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Phytochemical Components of *Phyllanthus niruri* and Dietary Inclusion on Immunity of Young *Catla catla*

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Abstract

Phytochemical components of *Phyllanthus niruri* the finest agents, strengthen the animal's immune system, regardless of whether they are ill or in a stressed polluted environment. Phytochemical components don't have the negative side effects of chemical agents or antibiotics. In the study, three extraction solvents water, ethanol, and methanol were utilized and a comparatively higher number of phytochemical components were obtained from the ethanolic extract than from the other two solvents. Nonspecific immune response in young *Catla catla* fed with a diet including ethanolic extract showed an increase in hemoglobin levels and blood cells, blood cells are a key part of the non-specific immune response.

***Key words*:** *Phyllanthus niruri*, Phytochemical substances, *Catla*, hemoglobin, RBC, WBC

Globally aquaculture industries are growing rapidly and meeting the protein demands of economically weaker sections. In India, aquaculture industries play a critical role in the Indian economy and are one of the multidisciplinary agricultural industries. The aquaculture industries are simultaneously threatened by the emergence of diseases, particularly in intensive aquaculture, which results in huge economic losses and large fatalities. Juvenile fishes become immunocompromised due to overcrowding in a small space for raising and poor water quality, and the microorganisms become opportunistic to spread disease. Hormones, antibiotics, vitamins and several other chemicals have been tested in aquaculture operations for various remedies Numerous techniques have been used to increase stress tolerance and immunity in the stocked members. Immunomodulation, particularly immunostimulatory or immunity enhancer, is one of these techniques focused on nowadays [1-2]. Recently concept of biomedicines is providing an alternative for enhancing the health of fish and fish products produced in aquacultural operations. They possess the potential of promoting growth of fishes and also improving their immune system and appetite stimulators Already, chemicals in the form of vaccinations or antibiotics have been used to control diseases, stimulate immunity, reduce mortality, and increase growth rates. Still, adverse effects have been noted in the emergence of microbial communities that are resistant to antibiotics, accumulation of chemical residues in the tissues of fish which may cause a serious problem to the consumers and the chemicals are expensive and not a healthy way to treat diseases [3]. They pollute the fish-culturing environment [4-5]. An alternative approach to improve immunity and stress tolerance is the use of natural products obtained from herbs, especially phytochemical components which have remarkable health benefits such as antimicrobial, anti-cancer, antioxidant, and anti-inflammatory actions [6-9].

Immunostimulants provide protection by inducing both specific and nonspecific immunity in the host [10-11], making them a formidable alternative to chemical and anti-chemotherapeutic drugs as well as antibiotics [12]. By employing different solvents and extracting methods, the phytochemical components can be obtained from different portions of the herbs [13-16]. *Phyllanthus niruri* (*P. niruri*) is a vital tropical medicinal plant used to treat jaundice in Ayurveda [17]. These substances are widely known for breaking down kidney stones [18]. T and B cell proliferation was stimulated in female mice by aqueous extracts of *Phyllanthus niruri*. When aqueous extract of *Phyllanthus niruri* was given intraperitoneally to adult *Oreochromis mossambicus*, both specific and nonspecific immunity were positively modulated [11], [19]. Papaya leaves and fruits have been extracted using a variety of solvents, and phytochemical screening has also been carried out [20]. In this study, we aimed to extract the phytochemical components using three solvents and to find which extract has many phytochemical components and use them to boost the immunity of young *Catla catla* fish.

MATERIALS AND METHODS

Sample extraction

Fresh leaves of *Phyllanthus niruri* were procured from a local medicinal plant vendor, which were then shade-dried for a week at room temperature, the dried leaves were ground as a fine powder. 30 grams of powder was taken and divided into three portions, each comprising 10 grams, and each portion was taken in a separate conical flask. To the first conical flask 50 ml of distilled water, to the second conical flask 50 ml of ethanol,

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and to the third conical flask 50 ml of methanol was added. Tightly closed conical flasks were left at room temperature for 72 hours. Whatman No. 1 filter paper was used to filter the suspension, and the filtrate was then concentrated in a rotary evaporator. The extracted sample was kept in an airtight jar.

