were then transferred immediately to sterile penicillin vial containing a pinch of lithium heparin powder, shaken gently and kept at 4 °C for haematological profiles; and for serum separation, the remaining blood samples were transferred to sterile Eppendorf tubes without anticoagulant and then stored at -20 °C for immunological assay.

Total serum protein of fish blood was determined by biuret method [19], albumin of fish blood serum was determined by BCG method [20], SGOT, SGPT and ALP were determined by Reitman and Frankel [21]. At the end of the experiment selected fishes from all the treatments (T1, to T8)) were anaesthetized using clove oil (50 µl of clove oil per liter of water) for 2-3 minutes. Fishes were then dissected and the tissues viz., liver and gills were immediately removed aseptically. A 5% tissue homogenate was prepared in chilled 0.25 M sucrose solution. The whole procedure was followed in ice cold condition. Homogenized samples were centrifuged at 10,000 rpm for 10 minutes at 4 °C. The supernatant was collected in 10 ml vials and stored in deep freezer (-20 °C) for enzyme assay. Superoxide Dismutase (SOD) was assayed according to the method of Kakkar *et al*., [22]. Catalase activity was assayed by the method of Luck [23].

2.11 Statistical Analysis

Statistical significance was analyzed using one-way analysis of variance (ANOVA) via SPSS 20.0 for Windows. Duncan's multiple range test was used for post hoc comparison of mean (P<0.05) between different acclimation temperatures. All data presented in the text, figures and tables are means ± standard error and statistical significance for all statistical tests was set at (P< 0.05).

3. Results

3.1 Proximate analysis during the experiment

Proximate analysis of fish feed (Table 2), experimental fish in the beginning (Table 3) and at the end (Table 4) were studied properly. Dry mater, Moisture, crude protein, ether extract, crude fibre, total ash, NFE and gross energy were studied through standard protocols during the feed preparation and found that all the nutrients were at optimum level considered for a normal growth of the fish (Table 2 & 3). But after 60 days of feeding trial protein level found high from 42 to 64% in the body of fish (Table 4).

3.2 Antioxidant properties of lapsi fruits during the experiment

Ethanol (80%) extract of lapsi fruits were prepared in the beginning to study the antioxidant properties like Phenolic, Flavonoids, ABTS, DPPH, FRAP and ascorbic acid (AA) in the Lapsi fruit extract. After the studies of different antioxidant properties like it has been concluded that lapsi fruits are highly rich in antioxidant compounds (Figure 3).

3.3 Growth profiles and Survivals

After 90 days of feeding trials weight gain%, SGR, FCR and Survival rate were measured and significant (P< 0.05) growth in T4 diet fed fish. A higher weight gain%, SGR, FCR and survival rate were 120.22±0.29f, 1.71±0.015f, 0.81±0.015a, 7.42±0.031f and cent-per-cent respectively as compared to weight gain%, SGR, FCR and Survival rate of control diet T1 (54.21±0.26c, 0.94±0.015c, 1.87±0.031d and 91.11±2.81a) fed fish. Growth profiles were measured and found better weight gain percentage in T4 (0.4% LE) diet fed fish as compared to other diets fed fish (Table 4). Similarly significant (P<0.05) SGR found in treated diets as compared to control and highest SGR found in T4 (0.4% LE) diet fed fish. Decreasing trends were observed in FCR as the dose of lapsi extract increased. The diets supplemented with lapsi powder in T7 and T8 when compared to T2 and T3 incorporated with lapsi extracts found that lapsi extract supplemented diet fed fish perform better growth as compared to lapsi powder supplemented diet fed fish. Similarly cent per cent survival rate were observed in T3 and T4 diet fed fish while the survival rates in other T1, T2, T5, T6, T7 and T8 were 91.1, 95.5, 97.7, 95.5, 93.3 and 97.7 respectively (Table 4).

3.4 Biochemical Profiles

3.4.1 Blood Protein Profiles

After the 90 days of feeding trials total serum protein, albumin, globulin and albumin/globulin ratio were studied and found that highest fivefold level total serum protein were increased in T4 diet fed fish as compared to control T1. Similar results were recorded in albumin and globulin. Thus better protein concentrations were observed in fish fed with diet containing 400 mg kg -1 of lapsi extract supplemented diets (Table 5).

3.4.2 Blood Enzyme Profiles

At the end of the final sampling the enzymes like SGOT, SGPT, ALP from serum and NBT from blood were analyzed and it was found decreasing trend line as compared to control diet fed fish T1. The lowest SGOT, SGPT, ALP and NBT were 0.19±00; 10.91±0.15; 21.79±0.29 and 0.62±0.36 in T4 diet of SGOT; SGPT and ALP and for NBT enzyme T8 diet fed fish as compared to control T1 0.65±0.01; 35.8±0.31; 72.59±0.62 and 1.53±0.16 respectively (Table 5).

3.4.3 Tissues Enzyme Profiles

After the 90 days of feeding trial fish were collected from each tank; liver and gills were removed after dissection and collected in tissue buffer solution. Then SOD and Catalase enzyme of liver and gills were assayed in kinetic mode in spectrophotometer Shimadzu 1800. Decreasing trends were observed in SOD of liver and the lowest 6.4±0.17 was recorded in T4 diet fed fish while SOD was highest 9.59±0.17 in control T1 diet fed fish. Similarly, SOD level in gills were also found decreasing trend up to T6 (1.52±0.18) and then it was in increasing order in T7 and T8 diet fed fish (Table 5). Similar results were recorded in Catalase enzyme of liver (CAT-L) and gills (CAT-G). CAT-L was minimum 5.38+0.05 in T4 diet fed fish as compared to control T1 (11.94±0.12) diet fed fish and it was 8.15±0.11 in CAT-G of T4 diet fed fish compared to control T1(15.46±0.13) diet fed fish (Table 5).

