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Effect of Phyllanthus niruri leaf extracts on growth parameters of freshwater fish Labeo rohita (Rohu)

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KEYWORDS

Phyllanthus niruri, Labeo rohita, aqueous leaf extract, growth parameter, phytochemical screening

ABSTRACT:

Introduction: Nowadays, herbal extracts of the medicinal plants are important in the aquaculture sector for the enhancement of growth and reproductive factors of freshwater fishes. In India, Labeo rohita is one of the most commonly cultured freshwater fish (rohu) for human consumption.

Objectives: To analyze the effect of aqueous extracts of P. niruri on the growth parameters of freshwater fish Labeo rohita

Methods: Phyllanthus niruri (Family: Euphorbiaceae) has various pharmacological properties due to their phytocompounds which are used to treat various ailments. After acclimatization, the L. rohita adult fishes (n=6) were treated aqueous leaf extracts of P. niruri (T1-5mg/gm, T2-10mg/gm, T3-20mg/gm, T4-40mg/gm and T5-80mg/gm) based diet for 30 days.

Results: Phytochemical screening showed positive results for alkaloids, phenols, terpenoids, saponins, steroids, tannins and flavonoids. Growth parameters such as WG, ADG, SGR, FCR and SR were studied. When compared to the control group rohu fishes, the treated group fish growth parameters were quite improved which resulted the enhanced growth in the aqueous leaf extracts of P. niruri treated L. rohita fishes.

Conclusions: The phytochemical makeup of the P. niruri leaf was responsible for the fishes increased growth. Therefore, it might be concluded that using this plant as an ingredient in fish food may increase aquaculture productivity.

1. Introduction

Aquaculture considered as the future need for safe, wholesome, and high-quality food presents a bright opportunity (Oliver, 2013). Diseases of various aetiologies are now widely acknowledged as a key obstacle to productive and sustainable farming with the development of large-scale commercial fish culture (Rao et al., 1992). India aggressively monitors diseases in extensive freshwater aquaculture due to recent economic losses. The immunological condition of fish is related to some nutrients, according to recent developments in immunonutrition studies (Priya et al., 2004; Kumar et al., 2005). This has caused fish nutritionists to focus on fish's immune protection in addition to its growth.

The ability to maintain the ideal balance between fish development and health is essential for sustainable aquaculture. The use of antibiotics and chemotherapeutics to treat fish ailments runs the danger of causing bioaccumulation, environmental contamination, and organisms that are resistant to them. Commercial vaccinations are pricey and specialised against particular infections, which makes them expensive for fish farming practises (Raa et al., 1992).

Strengthening fish defence mechanisms with preventative immunostimulant injection is one of the most promising strategies to disease management in aquaculture (Robertsen, 1999). In the drier areas, Phyllanthus niruri (Euphorbiaceae family) often develops as a winter weed. The Phyllanthus genus includes >600 species of biennial or annual herbs, trees and shrubs that can grow up to 60 cm in the tropical and subtropical climates. It has a wide range of pharmacological properties and phytochemicals (Calixto et al., 1998).

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It is also known as "quebra-pedras" which means stone-breaker, and used to treat urolithiasis patients in traditional Brazilian medicine (Kieley et al., 2008). Many nations have utilised the aerial section of Phyllanthus niruri L. in traditional medicine to treat a variety of illness conditions, including increasing libido or male fertility. Asthma, bronchial infections, liver disorders, diabetes, gonorrhoea, producing labour, and treating oedema, feverish discomfort, sore throat, female infertility, oliguria, and vaginitis are among the conditions that the herb is typically used to cure in India. According to Obianime and Uche (2009), they also utilised the herb to treat irregular menstruation, tachycardia, diarrhoea, spasmodic cough, itching, arthritis, otitis, swelling, skin ulcers, and male organ weakness.

Renal calculi are treated with Phyllanthus niruri L. tea in Brazil (Nishiura et al., 2004). It is used to treat hyperuricemia in traditional medicine in South Africa (Gupta and Vaghela 2019). It contains over 50 different chemicals, including carbohydrates, proteins, reducing sugar, amino acids, tannin, phenol, terpenoids, alkaloids, saponins, lignans, steroids resins, phyllanthin, cardio glycosides, flavonoids, hypophyllanthin, anthraquinones and glycosinoids (Bagalkotkar et al. 2006; Shanmugam et al., 2014; Narendra et al., 2012). Triterpenes are one of these chemicals, and studies have shown that they can reduce the cytotoxicity caused by calcium oxalate (Malini et al., 2000), as well as the excretion of substances that might cause kidney stones (Vidya et al., 2002) and signs of crystal deposition in the kidneys (Vidya et al., 2000).