Tests for phytochemical screening

The standard procedure was used to qualitatively analyze the presence of phytochemical components in the extracted samples of *Phyllanthus niruri*.

Mayer's test for alkaloids

A 2.0 ml extract was mixed with 2.0 ml concentrated hydrochloric acid and 2.0 ml Mayer's reagent, and then monitored for the appearance of a green or white precipitate, which would indicate the presence of alkaloids.

Cardiac glycoside-ferric chloride test

2.0 ml of glacial acetic acid, 0.5 ml of extract, and a few drops of 5% ferric chloride were added to the extracts. This had 1.0 cc of pure sodium hydroxide behind it. It was noted that the interface formed a brown ring, which denotes the presence of cardiac glycosides.

Sulphuric acid test for flavonoids

1.0 ml of extract was treated with a few drops of strong sulphuric acid, and there the development of orange colour.

Sulphuric acid test for glycosides

2.0 ml of extract were mixed with 1.0 ml of glacial acetic acid, 5% ferric chloride and a few drops of strong sulphuric acid to see if a greenish blue colour developed, which would suggest the presence of glycosides.

Phenols-ferric chloride test

1.0 ml of extract was mixed with 2.0 ml of distilled water and a few drops of 10% ferric chloride. The blue or green colour formation was seen, which denotes the presence of phenols.

Sulphuric acid test for quinones

To 1.0 ml of extract, 1.0 ml of concentrated sodium hydroxide was added, and the production of red colour, which denotes the presence of quinones.

Saponins-foam test

1.0 ml of extract was mixed with 5.0 ml of distilled water in a graduated cylinder for 15 minutes lengthwise. A 1.0 cm layer of foam was seen to form, which denotes the presence of saponins.

Salkowski's test for steroids test

2.0 ml of chloroform and 5.0 ml of extract, together with a few drops of strong sulphuric acid, were added. The mixture was then examined for the development of red colour, which denotes the presence of steroids.

Tannins test with ferric chloride

To 1.0 ml of extract, 2.0 ml of 5% ferric chloride was added, and the production of a dark blue or greenish-black colour, which denotes the presence of tannins.

Sulphuric acid test for terpenoids

0.5 ml of extract was mixed with 2.0 ml of chloroform, and then a small amount of concentrated sodium hydroxide was carefully added. At the interface, reddish-brown colour development was seen, indicating the presence of terpenoids.

Renin test

A very small amount of the extract was mixed with 10 ml of petroleum ether and the same amount of copper acetate solution. The combination was then violently agitated, and the presence of renin was determined by the appearance of the green colour.

Fish

Healthy young *Catla catla* (*C. catla*) were purchased from a local fish farmer in Thiruvallur district, Tamil Nadu, India. They were acclimatized for a week getting used to the lab environment. They were fed with a basic diet and vital water quality parameters were being monitored during the entire experimental period. In order to prepare dietary-supplemented feed, the powdered ethanolic extract was combined with the basic diet at a ratio of 6 gm/kg. The ingredients for the basic diet: Soybean meal -300 gm, grain meal - 150 gm, dry fish meal-410 gm, groundnut oil cake - 120 gm, and Vitamin -10 gm. Before adding vitamins, ingredients were autoclaved, dried, and pulverized. They were made into dough using distilled water and then prepared as tiny floating pellets.

Experimental setup

Acclimatized young *Catla catla* fish were split into two groups, each containing 30 fish. The second group received the experimental food at a ratio of 1 gm per 10 gm fingerlings, while the first group received the basal diet, which was designated the control. They fed for 21 days.