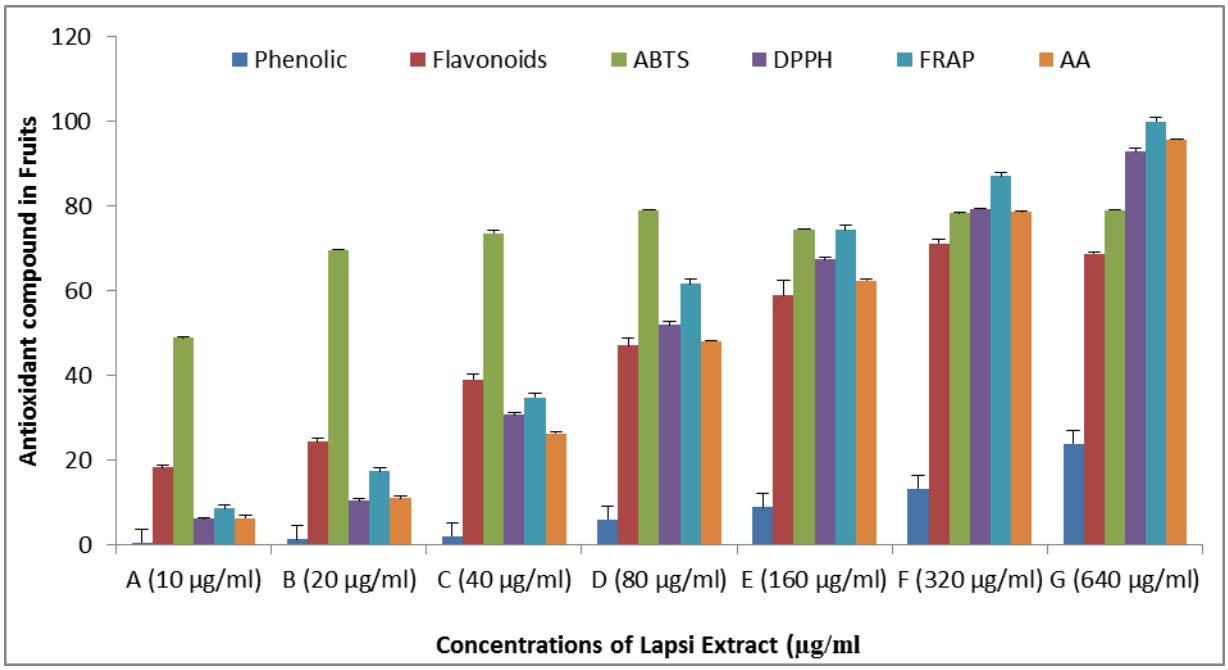

Fig 3: Various antioxidant properties found in Ethanol extract of Lapsi fruits.

4. Discussion

Fish is an important part of diet for a large proportion of the people living in the developing world. Fish food represents the primary source of animal protein for a billion of people in the 58 countries worldwide [24]. The contribution of fisheries is very promising and important for creating job opportunities for unemployed people, earning foreign exchange, alleviating poverty and improving nutritional status of the people [25]. Today's world population is estimated to be about 7 billion, which by 2030, 2 billion more people will be added in the world population mean that aquaculture will need to produce nearly double that, 85 million tons of fish per year just to maintain current consumption levels [26]. It is estimated that the total feed cost in culture accounts for 30 to 70% of production cost, depending upon the type of culture and intensity of feeding [27]. In the past, production was limited because techniques to reproduce the fish in captivity had not been refined or widely applied. Fingerlings were collected from the wild and transferred to ponds or cages for grow out. Since 1997 the production of Pangasius has increased greatly due to the application of hormone spawning techniques for controlled reproduction and the development of international markets for the product. The aquaculture potential of this species in tropical regions of the world outside of SE Asia would appear to be excellent [28].

Catfish of the family Pangasiidae especially *Pangasianodon hypophthalmus* is one of promising species for aquaculture because of its omnivorous feeding habit, adaptability to crowded conditions, hardiness and good market. *P. hypophthalmus* has immense economic importance in many countries of South and Southeast Asia, including India, Bangladesh, Thailand, Vietnam and Malaysia. *Pangasius* fillets have a stable market in about 70 countries in the world at present [29]. Vietnam is the world leader in *Pangasius* production (89% of world total *Pangasius* production) with an annual production of around 10 lakh tonnes. *Pangasianodon hypophthalmus* was introduced into India possibly during 1997 clandestinely via Bangladesh and adopted to culture in the state of West Bengal. It was estimated that over 200,000 tonnes of *P. hypophthalmus* catfish were produced in the country per annum. Its culture practice is growing rapidly in Andhra Pradesh, Bihar, Uttar Pradesh, Orissa, Maharashtra, Tamil Nadu, Karnataka, and Kerala and marketed in Bihar, Uttar Pradesh, Assam, Delhi, Punjab and Mumbai. Because of its remarkable growth rate (almost one kg in 90 days), there has been much enthusiasm among the fish breeder and farmers particularly in West Bengal and Andhra Pradesh for its culture and propagation. In India, the rapidly adopted *Pangasius* farming in Andhra Pradesh is facing major challenges including severe crises of steep decline in market price because of high production, lack of proper feed and inferior flesh quality (texture, colour, flavour and nutritional value) of fish. Protein is the most important component of diet and also the most expensive among all the feed ingredients. [17]

Present market status of *Pangasius* catfish is not socioeconomically beneficial to the farmer as cost of production is more than the farm gate price realized. In such case there is need to optimise the protein level in feed to bring down the cost of feed. Thus an experiment was conducted with the objectives to determine the effect of dietary supplementation of economically viable and locally available herbal plant lapsi *Choerospondias axillaris* (Roxb.) on survival, growth, biochemical and immune-haematological performance of striped catfish *Pangasius hypophthalmus* fingerlings.

