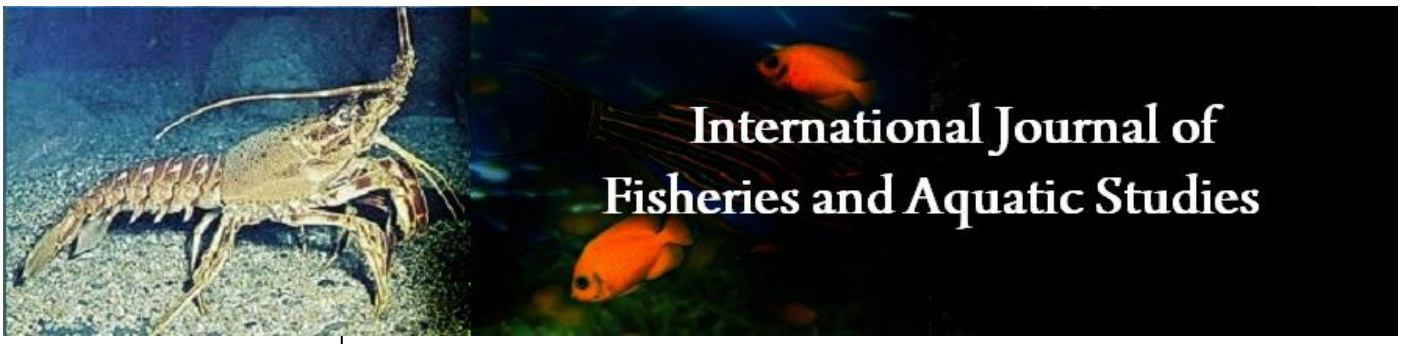

E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2023; 11(1): 26-30

www.fisheriesjournal.com
Received: 25-10-2022
Accepted: 28-11-2022

Rosidah
Department of Fisheries, Faculty of Fishery and Marine Science, Universitas Padjadjaran, Bandung, Indonesia

Corresponding Author:
Rosidah
Department of Fisheries, Faculty of Fishery and Marine Science, Universitas Padjadjaran, Bandung, Indonesia

A mini-review: Potential of *Phyllanthus niruri* L as immunostimulators in fish aquaculture

Rosidah

DOI: https://doi.org/10.22271/fish.2023.v11.i1a.2765

Abstract
Disease is the main factor causing crop failure in fish farming activities, which results in decreased production and of course is very detrimental economically for farmers. Therefore, it is necessary to control the disease with the right steps to avoid large losses. Preventive action is the right step, because it can be done as early as possible before the fish contract the disease, which of course costs less than treatment. One of the efforts to prevent disease is to increase the immune system of fish. Immunostimulants are compounds that can be used as an alternative therapy to increase the body's resistance to pathogen infections, because they can increase the work of the immune system through the activity of white blood cells and other phagocytic cells. Several research results have proven the effectiveness of various types of plants as immunostimulants, one of which is *Phyllanthus niruri* L. Several secondary metabolite compounds and filantin as specific compounds contained in *P niruri*, apart from having antibacterial and antioxidant effects, also function as immunostimulants. So the purpose of this article is to explain the potential of *P. niruri* L. as an immunostimulant in cultivated fish which can increase the body's resistance to disease. Based on the above studies, *P. niruri* L. can increase non-specific and specific fish body resistance, so it has the potential to be used as an alternative immunostimulant that can prevent several types of cultivated fish such as *Oreochromis mossambicus* and *Cyprinus carpio* L. fish from attack by pathogenic bacteria, including *Aeromonas hydrophila*.

