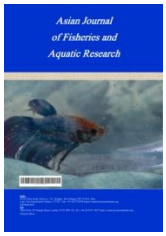

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A Mini- review : Effect of *Phyllantus niruri* L. on Growth and Health of Fish

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Phyllanthus niruri L. is one of the herbal ingredients that has been widely used to treat various diseases in humans such as intestinal infections, kidney stones, chronic liver disease, diabetes, hepatitis B, asthma, gonorrhea, bronchitis, syphilis and boost the immune system. The ability of *P. niruri* in overcoming various diseases is based on its phytochemical content and pharmacological properties. Based on these properties, the plant may possibly be used as a drug to treat diseases in fish. So the purpose of writing this article is to review the extent to which *P. niruri* can be used as an alternative medicine to treat disease and maintain fish health. Secondary metabolites and phyllanthine as specific compounds present in *P. niruri* have antibacterial effects for types of bacteria that usually attack fish such as *Aeromonas hydrophila, Edwardsiella tarda, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa.* In addition, it has the effect of being an antioxidant, immunostimulant and can be used for treatment and to increase fish growth. So it can be concluded that *P. niruri* can be used as an alternative material to maintain health and help fish growth.

Keywords: P. niruri; disease; treatment; immunostimulant; growth.

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1. INTRODUCTION

The drugs derived from herbal ingredients can be used as an alternative to replace antimicrobial agents that have a toxic effect and cause microbial resistance to the antibiotics used [1]. Plants contain several compounds that have pharmacological properties, namely as antimicrobials [2,3]. Apart from being an antimicrobial compound, the compounds contained in plants also have an antifungal, antioxidant, antiviral [4] and immunostimulant [5,6] effect which are of course very useful for human and animal health. In general, plants contain several secondary metabolites, namely alkaloids, flavonoids, steroids, saponins, terpenoids and tannins that can play a role in fighting various diseases [5,7].

Phyllanthus niruri L. belongs to the Euphorbiaceae family, is a tropical plant spread throughout the world, known as a medicinal plant with many benefits [8,9]. In Brazil *P. niruri* is known as Chanca Piedra, in southern India it is called Bhumyamalaki, in Malay it is called Dukong Anak and in Chinese it is called zhu zi cao [9]. Traditionally this plant has been widely used by people in tropical countries to treat various diseases including intestinal infections, kidney stones [10,11], chronic liver disease, diabetes, and prevent hepatitis B virus infection [11,12], asthma, gonorrhea, bronchitis, and syphilis [13,14] and enhances the immune system [15]. *P. niruri*'s ability to treat various diseases is due to its pharmacological properties, such as immunomodulator, antiviral, antibacterial, diuretic, anti-hyperglycemic and hepatoprotector [9,16]. In Indonesia, this plant is used to boost the immune system. This plant extract has been shown to be an immunomodulator that can increase peripheral blood proliferative activity and macrophage phagocytic activity [15].

Based on the phytochemical content and pharmacological properties of *P. niruri*, the plant can not only be used for the treatment of diseases in humans, it is also very possible to use the treatment of diseases in animals including fish. Therefore, the purpose of writing this article is to describe the *P. niruri* plant in an effort to help manage fish health, by looking at the phytochemical content of the plant, its antibacterial, antioxidant and growth effects as well as the immunostimulant effect on cultured fish that has been carried out by several researchers.

2. PHYTOCHEMICAL SCREENING OF *Phyllanthus niruri* L.

Many studies have been carried out on the chemical components of the plant *Phyllanthus niruri* L. Based on the results of qualitative phytochemical tests on *P. niruri* extract using different solvents produced several different types of compounds. But overall the compounds contained in the extract of *P. niruri* are alkaloids, flavonoids, tannins, saponins, steroids, phenols and terpenoids (Table 1).