5.1 Proximate Composition of Diets

The estimated crude protein contents of diets were found to be near the formulated levels. The crude protein content varied from 19.82±0.54 to 44.86±0.56%; ether extract content varied from 9.7±0.04 to 10.14±0.02%; total ash content from 4.8±0.003 to 5.6±0.004%, carbohydrate ranged between 34.95±0.72 and 60.2±0.35% and the moisture content is 5.82±0.004 to 6.7±0.002.Values of growth performance and feed utilization indices, in the present work, indicate an enhancement in growth and feed utilization for all fish groups fed lapsi fruits extract and powder at all inclusion levels compared to the control fish group. Many authors recorded the positive effects of administrating garlic in diets on growth

and feed utilization of many fishes including; African catfish, *Clarias gariepinus* ; [30] rainbow trout, *Oncorhynchus mykiss* ; [31, 32] Swordtail, *Xiphophorus helleri* [33] and Nile tilapia, *Oreochromis niloticus* [34-37].

5.2 Growth and Survival

From the present study, the result suggest that the dietary supplementation of Lapsi fruit pulp powder at all concentrations enhanced significantly (P<0.05) the growth and nutrient utilization, which is shown in improved weight gain, length gain, SGR, FCR and FER of *Pangasius* fingerlings. In overall, growth performance obtained was high as compared to control diet T1 (0.0 mg/kg of Lapsi extracts). The optimal growth was obtained in fish fed in treatment T4 (400mg/Kg Lapsi Extracts) in comparison to others. Similar result was shown by Turan [38] who used red Clover *Trifolium pretense*a herbal medicine as a growth promoting agent for Tilapia *O. niloticus*. Megbowon *et al*., [39] found better performance in terms of growth, nutrient utilization, high survival rate in the fish fed with different composition of garlic than control diet (A); agreeing with Metwally [36] and Shalaby *et al*., [34] who reported significant increased weight gain, FE, PER and SGR in the *O. niloticus* when fed with diet consisting of 30 g/kg and32 g/kg Garlic powder respectively. In addition, the use of 1% Livol (IHF 1000) as herbal medicine enhances maximum growth and improved nutrient digestibility of carp [40- 42].

Lapsi *Choerospondias axillaris* (Roxb.) is a medicinal plant used in Mongolia [43] and its lapsi fruit pulp powder (LFPP) is widely used for the treatment of cardiovascular diseases in clinic [44]. The constituents of LFPP have been investigated chemically and shown to include phenolic compounds and flavonoid content [45]. Phenol compounds are widely found in the secondary products of medicinal plants, as well as in many edible plants [46]. The ability of phenolic compounds to serve as antioxidants has been recognized, leading to speculation about the potential benefits of ingesting phenolic rich foods [47].

Similarly, herbs such as garlic, onion, marjoram, caraway, basil, anise, fennel, licorice, black seed and fenugreek have been tested for growth promoting activities [42, 48, 49], feed conversion [50-53] and improvement of protein digestibility and energy retention [51, 52] in aquatic animals. Shalaby *et al.,* [34] reported a significant increase in WG, FE, PER, and SGR of Nile tilapia fed diet containing 3% GP. Similarly, Diab *et al.,* [54] mentioned feeding diet with 2.5% garlic resulted in the highest growth performance in *O. niloticus*. In the same species, Abou-Zeid [55] found a positive improvement in biomass and SGR with garlic supplementation. Metwally [36] also mentioned that the best performance was obtained in Nile tilapia fed diet with 3.2% GP. A significant increase in growth, feed conversion and protein efficiency was shown in rainbow trout when fed diet with 1.0% garlic. [32] Abdel-Hakim *et al.* [56] reported that incorporation of garlic into Nile tilapia diets (diet with fresh garlic 3 g per kg) resulted in significant improvement in WG, feed conversion, protein efficiency and CF. The present results are also in agreement with those obtained by Khattab *et al.* [57] who found that the diet of Biogen increased feed intake, improved feed conversion ratio (FCR) and PER in Pangasius.

In the present research, the SGR was high in the fish with treatment T4 than in other treatments. This may be due to the positive effect of antioxidant compounds in the formation of collagen, which is necessary for normal growth as explained by Masumoto *et al.* [58] The present research was also satisfied with the result of Soliman *et al.* [59] He recorded significant lower (P<0.05) SGR in the *O. niloticus* juvenile fed without Ascorbic acid than in the fish fed with 125 mg of AA/100 gm. Shiau and Hsu [60] fed 90 mg/kg of L-Ascorbic acid and obtained highest weight gain of juvenile hybrid Tilapia *O. niloticus* x *O. aureus*. Supplementation of vitamin C at 1000 mg/kg diet enhanced the growth performance and feed utilization of experimental fingerlings of *B. sharpeyi* satisfied with Tewary and Patra [61] obtained maximum growth in Labeo rohita fingerlings fed with 1000 mg AA/Kg of supplemented diet. Alam *et al.,* [62] reported a similar result in *Heteropneusts fossilis*. However, utilization of vitamin C is different in different fish species [63-65]. Labh and Chakrabarti [66] had described the increment in average weight and specific growth rate of hybrid carp by the dietary addition of vitamin C at the rate of 400 mg/kg. From the present research, 400 mg/kg of Lapsi extracts shows the statistically significant (P<0.05) increase in growth performance of striped catfish *Pangasius*.