Hematological assessment

Blood was drawn from the caudal peduncle and pooled, sampling was done on day 1 and day 21 after the experiment began. Blood samples were used to count total red blood cells (RBC), and white blood cells (WBC) using a Neubauer counting chamber, differential leucocyte count (DLC) using Leishman's stain, and hemoglobin level by cyanmethemoglobin method. Data were provided as average with standard error after being statistically processed.

RESULTS AND DISCUSSION

Phytochemical components in the three extracts were listed in (Table 1). Comparing the three extracts, the one made with ethanol as the solvent has more phytochemical components. The ethanolic extract has a significant number of flavonoids. The phytochemical constituents in the ethanolic and methanolic extracts of *Phyllanthus niruri* are greater than those in the aqueous extract. The outcome indicates that the phytochemical components can be extracted more efficiently using polar solvents of methanol and ethanol than with non-polar aqueous solvents. Greater total phenols and flavonoids in the ethanolic extract of *Carica papaya* leaves were discovered, which is similar to what we reported [20]. In addition to registering flavonoids and terpenoids in the aqueous extract of papaya peel, some researchers have discovered saponin and steroids in the papaya peel, and seed aqueous extract [21]. The existence of phytochemical compounds was found in the methanolic extracts of *Parquetina nigrescens* and *Carica papaya* [13]. Some researchers have discovered the phytochemical compounds as secondary metabolites in the ethanolic and n-hexane extracts of papaya leaves [22].

The majority of phytochemicals with therapeutic value are extracted using ethanol, and they all have pharmacological activities. The phytochemicals function as reducing substances, hydrogen-free radical scavengers, antioxidants, and cell defenders. Papaya leaf extracts made with methanol, ethyl acetate, and chloroform, respectively, contained 60, 62, and 53 phytochemical components [23-25].

Table 1 Qualitative phytochemical analysis of extracts of *Phyllanthus niruri*

Test	Aqueous	Methanol	Ethanol
Alkaloids	+	++	++
Cardiac glycosides	+	++	++
Flavonoids	-	+	+++
Glycosides	+	++	+
Phenols	+	+	++
Quinones	+	++	++
Saponins	-	-	+
Steroids	+	+	++
Tannins	+	+	+
Terpenoids	+	+	++
Renin	-	-	+

+++Strong present, ++Positive, +Present, -Absent

A crucial method for predicting the immunological status directly is the measurement of hematological markers. In the current study, we observed a glaring difference in the blood parameters between the control and experimental groups, as shown in (Table 2). It was noted that all blood parameters rose as fingerlings were fed the experimental diet for an increasing number of days. In contrast to the control group, all blood parameters were seen to increase as the number of days that the fingerlings were fed the experimental diet rose. Hemoglobin levels rapidly increased; on day 1, they were 9.2±0.16 and 9.5±0.18 g% was recorded for both control and experimental respectively and the experimental group recorded a high of 15.5± 0.81 g% as opposed to the control group's 9.26±0.15 g% on 21st day, however, this gain was only gradual. A comparable result in juvenile *Clarias gariepinus* treated with ginger extract was reported [26]. This is in line with a study of researchers who found improved hemoglobin levels and other hematological markers in *Huso huso* treated with dietary ginger and garlic [27]. Koi carp fed methanolic extracts of rainbow trout and tetra supplemented with *Echinacea purpura* had considerably higher hemoglobin levels [28-29]. As a result, a rise in hemoglobin in the group that received *Phyllanthus niruri* ethanolic extract may be a sign of improved immune system function, and the phytochemical components may have the power to stimulate the hematopoietic organ to make hemoglobin. As a result, the fingerlings may withstand the pressure and recover from the infection.