Additionally, rats treated with a methanol-based leaf extract from *P. niruri*, which includes phyllanthin and lignans, showed uricosuric activity in hyperuricemic circumstances (Murugaiyah and Chan 2006). The current study assesses the phytochemical makeup of *P. niruri* and also looks at the impact of dietary *P. niruri* aqueous extract administration on the growth characteristics of adult *Labeo rohita* fish.

2. Methods

Experimental plant

The *Phyllanthus niruri* fresh plants were bought at a local botanical garden. Plant leaves cleaned with water for the removal of dirt before being immersed 20 mins in a Clorox mixture (10%), as per the procedures outlined by Panase et al. (2018a). They are dried by air in sterile circumstances. The leaves were weighed and combined in 1000 mL conical flasks with a 50% aqueous solution at a weight-to-volume ratio of 1:2.

The mixture were put in a muslin cloth for filtration, after being shaken for 24 hours at room temperature in an automated shaker. Phytocompounds were combined in a 5000ml flask and maintained at 4°C for seven days in a refrigerator. By using rotary evaporator, solvents were evaporated at 65°C in accordance with the procedure employed by Harikrishnan et al. (2009), and the dry weight was then calculated using a freeze-drying methodology. Until use, the powder form was maintained at 20°C. Alkaloids, phenols, sugar, terpenoids, saponins, steroids, tannins, quinones and flavonoids were all screened for by phytochemical screening of aqueous extracts of *P. niruri* leaves, as per the recommendations made by Harborne (1988), Monisha et al. (2017).

Fish treatment

Labeo rohita fingerlings were bought from a local fish farm. All of the fish were brought to the lab and were given 6 weeks to acclimatise in cement ponds (2x3x0.5m) with 300 L of water and a natural photoperiod. Every week, a system of continual air circulation and water was maintained. Throughout the acclimatisation period, parameters such as the dissolved oxygen, pH and temperature were maintained as 7.70±1.3 mg/L, 7.40±1.21 and 27.4±2.09°C respectively. Additionally, the fish feed was prepared based on rice and wheat bran for control fishes and phytoextract based feed for treatment (T1 to T5) and fed to the fishes twice daily (n=6), during the acclimatisation period 40% crude protein were fed.

Growth parameters

Fishes in each net cage were weighed initially and finally to adjust the feed volume. At 30th day, growth parameters were estimated, with the findings being reported. The following equations were used to calculate growth indices such as weight gain (WG), average daily gain (ADG), specific growth rate (SGR), feed conversion rate (FCR), and survival rate (SR) (Bagenal, 1978).

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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

3. Results

Phytochemical screening of P. niruri leaf extracts

Phytochemical screening of the plants is considered as one of the most important analysis in the green chemistry studies. Based on the chemical composition, the activity of the plant extracts was enhanced. Among the nine screening test, the aqueous leaf extracts of Phyllanthus niruri showed positive results for seven chemicals such as alkaloids, phenols, terpenoids, saponins, steroids, tannins and flavonoids which indicated the presence of this active compounds in the leaf extracts (Table 1). Sugar and Quinone analysis showed negative results which indicated the absence of these phytocompounds in the aqueous leaf extracts of Phyllanthus niruri.

Table 1. Phytochemical screening of aqueous extracts of Phyllanthus niruri leaves.

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

Effect of P. niruri on *L. rohita* growth

In control and five treatment groups fishes (n=6) were weighed initially (gm) and the prepared fish fed were applied to the tank twice daily. For control, rice and wheat bran based diet and for treatments, different concentrations (T1-5mg/gm, T2-10mg/gm, T3-20mg/gm, T4-40mg/gm and T5-80mg/gm) of leaf extracts based diet were fed to the fishes. At the end of 30th day, weight of each fishes in every group were weighed. Control fishes weight gain (WG) were observed as 100.6±5.6g whereas the ethanolic extracts of P. niruri treated fish WG were observed as 134.6±8.2, 145.8±6.9, 178.4±8.9, 199.3±9.2 and 216.6±5.6g for T1-5mg/gm, T2-10mg/gm, T3-20mg/gm, T4-40mg/gm and T5-80mg/gm respectively (Table 2).

Average daily gain (ADG) of the control fishes were observed as 3.53±0.58g whereas aqueous extracts of P. niruri treated fish ADG were observed as 4.48±0.89, 4.86±0.91, 5.94±0.96, 6.64±0.89 and 7.28±0.99g (Table 2) for T1-5mg/gm, T2-10mg/gm, T3-20mg/gm, T4-40mg/gm and T5-80mg/gm respectively.