5.3 Biochemical Performances in Relation to Immune Response

In intensive culture systems, fish are continuously exposed to stress, which can cause temporary homeostasis modifications leading to physiological adjustments. These responses aimed at mobilizing energy by adrenergic system stimulation, release catecholamine and increase adrenocorticotropic hormone and plasmatic cortisol [67]. These responses can be extended and cause chronic stress, which increases the imbalance. Low resistance and high disease susceptibility during a stress period, such as in winter, can cause fish death and, consequently, economic problems to producers.

Ascorbic acid requirements of some fishes have been investigated. Stickney *et al.,* [68] reported the fortification of 50 mg of ascorbic acid equivalent kg-1 diet as the level that allows for maximum weight gain and absence of deficiency signs in blue tilapia (*Oreochromis aureus).* A diet of 79 mg ascorbic acid per kg diet was found to be the requirement level for maximum weight gain of hybrid tilapia (*Oreochromis niloticus x Oreochromis aureus*) [69]. Studies have shown that high ascorbic acid concentration in tissue determines higher tolerance to ambient pollution and better resistance to bacterial infection [70]. Abdel-Tawwab *et al*., [71] found that a high level of acid ascorbic enhanced the weight gain, specific growth rate and survival rate of tilapia exposed to sub lethal dose of mercury.

The lapsi fruits are with rich in *vitamin C* content [12]. Vitamin C (ascorbic acid) is an essential nutrient in aqua-feeds. It is an indispensable nutrient required to maintain physiological processes such as normal growth, immunity and reproduction of different animals including fishes [72]. Ascorbic acid is water soluble and is essential for several metabolic functions including the antioxidant system. Most fishes, including tilapia, are not capable of vitamin C biosynthesis [73] due to the absence of the enzyme L-gulonolactone oxidase, which is responsible for synthesis of ascorbic acid [74]. L-ascorbic acid is extremely labile and the rate of degradation is a function of storage time, with the effect of temperature, oxygen, pH and light. Recent studies indicate that ascorbic acid derivatives that include sulfate and phosphates are more resistant to oxidation and retain ascorbic acid activity for in fish [75].

5.3.1 Serum Protein profile

In this experiment total serum protein, serum albumin,

globulin, albumin and globulin ratio were estimated. The result suggested that the dietary supplementation of lapsi fruit pulp extracts in different concentration had significant (P<0.05) higher protein profile i.e. total serum protein, serum albumin and globulin in all the treatments in comparison to control T1. Highest protein and albumin level were observed in T4 diet fed fish. This significant increase in the total protein and albumin levels suggests the stabilization of endoplasmic reticulum leading to protein synthesis. The herbal medicine (lapsi) boosts the immune system of *Pangasius* catfish. This may be due to the presence of vitamin C (AA) and other antioxidant compounds in the lapsi fruit pulp powder which acts as antioxidant and immunostimulant. In agreement with present findings, Ajeel and Al-Faragi [76] found that the use of Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) or mixture increased total plasma protein, albumin and globulin concentration significantly (P<0.05) and concluded that they protect the liver against deleterious agents and free radical- mediated toxic damages to the liver cells.

The same result was also agreed by Metwally [36]. By adding extract of ginger to fish diet increased the total protein level, the highest level of plasma protein was observed in those fishes fed with 1% ginger extract (Dugenci *et al.,* 2003) [77]. Hassan and Javed [78] also found higher concertration of sarcoplasmic protein in *Catla catla* corresponds with decrease of myofibrillar protein. Hamza *et al.,* [79] assessed the protein expression profile in the liver of 34 days old Pikeperch larvae fed with three isoprotic and isolipidic formulated diets and found that there was a significant modification of protein expression in the liver of fish. Sivaram *et al.*,[80] used Methanolic extracts of the herbals *O. sanctum, W. somnifera* and *Myristica fragrans* herbs and found significantly improved immune parameters such as phagocytic activity, serum bactericidal activity, albumin–globulin (A/G) ratio and leukocrit against *Vibrio harveyi* challenge in juvenile grouper, *Epinephelus tauvina*, larviculture. Likely, Sivagurunathan *et al.,* [81] studied and found increment in TEC, Hb, TLC, Lymphocyte, Neutrophils and 195 aponicas count fed with 2% of *P. emblica* in fish feed as immunostimulator.