According to several studies, *P. niruri* in addition to containing several bioactive molecules of flavonoids, tannins, alkaloids, steroids, also contains lignans, phyllanthin, hypophyllanthin, glycosides, ellagitannins, triterpenes, phenyl propanoids, ricinolic acid, niruriside and phyltetralin [21,22,23,24]. According to Sudarsono et al. [25] the *P. niruri* plant also contains essential oils, anthraquinones and arbutin. The leaves contain components of flavonoid compounds such as quercetin. The stem contains components of niruri, niruritenin and rutin. Lignin components such as phyllanthine, hypophilantin are present in all parts of the plant [26]. Triterpenoid compounds contain components of lupeol acetate and betasitosterol [27]. Based on the results of quantitative phytochemical tests, the total phenol content of the plant *P. niruri* L is equivalent to 28.05 g of gallic acid in 1 mg of plant extracts, while the flavonoid content in 1 mg of plant extracts is equivalent to 61.41 g of quercetin, while the flavonoid content in 1 mg of plant extracts is equivalent to 61.41 g of quercetin. 70% ethanol extract of *P. niruri* was 0.864% [17]. The Phytochemical analysis of the methanol extract revealed that *P. niruri* contains mainly flavonoids, alkaloids, tannins, saponins, coumarins, polyphenols, terpenoids and steroids compounds. [28,29].The type of solvent used for extraction affects the levels of secondary metabolites produced. *P. niruri* extracted using different solvents, namely 60% methanol, 60% ethanolacethon and 60% isoproponal produced different levels of secondary metabolites. The highest total flavonoids were obtained using 60% ethanol extract solution, which was 269.26±1.21 mg QE/g, while those using 60% methanol, 60% acetone and 60% Isoproponal solvents each produced 209.05±0 ,75, 247,60±1.23 and 212.70±1.14 mg QE/g. 60% acetone solvent resulted in the highest total phenol, tannin and antioxidant activity, respectively 188.77±1.05 mg GAE/g, 297.51±1.20 mg TAE/g, and 71.99±0.42

% [30]. The results of this study indicate that acetone is effectively used to extract *P. niruri* so as to produce the highest total phenol. This indicates that the polarity of the phenolic compounds of *P. niruri* extract corresponds to the polarity of acetone. Compounds obtained from extraction are based on the similarity of polarity to the solvent used [31].

3. ANTIBACTERIAL EFFECT OF *Phyllanthus niruri* L.

The phenolic compounds, flavonoids, saponins, alkaloids, and terpenoids contained in *P. niruri* have antibacterial activity against several bacteria that can infect cultured fish, such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Aeromonas hydrophila* and *Edwardsiella tarda* [18,32,19,33].

The results of the inhibitory test of *P niruri* solution using paper discs against the bacteria *Edwardsiella tarda*, showed a concentration of 50,000 ppm produced the highest average inhibition, which was 14.3 mm, while at concentrations of 25,000 ppm, 10,000 ppm and 5,000 ppm each produced average drag of 12 mm, 8.7 mm and 6 mm [34]. Methanol extract 80% P. niruri at a concentration of 50µg/ml (100µl) can inhibit the growth of *Staphylococcus aureus* bacteria with an inhibition zone diameter of 13 mm, while for *Escherichia coli* and *Pseudomonas aeruginosa* bacteria the diameter of the inhibition zone is 10 mm and 12 mm, respectively. [18]. The concentration of 50µL of *P. niruri* extract using various solvents can inhibit the growth of *Aeromonas hydrophila* bacteria. Chloroform solvent produced an inhibition zone diameter of 12 cm, while with ethanol, ethyl acetate and petroleum ether the same inhibitory zone was 16 mm [32] (Table 2). From this, it can be seen that 50µL *P. niruri* extract with ethanol, ethyl acetate and peroleum ether as solvents was more effective in inhibiting the growth of *A. hydrophila* bacteria. Meanwhile, 80% methanol extract of *P. niruri* at a concentration of 50µg/ml (100µl) was more effective in inhibiting the growth of *Staphylococcus aureus*. From the description above, it can be concluded that the type of solvent, concentration and type of bacteria determine the diversity of the diameter of the inhibitory zone.

Table 1. Phytochemical Screening of *Phyllanthus niruri* L.

solvents extract	Alkaloids	Flavonoids	Tannins	Saponins	Steroids	Phenols	Terpenoids	Ref
Ethanol 70%	+	+	+		+			[17]
Methanol 80%	+	+		+		+	+	[18]
Ethanol 96%	+	+	+	+				[19]
Ethanol 95%		+	+	+	+	+		[20]

Table 2. Antibacterial activity of *Phyllanthus niruri*

Extract solvents	Concentration Extract	Type of bacteria	Inhibition zone (mm)	Ref.
boiled solution	50.000 ppm	*Edwardsiella tarda*	14,3 mm	[34]
Methanol 80%	50µg/ml (100µl)	*Staphylococcus aureus*	13	[18]
Methanol 80%	50µg/ml (100µl)	*Escherichia coli*	10	[18]
Methanol 80%	50µg/ml (100µl)	*Pseudomonas aeruginosa*	12	[18]
Chloroform	50µL	*Aeromonas hydrophila*	12	[32]
Ethanol	50µL	*Aeromonas hydrophila*	16	[32]
Ethyl Acetate	50µL	*Aeromonas hydrophila*	16	[32]
Petroleum Ether	50µL	*Aeromonas hydrophila*	16	[32]
boiled solution	500.000 ppm	*Aeromonas hydrophila*	14,66	[35]
boiled solution	50.000 ppm	*Aeromonas hydrophila*	12	[35]
boiled solution	5.000 ppm	*Aeromonas hydrophila*	9	[35]

