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Improved growth parameters of Rohu fingerlings exposed to *Phyllanthus niruri* leaf extract

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Abstract

For the past three decades, the utilization of phytocompounds for the aquacultural growth and development are quite increased. Freshwater fish Rohu is one of the fine edible species in India. The medicinal plant *Phyllanthus niruri* has numerous bioactive compounds used to treat various human ailments. This study was planned to analyze the effect of *P. niruri* leaf extracts on the various growth parameters of freshwater fish, *Labeo rohita*. Adult fishes are acclimatized and exposed to 5mg/gm, 10mg/gm, 20mg/gm, 40mg/gm and 80mg/gm of aqueous extract of *P. niruri* for 30 days (n=6). Phytochemical analysis of the leaf extract showed the presence of phenols, saponins, alkaloids, tannins, terpenoids, flavonoids and steroids. Weight gain, average daily gain, specific growth rate, feed conversion ratio and survival rate of the control and five treated group fishes were studied. Our results concluded that bioactive compounds present in the aqueous leaf extracts of *P. niruri* enhanced the growth and feeding mechanism of the fishes which resulted in increased body weight, will eventually benefitted the aquacultural sector.

Keywords: *Phyllanthus niruri*, Rohu, Growth parameters, freshwater fish, phytocompound

Introduction

There will be an increasing demand for healthy, safe, and high-quality food in the future whereas aquaculture offers a promising future (Oliver 2013). With the growth of large-scale commercial fish culture, diseases of diverse aetiologies are now widely recognised as a major barrier to productive and sustainable farming (Rao et al., 1992). In light of recent economic losses, India actively monitors illnesses in its massive freshwater aquaculture. Recent advances in immunonutrition research have linked certain nutrients to fish immune status (Priya et al., 2004; Kumar et al., 2005). Because of this, fish nutritionists now concentrate not only on fish development but also on fish immune protection.

Sustainable aquaculture depends on the ability to keep fish development and health in the perfect balance. Antibiotics and chemotherapeutics can cause bioaccumulation, environmental contamination, and organisms that become resistant to them when used to cure fish diseases. Commercial vaccinations are expensive for fish farming operations since they are specialised against specific illnesses and are costly (Raa et al., 1992).

One of the most promising approaches to disease management in aquaculture is to fortify fish defence mechanisms using prophylactic immunostimulant injection (Robertson, 1999). Phyllanthus niruri (Family: Euphorbiaceae) frequently grows as a winter weed in dry places. More than 600 species of biennial or annual plants, trees, and shrubs that can reach heights of 60 cm in tropical and subtropical regions are found in the Phyllanthus genus. Numerous phytochemicals with pharmacological qualities are present in it (Calixto et al., 1998).

In traditional Brazilian medicine, it is also referred to as "Quebra-Pedras," which means stone breaker, and is used to treat patients with urolithiasis (Kieley et al., 2008). The aerial section of P. niruri L. has been used in traditional medicine for many years to treat a wide range of illness conditions, such as improving male fertility or libido; asthma; bronchial infections; liver disorders; diabetes; gonorrhoea; inducing labour and treating oedema; lowering fever; treating sore throats; female infertility; oliguria; and vaginitis. Obianime and Uche (2009) claim that they used the herb to treat irregular menstruation as well.

In Brazil, Phyllanthus niruri L. tea is used to treat renal calculi (Nishiura et al., 2004). In South Africa, traditional medicine uses it to treat hyperuricemia (Gupta and Vaghela 2019). According to Bagalkotkar et al. (2006), Shanmugam et al. (2014), Narendra et al. (2012), it has over 50 different chemicals in it, such as proteins, carbohydrates, reducing sugar, amino acids, tannin, phenol, terpenoids, alkaloids, saponins, lignans, steroids, resins, flavonoids, hypophyllanthin, phyllanthin, cardio glycosides, anthraquinones, and glycosinoids. Among these substances are triterpenes, which have been shown in studies to lessen the cytotoxicity of calcium oxalate (Malini et al., 2000), the excretion of substances that may cause kidney stones (Vidya et al., 2002), and kidney deposition symptoms (Vidya et al., 2000).

Furthermore, in hyperuricemic conditions, rats given a methanol-based leaf extract containing phyllanthin and lignans from *P. niruri* shown uricosuric activity (Murugaiyah and Chan 2006). The current study evaluates the phytochemical composition of *Phyllanthus niruri* and examines the effects of feeding adult *Labeo rohita* fish an ethanolic extract of P. niruri on their growth characteristics.