Higher concentration in protein profile of liver in the present study may be due to antioxidant property of lapsi fruit pulp powder. Pandey *et al.,* [82] concluded the use of Vitamin C and E as herbal drugs act as growth promoter and cure all kinds of diseases of fishes and other aquatic animals. Ibrahem *et al.,* [83] suggested that the vitamin C at the rate of 500 mg/kg for one month could be a potential, less expensive and positively affect innate immunity and resistance of Nile Tilapia (*O. niloticus*) in aquaculture. Lovell [84] use of vitamin C reduces the deficiencies sign in fishes whereas supplementation of vitamin C ranged from 25 to 50 mg/kg as explained by Lim and Lovell [85] improved wound healing three times in channel catfish. Kumarai and Sahoo [86] recommended increasing concentration of vitamin C ten times in the diet explained by Lim and Lovell [85] stimulates the immunity against bacterial infection in Asian catfish *Clarias batrachus*. Such improvement was also observed in *O. niloticus* (Soliman *et al.,* 1994) [87]. Labh and Chakrabarti [66] also noted marked antioxidant and immunostimulants properties of vitamin C (LATP-Ca) in fishes. This result was also supported by Merchie *et al*., [88]. They found a use of 2000 mg ascorbic acid per kilogram promote the resistance of shrimp postlarval to stress condition and bacterial infection *Penaeus vannamei.* Ndong and Fall [89] demonstrated that juvenile hybrid Tilapia fed with Gralic (0.5g/kg) enhanced TLC, respiratiory burst, phagocytic activity, phagocytic index and lysozyme activity as compared to diet T2 and control diet. According to El-Sayed *et al*., [90] herbal compounds such as phenolics, polyphenols, alkaloids, quinines, terphenoids, lectines and polypeptides have shown to be very effective alternatives to antibiotics and other synthetic compounds as growth promotion, immunostimulation, antistress, antibacterial, antifungal, antiviral, appetite stimulators and aphrodisiac.

5.3.2 Blood Enzyme Profile and Immune Response

The functional state of the liver was determined by estimating the immunological parameters such as Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Serum Alkaline Phosphatase (SALP), were estimated by enzymatic UV kinetic methods based on the reference method of International Federation of Clinical Chemistry. Decreasing trends were observed in all the parameters means as the dose of lapsi extracts were increased in the diets the concentrations level of SGOT, SGPT, alkaline phosphatase (ALP) and NBT were decreased. Better results were observed in T4 diet fed fish. The reduction in the levels of SGPT and SGOT toward the normal value indicated that the extract protected the structural integrity of hepatocyte cell membrane by stimulating the regeneration process of damaged cells. Reduction in ALP levels implies that the extract restored the stability of the biliary function during injury with CCl4 Serum glutamic oxaloacetic transaminase (SGOT) or Aspartate aminotransferase (AST) and Serum glutamate pyruvate transaminase (SGPT) or alanine aminotransferase (ALT) belong to the plasma non-functional enzymes, which are normally localized within the cells of liver, heart, gills, kidneys, muscle and other organs. Their presence in blood plasma may give information on tissue injury or organ dysfunction [91]. The diagnostic significance of aminotransferase (SGOT and SGPT) has been well recognized. These enzymes are liberated into the blood in pathological situations and therefore are of clinical importance. In the environmental studies also blood and tissues level of SGOT and SGPT have been measured to assess the toxic impact of aflatoxicosis and ochratoxicosis. [92] An inverse relationship was found between the SGOT, SGPT levels and the dose of vitamin C in the diet of fish regardless of species and weight. The concentration of alkaline phosphatase in the blood increased as the dose of vitamin C increased in the diet of fish. SGOT and triglycerides are indicators of liver function. Ren *et al*.,) [93] suggested that when Japanese eel, *Anguilla japonica* were fed with two levels of vitamin C 32 or 762 mg AA/kg diet for 3 weeks, SGOT of serum from fish fed diets containing 762 mg AA/kg was significantly lower than those of fish fed diet containing 32 mg AA/kg regardless of diet.

As the alternative to chemotherapy, application of natural products, like plant extracts, in aquaculture is new and developing venture which needs further research in fish [48, 49,]. Phagocytosis and killing activity by phagocytic cells is an important defense mechanism against pathogenic bacteria. [94, 95] Fish phagocytes are able to produce superoxide anion (O_2^-) during a process called respiratory burst. [94, 96, 97, 98] It is considered that these oxygen forms are toxic for bacterial pathogens [99, 96]. The respiratory burst (NBT) activity can be quantified by the Nitroblue Tetrazolium (NBT) assay, which measures the quantity of intracellular superoxide radicals produced by leukocytes [96, 98]. Herbal based

immunostimulants can enhance the respiratory burst activity of fish phagocits. For instance, Rao *et al.* [95] reported that Superoxide anion production by the blood leucocytes was enhanced in *Labeo rohita* after feeding the fish with *Achyranthes aspera* seed. Ardo *et al*., [98] also reported that feeding Nile tilapia (*Oreochromis niloticus*) with two herbal extracts (*Astragalus membranaceus* and *Lonicera japonica*) alone or in combination significantly enhanced phagocytic and respiratory burst activity of blood phagocitic cells. Similarly, the plant extracts we used in this study could enhance respiratory burst (NBT) activity in treatment groups compared to control group.

There is a relationship between tissue ascorbate and the fish health [100]. After 8 weeks of feeding trials in the present experiment a direct relationship was found between the dose of lapsi fruit pulp (LP) in the diet of tilapia and the concentration of vitamin C in the brain and liver of fish. Vitamin C level in brain and liver were significantly (P<0.05) higher in the tilapia fed with diet D3 followed by tilapia fed with diet D2, D1 and minimum in tilapia fed with diet C. Like brain, vitamin C concentration in liver was 16.78 to 78.15% higher in the tilapia fed with D3 diet compared to the tilapia fed with other diets. These results strongly indicate that vitamin C significantly affects the growth, survival and immunity of juvenile *O. karongae* [101]. Weight gain increased with dietary level of vitamin C is considered by many nutritionists to be the most important and meaningful response in nutritional requirement studies [68]. The diet without ascorbic acid supplementation decreased the specific growth rate (0.32% per day) of juvenile *O. karongae* and this is in accordance with studies conducted by Ai *et al.*, [101] who also observed declining specific growth rate with ascorbic acid deficient diet for seabass (*Scophthalmus maximus*).