Thin layer chromatography results showed that *P. niruri* ethanol extract was able to inhibit the growth of *Staphylococcus aureus* bacteria with an Rf value of 0.46. Analysis with infra red spectrophotometer showed that the ethanol 96% extract has hydroxyl (-OH) and carbonyl (C=O) functional groups. This shows that the compounds that play a role in inhibiting *S. aureus* are alkaloids and tannins [19]. The results of the antibacterial test of *P. niruri* extract against *E. tarda* bacteria using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) test showed that the extract concentration of 0.0313 g/ml was the best concentration, because it was the lowest concentration that could inhibit growth of *E. tarda* bacteria [25]. Quercetin is a flavonoid compound of the flavonol type which has antimicrobial and antiviral roles [36]. Phenols, flavonoids, and tannins are the main class of phytochemicals that have antimicrobial activity [37]. From the foregoing description, it can be concluded that *P. niruri* is effective in inhibiting the growth of pathogenic bacteria in fish. Inhibiting bacterial growth is determined by the type of bacteria and the concentration of antibacterial compounds contained in herbal ingredients.

4. TREATMENT EFFECTS OF *Phyllanthus niruri* L. ON FISH

The results of the LD_{50} test of *P niruri* solution on *Clarias gariepinus* fish showed that at concentrations of 40,000 and 60,000 ppm with a 24-hour immersion time it caused 10% death. When tested to treat *Clarias gariepinus* fish infected with *Edwardsiella tarda* with a density of 1 ml x 10 -9 bacterial cells/ml/100 g of fish weight through immersion for 30 minutes, it turned out that a concentration of 7000 ppm was quite effective, with a survival rate of 86, 67% [34]. Based on the in vivo test results, a solution of *P. niruri* with a concentration of 5,000 mg/L can treat *Cyprninus carpio* fish infected with *Aeromonas hydrophila* bacteria through immersion. The effective immersion time was 5 hours, with the highest survival rate of 76.67% [35]. From the description above, it can be concluded that *P. niruri* can be used to treat fish infected by bacterial diseases. In carrying out treatment, each type of fish and bacteria has a different response to the concentration of herbal ingredients and the length of treatment time. Therefore, to get an effective treatment, it is necessary to pay attention to the type of bacteria, the type of fish, the concentration of herbal ingredients, and the length of time for treatment.

5. ANTIOXIDANT EFFECT OF *Phyllanthus niruri* L.

Several secondary metabolites contained in the *P. niruri* plant, apart from being very useful for treating various diseases, can also be used as antioxidants [38]. Flavonoid compounds are one of the secondary metabolite compounds contained in the *P. niruri* plant, besides having antithrombotic and anti-inflammatory effects, they also have antioxidant and anti-radical effects [39].

Quercetin is a flavonoid compound of the flavonol type [36] which is a strong anti-radical, able to fight free radical damage, is also a strong anti-radical against peroxyl radicals, superoxide anions, and hydroxyl and is able to scavenge free radicals directly [40]. The results of the total antioxidant activity test on *P. niruri* extract showed significant results. A total of 1 mg/ml of P. niruri extract showed total antioxidant with antioxidant activity equivalent to 216.28 g of ascorbic acid which was further confirmed by the DPPH radical scavenging test. The antioxidants contained in *P. niruri* extract were able to reduce DPPH. The IC50 value of the growing extract *P niruri* produced for scavenging activity of DPPH free radicals was 10.53 g, while for ascorbic acid it was 8.90 g. As much as 1 mg of *P. niruri* for its antioxidant activity its strength is comparable to 152 ascorbic acid. Here, *P. niruri* extract shows high antioxidant activity and is able to reduce ferric chloride and scavenge DPPH radicals. The ability of this plant extract is based on several secondary metabolite compounds contained in these plants including phenolic compounds and flavonoids. Based on the results of the study showed the higher the amount of phenols and flavonoids, the higher the antioxidant activity and scavenge free radicals [18]. Chakraborty et al. [41] reported Fish that are given antioxidants sourced from plants as feed additives can help improve the physiological condition of fish in general. From this it can be seen that not all of the metabolite compounds contained in *P. niruri* are strong antioxidants, flavonoids and phenols are powerful antioxidants. These antioxidants can be used to help maintain fish health, because they can improve the physiological condition of fish.