Materials and methods

Experimental plant

Fresh *Phyllanthus niruri* plants were purchased from a nearby botanical garden. According to Panase et al. (2018a) instructions, plant leaves were washed with water to remove dirt before being submerged in a 10% Clorox mixture for 20 minutes. In sterile conditions, air is used to dry them. The leaves were weighed and mixed at a 1:2 weight-to-volume ratio with a 50% ethanol solution in 1000 mL conical flasks.

After being shaken for 24 hours at room temperature in an automated shaker, the mixture was placed in a muslin cloth for filtering. Mixtures of phytocompounds were kept in a refrigerator at 4°C for seven days using a 5000ml flask. According to the method used by Harikrishnan et al. (2009), solvents were evaporated at 65°C using a rotary evaporator, and the dry weight was then determined using a freeze-drying process. The powder was kept at 20°C until it was used. Following the guidelines provided by Monisha et al. (2017) and Harborne (1988), ethanolic extracts of *P. niruri* leaves were phytochemically tested for phenols, terpenoids, alkaloids, steroids, saponins, tannins, quinones, flavonoids and sugar.

Fish treatment

Labeo rohita fingerlings were procured from a nearby fish farm. Once at the lab, the fish were all placed in cement ponds of 2 by 3 by 0.5 metres, with 300 litres of water and a natural photoperiod, and allowed to acclimatize for six weeks. A system of constant water and air circulation was maintained once a week. Parameters including temperature, pH, and dissolved oxygen were kept at 7.40±1.21, 27.4±2.09°C, and 7.70±1.3 mg/L, respectively, during the acclimatisation period. Additionally, twice a day (n = 12) the fish were fed 40% crude protein during the acclimatisation period. The fish feed was made based on rice and wheat bran for the control fish and phytoextract based feed for the treatment (T1 to T5).

Growth parameters

Weighing the fish in each net cage both at the beginning and the end allowed us to modify the feed volume. Growth parameters were estimated at the 30-day mark, and the results were marked. Growth indicators like weight gain (WG), average daily gain (ADG), specific growth rate (SGR), feed conversion rate (FCR), and survival rate (SR) were computed using the following formulas (Bagenal, 1978).

Weight gain (WG) = final weight (g) – initial weight (g)

Average daily gain (ADG) = [WG / experimental days]

Specific growth rate (SGR) = [ADG] × 100

Feed conversion ratio (FCR) = [Total feed fed (g) / weight gain (g)]

Survival rate (SR) = [number of survived fish / initial number of fish] × 100

Results and Discussion

Leaf extract phytochemical screening

In studies on green chemistry, phytochemical screening of plants is regarded as one of the most crucial analyses. The plant extracts' improved efficacy was based on their chemical composition. *Phyllanthus niruri* Linn. is a member of the Euphorbiaceae family of plants. A variety of *P. niruri* components have been used to identify the active phytochemicals, including flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins, and saponins. Several clinical trials have shown the medicinal benefits of this herb's extracts (Paithankar et al. 2011).

Among the nine screening tests, seven substances including alkaloids, phenols, terpenoids, saponins, steroids, tannins, and flavonoids showed good results for the ethanolic leaf extracts of *Phyllanthus niruri* indicating the existence of these active components in the leaf extracts (Table 1). The ethanolic leaf extracts of *Phyllanthus niruri* showed negative results for Sugar and Quinone tests.

Table 1. Phytochemical screening of ethanolic extracts of *Phyllanthus niruri* leaves.

Phytochemicals	Result
Alkaloids	++
Phenols	++
Flavonoids	++
Terpenoids	++
Saponins	++
Steroids	++
Tannins	++
Quinones	-
Sugar	-

Some plants has very diverse chemical compositions depending on whether part of the plant (leaves, stalks, or seeds) is used and how it is extracted from the plant using methods or solvents. Three phytochemical components were found in a 50% ethanolic extract of *A. graveolens* investigation were steroids, pseudotannins, and phenolics. Nonetheless, additional measurable components, such as sugars, flavonoids, alkaloids, steroids, and glycosides, are present in the phytochemical components present in methanolic extracts of seeds (Al-Snafi, 2014).

Effect of P. niruri on L. rohita growth

Fish (n=12) in the control and five treatment groups were first weighed (g) and the prepared fish food was added to the tank twice a day. The fish were fed a diet based on rice and wheat bran as a control and various concentrations (T1-5 mg/gm, T2-10 mg/gm, T3-20 mg/gm, T4-40 mg/gm, and T5-80 mg/gm) of leaf extracts as treatments. Weighing was done on each fish in each group at the end of the thirty-first day. Fish treated with ethanolic extracts of P. niruri showed weight gains (WG) of 108.6±5.6g for control fish, 142.6±6.2, 151.8±4.9, 183.4±4.9, 206.3±5.2 and 225.6±3.6g for T1-5mg/gm, T2-10mg/gm, T3-20mg/gm, T4-40mg/gm and T5-80mg/gm, respectively (Table 2).