Kim *et al.*, [102] suggested that the presence of unknown factors in various medicinal herbs led to outstanding results in fish growth trails. The present findings may indicate the presence of antioxidant properties in lapsi fruit pulp stimulated the growth and nutrients utilization in *Pangasius*. It has been shown that antioxidant requirement for normal growth of trout range from 10 to 20 [103] although the NRC recommended 50mg vitamin C/ Kg diet for an optimal performance of trout. Likely, the present study has shown 200 mg/Kg of lapsi powder containing vitamin C gives the optimal growth in Nile Tilapia. Similarly, Gammanpila *et al.*, [104] resulted that female Nile Tilapia exhibited significantly lower weight gain due to vitamin C deficient in the diet compared to diet supplemented with higher vitamin C. He also found the higher survival rate in the fish fed with high dose of vitamin C. The same thing was agreed by who concluded inclusion of vitamin C (AA) between 100 to 200 mg/Kg in the diet of *H. longifilis* increase survival rate and growth at a top level significantly (P<0.05). Dada [105] fed herbal powder (Superliv) to *Oreochromis niloticus* and found that specific growth rate (1.33 ± 0.33% per day) and best FCR (0.85 ± 0.03) were obtained in the 10 g/ Kg herbal meal diet treatment whereas present result shows maximum SGR to be 1.71±0.015f% per day and best FCR (0.81±0.015) in the fish fed with 400 mg/kg of lapsi fruit extracts. The best result obtained was due to presence of antioxidants in the supplemented diet.

5.3.3 Tissue Enzyme Profile and Immune Response

The functional state of the liver was determined by estimating the biochemical parameters such as SOD in liver and Gills; Catalase in liver and gills were estimated by enzymatic UV kinetic methods based on the reference method of International Federation of Clinical Chemistry. Decreasing trends were observed in all the parameters means as the dose of lapsi extracts were increased in the diets the concentrations level of SOD in liver, SOD in gills, Catalase in liver and catalase in gills were decreased. Better results were observed in T4 diet fed fish. The reduction in the levels of SOD and Catalase toward the normal value indicated that the extract protected the structural integrity of hepatocyte cell membrane by stimulating the regeneration process of damaged cells.

A large number of plants including mistletoe (*Viscum album*), nettle (*Urtica dioica*) and ginger (*Zingiber officinale*) have been used in traditional medicine for the treatment and control of several diseases [106]. Three of such plants are mistletoe (*Viscum album*), nettle (*Urtica dioica*), and ginger (*Zingiber officinale*). Some of the medicinal plants have been used as as the phytogenic basis immunostimulatory preparations. Such preparations have been used, as such as adjuvant therapy, in cancer and AIDS [88, 107, 108]. In Mongolian medicine lapsi is used in the treatment of myocardial ischemia, calming nerves, ameliorating blood circulation and improving microcirculation [5]. Epidemiological studies have shown that the use of antioxidants may decrease the probability of cardiovascular diseases. Lapsi (LFPP) has been reported to be effective in treating cardiovascular diseases in humans. It is well known that fish treated with immunostimulants show increased phagocytosis as well as respiratory burst activity. [109-112]

Lapsi (*C. axillaris*) fruit contains 71% (w/w) of edible parts and 29% of stones. The edible part contains 83% water, 165 mg nitrogen per 100 g, 3.4% total sugars and 6.76% of titratable acidity. Ascorbic acid content of the market collected samples was lower (6.7%) and total amino acid content was 317 mg per 100 g for 21 essential amino acids. Lapsi fruits are very rich in arginine (106 mg per 100 g), glutamic acid (36 mg per 100 g), glutamine (32 mg per 100 g), glycine (28 mg per 100 g), lysine (8 mg per 100 g) and tyrosine (20 mg per 100 g) and also 563 mg per 100 g contained high concentrations of phenolic compounds (Poudel *et al*., 2003) [113]. These constituents of lapsi enhance growth of Nile tilapia and protect them in adverse environments. This work provides a new perspective for the use of medicinal plants as adjuvant therapy added to fish food to prevent diseases. Thus, application of immunostimulants before outbreaks of disease, might avoid high mortalities.

Table 2: Proximate composition of diets (% dry matter basis)

Ingredients (g/kg)	T1 (0.0% LE)	T2 (0.1% LE)	T3 (0.2% LE)	T4 (0.4% LE)	T5 (0.8% LE)	T6 (1.6% LE)	T7 (0.1% LP)	T8 (0.2% LP)
Dry Matter	91.63 ±0.09a	91.71 ±0.10ab	91.72 ±0.01ab	91.76 ±0.01abc	91.79 ±0.01bc	91.83 ±0.01bc	91.87 ±0.01bc	91.90 ±0.01c
Moisture	8.37 ±0.09c	8.29 ±0.10bc	8.28 ±0.01bc	8.24 ±0.01abc	8.21 ±0.01ab	8.17 ±0.01ab	8.13 ±0.01ab	8.10 ±0.01a
Crude protein	34.94 ±0.05c	35.22 ±0.04c	36.48 ±0.05e	37.74 ±0.05f	35.92 ±0.14d	36.27 ±0.05e	32.38 ±0.13a	34.38 ±0.23b