Keywords: Fish, *Phyllanthus niruri* L, immune system, disease

1. Introduction
Fish farming activity is one of the activities that can support food security which is developing very rapidly [1]. Even the production of the world's fisheries sector is highly dependent on aquaculture activities, both freshwater, brackish water and sea [2]. However, aquaculture activities are often faced with quite formidable challenges, which can threaten the sustainability of production, namely crop failure, one of which is caused by disease [3]. Among the causes of diseases that are very detrimental economically to fish farmers are those caused by pathogenic microorganisms in the form of bacteria and viruses. Both types of pathogenic microorganisms easily spread from one cultivation site to another and this condition is very dangerous [4]. Therefore it is necessary to make efforts to control the disease as early as possible in cultivated fish in the right way, one of which is to take precautions, so that the fish can avoid disease. Efforts to prevent disease are relatively easier and cheaper than treatment efforts, because it is done before the fish are infected by the disease. Increasing the non-specific immune system in fish is one of the efforts that can be made so that fish are resistant and protected from disease. There are several ways to increase the non-specific resistance of fish against disease, including by administering immunostimulants. According to Roitt, (2017) [5] immunostimulant is a compound that can be used as an alternative therapy to increase non-specific fish body resistance to pathogenic microorganism infections, through the mechanism of increasing the work of the immune system, namely increasing the activity of white blood cells and other phagocytic cells, so that the physiological condition of the body is not disturbed. Some materials can be used as a source of immunostimulant, including plants. In general, plants contain several secondary metabolite compounds that have pharmacological properties, including as immunostimulants [6, 7]. Secondary metabolite compounds contained in plants include alkaloids, flavonoids, steroids, saponins, terpenoids and tannins which can play a role in fighting various diseases [6, 8].

Phyllanthus niruri belongs to the Euphorbiaceae family, is a tropical plant that is spread all over the world, known as a medicinal plant with many benefits [8, 9]. This plant contains several secondary metabolites and phyllantine as specific compounds found in P. niruri. The pharmacological properties possessed by *P. niruri* include being an immunomodulator which can enhance the immune system or the immune system against a disease [11, 10, 12]. This plant extract has been shown to be a strong immunomodulator that can increase blood peripheral proliferative activity and phagocytic activity of macrophages [11]. Based on the phytochemical content and pharmacological properties of *P niruri*, the purpose of writing this article is to explain the potential of *P. niruri* as an immunostimulant in cultured fish which can increase the body's resistance to disease.

2. Classification and morphology of *Phyllanthus niruri* L.

Phyllanthus niruri L. (Figure 1) belongs to the Euphorbiaceae family, a plant that is distributed in tropical and subtropical regions. In Malay this plant is called dukong anak, in China it is known as zhu zi cao, in Brazil it is called chanca Piedra and in southern India it is called Bhumyamalaki [10]. This plant lives on land to an altitude of 1000 m DPL, in rocky places, damp places such as along waterways, or among grass and shrubs [Dalimarta]. The classification of *Phyllanthus niruri* L. is as follows:

Kingdom	:	Plantae
Division	:	Spermatophyta
Sub Divisi	:	Angisopermae
Class Order	:	Dicotyledonae
Family	:	Dicotyledonae
Genus	:	Euphorbiaceae
Species	:	Euphorbiaceae
	:	*Phyllantus niruri* L. [14 Kardinan, 2004]

Phyllanthus niruri L. is a wild herb plant, originating from Asia and has now reached the African, Australian and American continents, having light green or dark green stems. Each branch or twig consists of 8-25 leaves. The leaves are green, measuring 0.5-2 x 0.25-0.5 cm [14]. It has white male and female flowers. Male flowers appear under the leaf axils, while female flowers appear above the leaf axils [15]. The ripe meniran anthers will burst longitudinally [14].