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)**
Control	215.4±14.7	315.4±16.7	100.6±5.6	3.53±0.58
T1-5mg/gm	212.9±16.4	345.3±17.6	134.6±8.2	4.48±0.89
T2-10mg/gm	213.7±17.3	357.9±16.4	145.8±6.9	4.86±0.91
T3-20mg/gm	217.4±13.9	390.3±14.8	178.4±8.9	5.94±0.96
T4-40mg/gm	215.3±14.8	413.2±16.8	199.3±9.2	6.64±0.89
T5-80mg/gm	216.4±14.1	432.4±17.4	216.6±5.6	7.28±0.99

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Survival growth rate (SGR) of the control fishes were observed as 353.6±21.5% whereas ethanolic extracts of P. niruri treated fish ADG were observed as 448.6±17.5, 486.9±19.7, 594.5±25.4, 664.8±28.6 and 728.4±46.4% for T1-5mg/gm, T2-10mg/gm, T3-20mg/gm, T4-40mg/gm and T5-80mg/gm respectively (Table 3). Feed conversion ratio (FCR) and survival rate (SR) of the control fishes were observed as 9.3±0.14g and 99.8±1.2% respectively. Treatment groups FCR and SR were observed as 6.69±0.11g and 99.6±1.6% (T1-5mg/gm), 6.17±0.15g and 99.3±1.5% (T2-10mg/gm), 5.05±0.21g and 99.7±1.1% (T3-20mg/gm), 4.51±0.19g and 99.8±1.2% (T4-40mg/gm), 4.15±0.18g and 99.9±1.0% (T5-80mg/gm) respectively.

Table 3. Mean±SD values of growth parameters of adult Labeo rohita feed with P. niruri leaf based diet for 30 days

Groups	SGR (%)	FCR (g)	SR (%)
Control	353.6±21.5	9.3±0.14	99.8±1.2
T1-5mg/gm	448.6±17.5	6.69±0.11	99.6±1.6
T2-10mg/gm	486.9±19.7	6.17±0.15	99.3±1.5
T3-20mg/gm	594.5±25.4	5.05±0.21	99.7±1.1
T4-40mg/gm	664.8±28.6	4.51±0.19	99.8±1.2
T5-80mg/gm	728.4±46.4	4.15±0.18	99.9±1.0

4. Discussion

The therapeutic capabilities of a wide range of phytochemicals found in the Euphorbiaceae plant Phyllanthus niruri Linn. Different components of P. niruri have been used to identify the active phytochemicals, including flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins, and saponins. Numerous clinical trials have demonstrated the medicinal benefits of this herb's extracts (Paithankar et al. 2011).

Depending on the component of the plant that is used (leaves, stalks, or seeds) and the techniques or solvents that are used to extract them from the plant, celery has quite different chemical compositions. In this investigation, three phytochemical components—steroids, pseudotannins, and phenolics—were identified in an A. graveolens extract that was 50% ethanolic. However, the phytochemical components found in methanolic extracts of seeds contain more quantifiable elements, including sugars, flavonoids, alkaloids, steroids, and glycosides (Al-Snafi, 2014).

Kollab and Alamen (2018) found that the A. graveolens leaf extract produced using ethyl alcohol and aqueous solvents contained flavonoids, phenols, tannin, saponins, steroids, alkaloids, and carbohydrates. However, terpenoids were only found in the ethyl alcohol extract, while phlobatanins, Apanthraquinons, and glycosides were not present in this plant species. A. graveolens leaf extract in a 50% ethanolic solution hasn't been studied for its effects on Labeo chrysophekadion, despite several reports of some herbal extracts' advantages for fish development and immunological stimulation.

When fish are fed on diets that differ in "digestible energy" and "digestible protein" at different rates, food intake is regulated to maintain a constant energy intake or, in fact, that energy need remains constant. According to Citarasu (2010), the active plant ingredients in food may cause the secretion of digestive enzymes, which will increase hunger and food intake. Spirulina enriched diets were also found to increase feed intake and nutritional digestibility in rainbow trout (Smith 1981), red swordtail (James et al., 2006), and blue gourami (Rinna Hamlin et al., 2013).

There have been studies on the effects of herbal extracts on other fish species. For instance, hybrid catfish, Clarias microcephalus, C. gariepinus, were fed with 300g/kg of Euphobia hirta leaf extract, and it was found that this improved growth performance, haematological results, and some organosomatic indices (Panase et al., 2018a). Additionally, Anabas testudineus can benefit from the usage of Polygonum minus leaf extract to improve haematological indicators and growth performance (Panase and Tipdacho, 2018). Additionally, Houttuynia cordata leaf extract can be employed to enhance the growth performance of the hybrid catfish Clarias microcephalus and C. gariepinus, according to Panase et al. (2018b).

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5. Conclusion

Based on the results of the current study, it can be inferred that adding ethanolic leaf extracts of P. niruri to feed (diet) encourages better consumption, boosts absorption, and accelerates the growth of the fish, L. rohita than control fishes. As a result, this plant can be taken into account while creating freshwater fish diet. The phytochemical makeup of the P. niruri leaf was responsible for the fishes increased growth. Therefore, it might be concluded that using this plant as an ingredient in fish food may increase aquaculture productivity.

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