Ether Extract	12.35 ±0.07c	12.71 ±0.05d	13.05 ±0.07de	13.40 ±0.07ef	13.74 ±0.07f	13.42 ±0.27ef	10.40 ±0.08a	10.40 ±0.11b
Crude Fibre (CF)	7.51 ±0.23	7.62 ±0.14	7.63 ±0.20	7.74 ±0.06	7.50 ±0.26	7.43 ±0.21	7.54 ±0.27	7.86 ±0.05
Total Ash	13.54 ±0.14d	13.47 ±0.08d	12.54 ±0.12c	12.24 ±0.05bc	12.13 ±0.51bc	11.72 ±0.31b	10.84 ±0.29a	10.64 ±0.08a
NFE (Carb'te)	31.66 ±0.22d	30.97 ±0.03bcd	30.31 ±0.30b	28.89 ±0.16a	30.72 ±0.33bc	31.17 ±0.17cd	39.83 ±0.37f	36.72 ±0.11e
Gross energy (cal-1gj)	332.29 ±0.62a	335.27 ±0.50b	338.08 ±0.55c	340.94 ±0.55d	343.81 ±0.55e	346.67 ±0.55f	319.54 ±0.55g	352.40 ±0.55h

Table 3: Proximate analysis of tissue in the beginning of the experiment

Ingredients (g/kg)	**T1 (0.0% LE)**	**T2 (0.1% LE)**	**T3 (0.2% LE)**	**T4 (0.4% LE)**	**T5 (0.8% LE)**	**T6 (1.6% LE)**	**T7 (0.1% LP)**	**T8 (0.2% LP)**
Crude protein	41.15 ±1.30	42.80 ±1.07	42.80 ±1.07	41.20 ±1.89	42.14 ±0.21	42.00 ±0.08	42.12 ±0.14	42.04 ±0.01
Crude Fibre	0.26 ±0.02	0.24 ±0.02	0.24 ±0.02	0.19 ±0.04	0.19 ±0.01	0.23 ±0.01	0.24 ±0.01	0.23 ±0.00
Ether Extract	6.36 ±1.87	5.31 ±0.94	5.31 ±0.94	5.21 ±0.54	4.58 ±0.47	6.07 ±0.34	6.56 ±0.54	6.25 ±0.05
Total Ash	7.14 ±0.28	7.39 ±0.20	7.39 ±0.20	7.22 ±0.05	7.48 ±0.12	7.20 ±0.05	7.12 ±0.08	7.17 ±0.01
Moisture	8.05 ±0.32	8.50 ±0.24	8.50 ±0.24	7.92 ±0.20	8.68 ±0.07	8.16 ±0.06	8.07 ±0.10	8.12 ±0.01
Dry Matter	91.95 ±0.32	91.50 ±0.24	91.50 ±0.24	92.08 ±0.20	91.32 ±0.07	91.84 ±0.06	91.93 ±0.10	91.88 ±0.01
NFE (CHO)	45.08 ±2.82	44.26 ±1.52	44.26 ±1.52	46.19 ±1.49	45.61 ±0.42	44.51 ±0.37	43.96 ±0.60	44.31 ±0.06
ENERGY	476.40 ±3.20	472.13 ±6.69	472.13 ±6.69	470.07 ±2.11	466.95 ±1.98	475.90 ±2.17	479.11 ±3.51	477.10 ±0.35

Table 4: Proximate analysis of tissue at the end of the experiment

Ingredients (g/kg)	**T1 (0.0% LE)**	**T2(0.1% LE)**	**T3 (0.2% LE)**	**T4 (0.4% LE)**	**T5 (0.8% LE)**	**T6 (1.6% LE)**	**T7 (0.1% LP)**	**T8 (0.2% LP)**
Crude protein	42.06 ±0.47a	52.80 ±1.07c	62.18 ±1.57d	64.53 ±1.66d	62.14 ±0.21d	62.00 ±0.08d	52.12 ±0.14c	45.71 ±0.68b
Crude Fibre	3.76 ±0.70ab	5.39 ±0.17bc	6.14 ±0.12cd	7.25 ±0.03cde	8.60 ±0.21e	7.54 ±0.09de	5.88 ±1.50cd	2.31 ±0.01a
Ether Extract	7.23 ±0.42a	10.72 ±0.09c	10.49 ±0.19abc	13.69 ±0.12bc	13.62 ±0.14c	11.94 ±2.96c	9.25 ±0.05ab	11.56 ±0.46c
Total Ash	7.21 ±0.33a	7.39 ±0.20ab	7.08 ±0.14a	7.22 ±0.05ab	7.48 ±0.12b	7.20 ±0.05c	7.12 ±0.08b	7.17 ±0.01a
Moisture	8.11 ±0.36	8.50 ±0.24	8.02 ±0.46	7.92 ±0.20	8.68 ±0.07	8.16 ±0.06	8.07 ±0.10	8.12 ±0.01
Dry Matter	91.89 ±0.36	91.50 ±0.24	91.98 ±0.46	92.08 ±0.20	91.32 ±0.07	91.84 ±0.06	91.93 ±0.10	91.88 ±0.01
NFE (CHO)	39.74 ±1.01c	23.70 ±1.10c	14.11 ±1.76b	7.32 ±1.61a	8.16 ±0.43a	11.33 ±3.03ab	25.63 ±1.47c	33.25 ±1.01d
ENERGY	467.88 ±2.96a	495.74 ±0.84bc	506.73 ±2.65cd	522.77 ±2.04d	512.21 ±1.03cd	508.23 ±5.66cd	485.75 ±6.13ab	503.29 ±3.20bcd

Table 4: Growth Performance of *P. hypophthalmus* fed graded level of lapsi diets