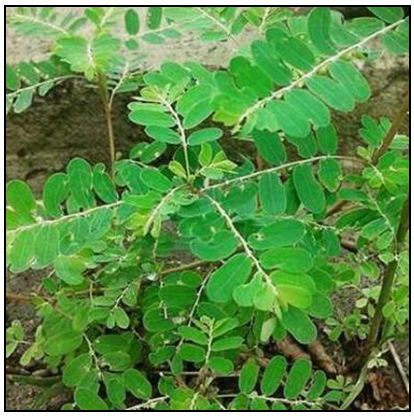

Fig 1: *Phyllanthus niruri* L. Plant

3. The content and benefits of *Phyllanthus niruri* L.

In general, the *Phyllanthus niruri* L. plant contains several complete chemical compounds, so it is very beneficial for health. *P. niruri* contains alkaloids, flavonoids, tannins, saponins, steroids, phenols and terpenoids [16, 17, 18, 19]. Triterpenoid compounds contain components of lupeol acetate and betasitosterol [20]. Apart from containing bioactive compounds of flavonoids, tannins, alkaloids, steroids, *P. niruri* also contains molecules of lignans, phyllanthin, hypophyllanthin, glycosides, ellagitannins, triterpenes, phenyl propanoids, ricinolic acid, niruriside, phyltetralin, essential oils, anthraquinones and arbutin [21, 22, 23, 24, 25]. Components of flavoniod compounds such as quercetin are mostly found in the leaves, while the stem contains niruri components, niruritenin, rutin. Lignin compounds such as phyllanthin and hypophyllanthin are present in all parts of the plant [26]. Quantitatively, the Phyllanthus niruri L plant contains a total phenol equivalent to 28.05 g of gallic acid in 1 mg of plant extract, while the content of flavonoids in 1 mg of plant extract is equivalent to 61.41 g of quercetin, while the filantin content in 70% P niruri ethanol extract of 0.864% [16]. Traditionally this plant has been widely used by people in tropical countries to treat various types of diseases including intestinal infections, kidney stones [27, 28], chronic liver disease, diabetes and preventing hepatitis B virus infection [28, 29], asthma, gonorrhea, bronchitis, and syphilis [30, 31] and boosting the immune system [11]. The ability of *P. niruri* to treat various diseases is due to its pharmacological properties, including as an immunomodulator, antiviral, antibacterial, diuretic, anti-hyperglycemic and hepatoprotector [10, 12]. In Indonesia, this plant is used to boost the immune system. This plant extract has been shown to be an immunomodulator that can increase blood peripheral proliferative activity and macrophage phagocytic activity [11].

4. Applications of *Phyllanthus niruri* L. as immunostimulant in culture fish

Several studies have been carried out to prove the effect of *P. niruri* as an immunostimulant that can increase the resistance of humans and animals including fish against disease. Several parameters can be used as indicators of an increase in the immune system or an increase in body resistance after being treated with compounds that act as immunostimulants, including antibody titres, white blood cells including their components, namely neutrophils, monocytes, lymphocytes and macrophages. Meanwhile, cytokines, antibodies, and complement proteins are included in the immune molecule. Synchronous interactions between the immune system and immune molecules can induce a good immune response, which can lead to resistance to pathogenic infections [32]. Antibody titer is one of the parameters to determine the effectiveness of an immunostimulant substance, by looking at the ability of serum proteins containing antibodies to collect and destroy antigens that enter the body [33].

Two sets of Oreochromis mossambicus fish groups, each consisting of 6 groups (n = 8 per group) were used as test fish to prove the ability of *P. niruri* as an immunostimulant by observing the levels of an body titers and neutrophil levels. The part of the *P. niruri* plant used was the leaf in the form of an aqueous extract. The extract was given to fish by intraperitoneal injection with various doses, namely 20, 2, 0.02 and 0.002 mg/kg fish body weight. To observe the activity of antibody titers, the first group of fish, after two days of administration of *P. niruri* leaf extract, were challenged with Sheep red blood cells (SRBC) which function as antigens. Sheep red blood cells (SRBC) are given by intraperitoneal injection with the first dose of 5% of 0.1 mL and a second dose of 25% (booster) of 0.1 mL given three

days after the first dose, then observed for 60 days from the first injection. As for observing neutrophil activity, fish in the second set were challenged with 100 µL HA-BSA (heat aggregated-bovine serum albumin) with a concentration of 5 mg via intraperitoneal injection, then observations were made for 12 days. The results showed that administration of *P. niruri* aqueous extract resulted in a significant increase compared to controls in the activation of antibody and neutrophil responses. Of the various doses tested, the group of fish that were given *P. niruri* leaf aqueous extract at a dose of 20 mg/kg body weight gave the maximum increase in primary and secondary antibody responses on the 15th day of observation. Whereas the group of fish that were given P. niruri leaf aqueous extract at a dose of 0.02 mg/kg body weight resulted in a maximum increase in neutrophil levels on day 6 [34]. Tilapia fish (*Oreochromis niloticus*) that have been given *P. niruri* leaf extract through injection are able to survive against *Aeromonas hydrophila* bacteria. A concentration of 50 mg was significantly the best concentration with the highest survival of tilapia, which was 76.7% [35 2014]. carp (*Cyprinus carpio*) after being given feed mixed with P. niruri powder for 60 days was then challenged with A. hydrophila bacteria with a density of 10^3 CFU with the cohabitation method through water in a rearing aquarium, able to survive the attack of these bacteria. Giving *P. niluri* powder as much as 2% (10 g/500 g feed) showed the highest fish resistance against attack by *A. hydrophila* bacteria, which was 100% [36] (Table 1).