Table 2. Mean±SD values of growth parameters of adult *Labeo rohita* feed with *P. niruri* leaf based diet for 30 days

Groups	Initial Wt. (g)	Final Wt. (g)	WG (g)	ADG (g)	SGR (%)	FCR (g)	SR (%)
Control	305.4±8.7	405.4±6.7	108.6±5.6	5.13±0.26	416.6±17.5	11.3±0.14	99.8±1.2
T1-5mg/gm	302.9±8.4	435.3±8.6	142.6±6.2	6.22±0.33	590.6±22.5	8.69±0.11	99.6±1.6
T2-10mg/gm	303.7±11.3	447.9±7.4	151.8±4.9	7.19±0.36	669.9±16.7	8.99±0.15	99.3±1.5

T3-20mg/gm	307.4±10.9	480.3±6.8	183.4±4.9	7.89±0.48	734.5±24.4	9.36±0.21	99.7±1.1
T4-40mg/gm	305.3±9.8	503.2±8.8	206.3±5.2	8.55±0.58	848.8±16.6	10.47±0.19	99.8±1.2
T5-80mg/gm	306.4±8.1	522.4±9.4	225.6±3.6	9.48±0.48	904.4±24.4	12.15±0.18	99.9±1.0

Fish eat to maintain a constant energy intake, or rather, a constant energy demand, when they are fed diets that vary in "digestible energy" and "digestible protein" at varying rates. Citarasu (2010) asserts that the digestive enzymes secreted by food's active plant components may enhance appetite and meal consumption. Additionally, it has been discovered that feeding rainbow trout (Smith, 1981), red swordtail (James et al., 2006), and blue gourami (Rinna Hamlin et al., 2013) supplemented diets increased feed intake and nutritional digestibility.

The fish treated with *P. niruri* had average daily gain (ADG) values of 6.22±0.33, 7.19±0.36, 7.89±0.48, 8.55±0.58, and 9.48±0.48g for T1–5 mg/gm, T2–10 mg/gm, T3–20 mg/gm, T4–40 mg/gm, and T5-80 mg/gm, respectively, while the control fish had an ADG of 5.13±0.26g. The fish treated with *P. niruri* had survival growth rates (SGR) of 416.6±17.5%, while the fish treated with ethanolic extracts had ADGs of 590.6±22.5, 669.9±16.7, 734.5±24.4, 848.8±16.6 and 904.4±24.4% for T1-5mg/gm, T2-10mg/gm, T3-20mg/gm, T4-40mg/gm, and T5-80mg/gm, respectively (Table 2).

The control fishes had a feed conversion ratio (FCR) of 11.3±0.14g and a survival rate (SR) of 99.8±1.2%, respectively. Table 2 displays the observed values for treatment groups FCR and SR: 8.69±0.11g and 99.6±1.6% (T1-5mg/gm), 8.99±0.15g and 99.3±1.5% (T2-10mg/gm), 9.36±0.21g and 99.7±1.1% (T3-20mg/gm), 10.47±0.19g and 99.8±1.2% (T4-40mg/gm), 12.15±0.18g and 99.9±1.0% (T5-80mg/gm) respectively.

Studies on the effects of herbal extracts on different kinds of fish have been conducted. For example, feeding 300g/kg of *Euphobia hirta* leaf extract to hybrid catfish, *Clarias microcephalus, C. gariepinus*, was observed to increase growth performance, haematological outcomes, and some organosomatic indicators (Panase et al., 2018a). Furthermore, *Polygonum minus* leaf extract can help *Anabas testudineus* by enhancing growth performance and haematological markers (Panase and Tipdacho, 2018). Furthermore, Panase et al. (2018b) report that *Houttuynia cordata* leaf extract can be utilised to improve the hybrid catfish *Clarias microcephalus* and *C. gariepinus* growth performance.

Conclusion

The current study's findings suggest that supplementing feed (diet) with ethanolic leaf extracts of *Phyllanthus niruri* promotes better consumption, increases absorption, and speeds up the growth of *L. rohita* fish compared to control fish. Consequently, this plant might be considered while designing a diet for freshwater fish. The *P. niruri* leaf's phytochemical composition was the cause of the fish's accelerated growth. Consequently, it stands to reason that adding this plant to fish meals could boost aquaculture output.

Conflict of Interest: None

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