Total red blood cells (RBC) counts are a trustworthy measure of stress and illness. Between days 1 and 21, the experimental group's total RBC increased from 2.85±0.83 to 5.34±0.10 million cells/cu. mm, which was greater than the control on that day (2.9±0.04 million cells/cu. mm). RBCs aid in the delivery of oxygen to all tissues, which need more oxygen for cellular respiration. When Nile tilapia was fed a diet supplemented with green tea, similar elevated RBCs were seen in those fish [30]. Similarly, when African catfish fed with Mangosteen [31], *Huso huso* fed with ginger and garlic extract [27], and Rainbow trout provided a supplement comprising powdered ginger rhizome [32], erythrocyte counts were significantly higher. When *Phyllanthus emblica* was added to the diet of *Tilapia mossambicus*, it stimulated the synthesis of red blood cells and increased the overall number of RBCs in the fish [33]. The fact that the fingerling showed a rise in haemoglobin and total RBC indicates that the medicinal herb *Phyllanthus niruri* has an immunostimulatory effect. These two are able to improve fish fingerlings' ability to resist illness and handle stress. The experimental group's total WBCs have increased, whereas the control groups have not. The total WBC for fingerlings fed experimental feed jumped from 4.93±0.82 × 10^3 cells/cu.mm to 7.23±0.20 × 10^3 cells/cu.mm. While the total WBC for the basal diet-fed control group was 4.95±0.05 × 10^3 cells/cu.mm. The experimental group's lymphocyte, neutrophil, and monocyte counts were shown to be higher in differential leucocyte counts. Despite playing a significant protective effect, eosinophils and basophils were found to be less abundant in the experimental group than in the control group. The host is protected by lymphocytes, which are the body's main immune cells. WBC levels for *Labeo rohita* fingerlings fed *Mangifera indica* kernels increased [34]. It was found that feeding juvenile *Clarias gariepinus* with ginger produced similar outcomes in terms of an increase in lymphocyte counts [26].

Increased leucocyte count was seen in common carp fed *Epilobium hirsutum* both before and after *Aeromonas hydrophila* infection [35]. *Labeo rohita* fed with a diet that included extract of *Phyllanthus amarus* expressed improved health status and resistance against *Aeromonas hydrophila* [36].

Table 2 Comparison of hematological parameters in *Catla catla* fingerlings fed with experimental feed and basal feed

Parameters	1st day		21st day	
	Control	Experimental	Control	Experimental
Haemoglobin (g %)	9.2 ± 0.16	9.5 ± - 0.18	9.26 ± 0.15	15.05 ± 0.81
Total RBC (Million cells/cu.mm)	2.83 ± 0.05	2.85 ± 0.83	2.9 ± 0.04	5.34 ± 0.10
Total WBC (x10^3 cells/cu.mm)	4.91 ± 0.75	4.93 ± 0.82	4.95 ± 0.05	7.23 ± 0.20
DLC				
Lymphocyte (%)	40.66 ± 0.81	41.5 ± 1.04	41.1 ± 0.75	44.5 ± 0.84
Neutrophil (%)	32.17 ± 0.41	32.67 ± 0.52	34.5 ± 0.84	36.33 ± 0.82
Monocyte (%)	10.67 ± 0.52	10.83 ± 1.33	11.67 ± 1.97	12.33 ± 1.03
Eosinophil (%)	5.66 ± 0.82	5.83 ± 0.41	5.50 ± 0.84	3.5 ± 1.22
Basophil (%)	7.67 ± 0.52	8.83 ± 0.41	6.67 ± 1.51	2.83 ± 0.75

CONCLUSION

Our analysis of the data led us to the conclusion that among the three extracts, ethanolic extract has numerous numbers of phytochemical compounds and *Catla catla* fingerlings fed a meal containing ethanolic extract exhibited the best immunity, which was further substantiated by the identification of elevated non-specific immunological markers in the blood. The non-specific immunity of the group is therefore improved by the herb's extract. Further, these improved immunological characteristics may offer protection against opportunistic microbes in a stressed environment and disease-causing pathogens in immunocompromised conditions for the host.

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