The results of research conducted by Ma'at *et al*., (1996) [37] showed that *P. niruri* had an immunomodulating effect *in vitro*. P. niruri extract (50-200 mg/kg) can elicit both cellular and humoral immune responses. As the results of research by Nworu *et al*., (2010) [38] showed *that P. niruri* water extract (12.5-200 g/ml) could induce lymphocytes and macrophages in experimental animals.

Table 1: Applications of *Phyllanthus niruri* L. Sebagai Immunostimulant

Jenis Ikan	Treatment	Metode Uji Tantang	Hasil	Ref
Oreochromis mossambicus	Administration of *P. niruri* aqueous extract via intraperitoneal.	Injection Fish were injected intraperitoneally with Sheep red blood cells (SRBC). The first dose of 0.1 mL of 5% and the second dose (booster) of 0.1 mL of 25%	*P. niruri* extract dose of 20 mg/fish body weight resulted in a maximum increase in antibody levels on day 15.	[34]
Oreochromis mossambicus	Administration of *P. niruri* leaf aqueous extract by intraperitoneal injection.	Fish were injected intraperitoneally with HA-BSA (heat aggregated-bovine serum albumin) with a concentration of 5 mg as much as 100 µL.	A dose of P. niruri extract of 0.02 mg/kg body weight resulted in a maximum increase in neutrophil levels on the 6th day.	[34]
Oreochromis niloticus	Administration of *P. niruri* leaf aqueous extract by injection.	Infecting fish by injection with *Aeromonas hydrophila* bacteria.	The concentration of 50 mg significantly resulted in the highest survival, which was 76.7%.	[35]
Cyprinus carpio	Giving water extract of *P. niruri* leaves through feed.	Infection of fish with *A. hydrophila* 10^3 CFU in cohabitation through water media in rearing aquariums.	The concentration of 2% (10 g/500 g of feed) resulted in the highest fish survival, namely 100%.	[36]

In Table 1 it can be seen that the *P. niruri* plant used as an immunostimulant is the leaf part, in the form of extract or powder (flour) and the method of administration is also by injection or through feed. Testing the ability of these plants as immunostimulants in fish can be carried out using various challenge test methods and various observational indicators, among others by looking at antibody levels, neurophil levels and survival of fish after being challenged with pathogenic bacteria. The type of fish tested, the challenge test method and the dosage used influenced the results obtained.

Based on the results of several studies above, it proves that *P. niruri* can be used as an immunomodulator that can induce fish resistance to pathogenic bacteria. As according to the POM Agency (2006) [39] that *P. niruri* is an immunomodulator, which can improve or enhance the immune system. The main component and is an active compound as an immunostimulant which is contained in *P niruri* flavonoids. The mechanism of flavonoids as an immunostimulant is to activate the body's cells by sending intracellular signals to cell receptors, so that the body's cells work optimally. Apart from flavonoids, other compounds contained in *P. niruri*, including phyllanthin and hyphophyllanthin are two compounds that have anti-inflammatory activity so that they can strengthen immunity. Another opinion states that *P. niruri* has an effect as an immunomodulator through activation and augmentation of the cellular immune system. Specifically *P. niruri* can activate neutrophils, macrophages, monocytes, T and B lymphocytes. The increase in phagocytosis by neutrophils indicates an accelerated process of eradicating pathogenic microbes, especially for extracellular pathogens, such as viruses, bacteria or fungi. *P. niruri* can induce an increase in monocytes and macrophages, which are able to lyse infecting intracellular pathogenic cells and expose these pathogenic cells to other immune components in the extracellular compartment. In addition, P niruri can modulate cytokine secretion, including stimulating IFN-γ, TNF-α, IL-4, IL-6, IL-12 and suppression of IL-10. This shows that *P. niruri* can induce the body's defense, by engaging the cellular immune system against foreign pathogens [40]. On the basis of the immunostimulatory properties possessed by *P. niruri* from a series of non-specific (neutrophil), specific (antibody) parameter tests and other tests, namely the survival rate of fish challenged with pathogenic bacteria can be used either as a routine feed supplement to activate the immune system of cultivated fish, other than that it can be used as an adjuvant in the use of vaccines.

Conclusion

Based on the above studies, Phyllanthus niruri L. with various preparations can increase non-specific and specific fish body resistance, so it has the potential to be used as an alternative immunostimulant that can prevent cultivated fish such as Oreochromis mossambicus and Cyprinus carpio fish from attack by pathogenic bacteria, namely Aeromonas hydrophila.

Competing interests
The author has declared that no competing interests exist.

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