6. GROWTH EFFECT OF *P niruri* L

Researches have shown that several medicinal plants are successfully used as supplement to trigger growth and feed conversion in fish and

shrimp, including garlic [42], turmeric [43], black cumin [44], *Aloe vera* [45], *Andrographis paniculata* [46] and so on. Likewise *P. niruri* apart from being an antibacterial, antioxidant and immunostimulant, can also trigger fish growth. Sunitha [47] reported that carp (*Cyprinus carpio*) fed with a diet mixed with *P. niruri* powder for 60 days showed better growth compared to controls. Among the treatments given, feeding mixed with *P. niluri* powder as much as 2% (10 g/ 500 g) showed the highest fish growth rate in terms of weight parameters (Weight : 1.15± 0.16 g, growth : 0.55± 0.04 g and SGR : 0.92± 0.07% and length (3.75± 0.24 cm).While the fish fed with *P niluri* powder at 1% (5g/500 g), resulted in lower weight and length, namely weight :1.05± 0.09, growth :0.49± 0.03 and SGR : 0.82± 0.07% and the length is 3.53± 0.25 cm. *P. niruri* as an additive mixed into feed, with a duration of administration for 60 days can increase the growth of *Carassius auratus* fish. Experimental results show a concentration of 1.5 percent produces the highest average weight gain, which is 1.769 g [48]. From this it can be seen that *P. niruri* can be used to increase fish growth. Different types of fish showed different growth responses to the concentration of herbal ingredients given.

7. EFFECT OF *Phyllanthus niruri* L. AS AN IMMUNOSTIMULANT

Research on the effects of *P. niruri* as an immunostimulant showed, that it can increase the body's resistance in both humans and animals to disease attacks. Observation of antibody titer is one of the parameters to determine the effectiveness of a substance that acts as an immunostimulant, by looking at the ability of serum proteins containing antibodies to collect and destroy antigens that enter the body [49]. Fish *Oreochromis mossambicus*, which had been treated with aqueous extract of *P. niruri* leaves at various doses, and then challenged using sheep red blood cells (SRBC) as an antigen, showed an increase in the response of the primary antibody titer.

The highest significant increase occurred at a dose of 20 mg. On day 15 the secondary antibody response was higher at all doses (20, 2, and 0.002 mg), when compared to the control. As for the neutrophil activation test using hot aggregated serum albumin (HA BSA), the results obtained showed that all doses could increase neutrophil activity, but the highest number of activated neutrophils was in fish given *P. niruri* leaf aqueous extract of 0.02 mg, followed by 0.02 mg. 0.2 and the lowest was given the extract at a dose of 0.002 mg [50]. Neutrophils are part of the white blood cells that are part of the immune system. In addition to neutrophils, the white blood cells that are part of the immune system are monocytes, lymphocytes and macrophages. While cytokines, antibodies, and complement proteins are included in immune molecules. Synchronous interactions between the immune system and immune molecules can induce a good immune response, which can lead to resistance to pathogenic infections [51]. The results of research conducted by Ma'at et al. [52] showed *P. niruri* in vitro to have an immunomodulating effect. *P. niruri* extract (50–200 mg/kg) can elicit both cellular and humoral immune responses. Nworu et al. [53] also showed that aqueous extract of *P. niruri* (12.5–200 g/ml) could induce lymphocytes and macrophages in experimental animals.

Tilapia (*Oreochromis niloticus*) which had been given *P. niruri* leaf extract by injection was able to survive the attack of *Aeromonas hydrophila* bacteria. Dose of 50 mg was the best concentration by producing the highest (76.7%) tilapia survival [54].

Cyprinus carpio L. after being given feed mixed with *P. niruri* powder for 60 days then challenged with *A. hydrophila* bacteria with a density of 10^3 CFU, able to withstand the attack of these bacteria. Giving *P. niluri* powder as much as 2% (10 g/500 g of feed) showed the highest resistance of fish to attack by *A. hydrophila* bacteria, which was 100% [47].

The POM [55] stated that *P. niruri* is one of the immunomodulatory drugs, namely drugs that can improve or enhance the immune system. Compounds that play a role in this are the flavonoid group which is the main component in *P. niruri*. Flavonoids act on body cells by sending intracellular signals to cell receptors, so that cells work optimally. Compounds contained in meniran, including phyllanthin and hyphophyllanthin are two compounds that have anti-inflammatory activity so that they can strengthen immunity.

P. niruri has an immunomodulatory effect through activation and augmentation of the cellular immune system. Specifically, *P. niruri* can activate neutrophils, macrophages, monocytes, T and B lymphocytes. The active process of phagocytosis by neutrophils indicates

an acceleration of the 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 suggests that *P. niruri* can inhibit the body's defenses, by engaging the cellular immune system against foreign pathogens [56] . From the description above, it can be concluded that *P niruri* can be used as an immunostimulant that can increase the body's resistance to disease (pathogenic bacterial attack). The amount of *P. niruri* concentration and the type of fish determine the success in increasing the fish's body resistance to disease attacks.

8. CONCLUSION

Phyllanthus niruri has the potential to be used as an alternative supplement for the health of various fish, because it has antibacterial, antioxidant, treatment, growth and immunostimulant effects.

NOTE

The study highlights the efficacy of "Herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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