Titles	**T1 (0.0% LE)**	**T2 (0.1% LE)**	**T3 (0.2% LE)**	**T4 (0.4% LE)**	**T5 (0.8% LE)**	**T6 (1.6% LE)**	**T7 (0.1% LP)**	**T8 (0.2% LP)**
Initial Weight	7.69 ±0.15	7.59 ±0.14	7.61 ±0.13	7.94 ±0.02	7.55 ±0.17	7.69 ±0.18	7.79 ±0.19	7.75 ±0.06
Final Weight	11.86 ±0.22 b	13.94 ±0.29 c	14.74 ±0.25cd	17.48 ±0.03e	14.71 ±0.29cd	15.28 ±0.31d	9.27 ±0.51 a	11.39 ±0.21 b
Weight Gain	4.17 ±0.07c	6.35 ±0.14d	7.13 ±0.12e	9.54 ±0.02f	7.16 ±0.12e	7.58 ±0.15e	1.48 ±0.32a	3.63 ±0.13b
Weight Gain%	54.21 ±0.26c	83.57 ±0.27d	93.68 ±0.19e	120.22 ±0.29f	94.86 ±0.92e	98.55 ±0.58e	18.85 ±0.76a	46.88 ±0.65b
SGR (%/day)	0.94 ±0.015c	1.32 ±0.014d	1.43 ±0.013e	1.71 ±0.015f	1.45 ±0.031e	1.493 ±0.15e	0.37 ±0.071a	0.83 ±0.19b
FCR	1.87 ±0.031d	1.23 ±0.027c	1.09 ±0.019b	0.81 ±0.015a	1.09 ±0.017b	1.03 ±0.018b	2.77 ±0.031f	2.11 ±0.015e
FCE	3.34	5.16	5.78	7.42	5.85	6.083	1.16	2.89

(%)	±0.031c	±0.031d	±0.031e	±0.031f	±0.031e	±0.031e	±0.031a	±0.031b
Survival (%)	91.11 ±2.81a	95.55 ±2.83abc	100 ±1.07c	100 ±1.43c	97.77 ±2.81bc	95.55 ±2.83abc	93.33 ±1.52ab	97.77 ±2.81bc

Table 5: Biochemical Performance of *P. hypophthalmus* fed graded level of lapsi diets

Titles	**T1 (0.0% LE)**	**T2 (0.1% LE)**	**T3 (0.2% LE)**	**T4 (0.4% LE)**	**T5 (0.8% LE)**	**T6 (1.6% LE)**	**T7 (0.1% LP)**	**T8 (0.2% LP)**
Serum Total protein (g)	2.89 ±0.09a	7.85 ±0.08c	11.92 ±0.42e	13.78 ±0.63f	12.09 ±0.07e	9.86 ±0.07d	5.03 ±0.28b	8.14 ±0.05c
Albumin (g)	1.05 ±0.04a	2.82 ±0.10d	3.57 ±0.05e	3.25 ±0.07e	2.63 ±0.03cd	2.40 ±0.06bc	1.22 ±0.11a	2.16 ±0.25b
Globulin	1.84 ±0.07a	5.03 ±0.14c	8.35 ±0.37e	10.54 ±0.62g	9.46 ±0.05f	7.46 ±0.13e	3.80 ±0.37b	5.97 ±0.23d
A-G Ratio	0.58 ±0.03c	0.56 ±0.03c	0.43 ±0.02b	0.31 ±0.02a	0.28 ±0.00a	0.32 ±0.01ab	0.33 ±0.05ab	0.37 ±0.06ab
SGOT (IU/L)	0.65 ±0.01e	0.46 ±0.00d	0.38 ±0.01d	0.19 ±0.00a	0.22 ±0.01b	0.27 ±0.01c	0.55 ±0.02e	0.54 ±0.00f
SGPT (IU/L)	35.80 ±0.31f	26.03 ±0.15e	21.38 ±0.42d	10.90 ±0.15a	12.53 ±0.33b	15.19 ±0.42c	31.00 ±1.06f	30.88 ±0.10g
ALP (IU/L)	72.593 ±0.62f	52.063 ±0.29e	42.763 ±0.83d	21.793 ±0.29a	25.06 ±0.65b	30.383 ±0.83c	62.003 ±2.12f	58.757 ±0.2g
NBT (mg NBT formazan/ml)	1.539 ±0.16e	0.935 ±0.01d	0.7357 ±0.02c	0.626 ±0.01bc	0.537 ±0.03ab	0.4413 ±0.01ab	0.45 ±0.01ab	0.364 ±0.02a
SOD-L (U/mg prot.)	9.59 ±0.17d	8.54 ±0.21e	7.5767 ±0.19b	6.46 ±0.17a	6.56 ±0.17a	7.65 ±0.18b	8.54 ±0.21c	7.57 ±0.19b
SOD-G (U/mg prot)	6.46 ±0.17e	5.41 ±0.21d	4.44 ±0.19c	3.33 ±0.17c	2.43 ±0.17b	1.52 ±0.18a	5.41 ±0.21d	4.44 ±0.19c
CAT-L (U/mg prot)	11.94 ±0.12f	11.33 ±0.12e	9.7 ±0.12c	5.38 ±0.05a	6.89 ±0.05c	6.51 ±0.05b	11.55 ±0.18c	10.73 ±0.12d
CAT-G (U/mg prot)	15.46 ±0.13g	14.36 ±0.13f	13.7 ±0.13e	8.15 ±0.36a	10.62 ±0.18c	9.51 0.2b	12.53 ±0.19d	12.16 ±0.13a

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