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Phytochemical Screening of *Phyllanthus niruri* collected from Kerala Region and its Antioxidant and Antimicrobial Potentials

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Abstract

Various compounds found in the plant have shown antioxidant and antimicrobial activity. In this study, the phytochemical screening, antioxidant and antimicrobial activity of *Phyllanthus niruri* was evaluated. *P. niruri* has shown various pharmacological activity and commonly used in traditional medicine throughout the world. Phenol and flavonoid content were determined quantitatively. The antioxidant property was determined using total antioxidant assay, DPPH scavenging activity and reducing power assay. Antibacterial activity was determined using agar gel diffusion method. Phenol and flavonoid in *P. niruri* were found to be equivalent to 28.05µg and 61.41µg of gallic acid and quercetin, respectively. The total antioxidant activity and ferric reducing power of the 1mg plant extract were found to be 108.14µg and 152µg equivalents of ascorbic acid. The IC_{50} value of plant extract was 10.53µg as compared to the IC_{50} value of 8.90µg of ascorbic acid. It was found to inhibit the growth of *Escherichia coli, Lactobacillus acidophilus, Pseudomonas aeruginosa and Staphylococcus aureus*. It can be concluded that *P. niuri* is rich in phytochemicals that have antioxidant and antimicrobial activity. It also corroborates the use of *P. niruri* in traditional medicine.

Keywords: *Phyllanthus niruri*, antioxidant, antimicrobial, phytochemicals

INTRODUCTION

Sustaining life in absence of oxygen is impossible. But the importance of oxygen is contradicted by deleterious effects of free radicals and reactive oxygen species like hydroxyl radical, superoxide anion radical, hydrogen peroxide generated from oxygen. Free radicals are highly reactive molecules having an unpaired electron which are produced inside cells during different metabolic processes such as reactions involved in respiration, inflammation, phagocytosis, arachidonate pathway [1]. Free radicals are also generated by exposure to cigarette smoke, ozone, environmental pollutants, and radiation. These molecules initiate a cascade reaction causing cellular damage and disruption of homeostasis by damaging important biomolecules like DNA, lipid, and proteins [2, 3]. Reactive oxygen species also induce apoptosis, necrosis, oxidation of low-density lipoproteins and oxidative stress [4]. Though ROS are necessary for protection against viruses and bacteria, but their excess has fatal consequences [5]. Studies have inferred that these molecules are associated with initiation and progression of cancer, arthritis, heart diseases, atherosclerosis, aging, Parkinson's disease, Alzheimer's disease and a myriad of other inflammatory diseases [6, 7].

Antioxidants scavenge the reactive oxygen species, thus ameliorating or inhibiting the oxidation of different macromolecules and eventually preventing further cellular damage [8]. As ROS produced under oxidative stress have serious consequences, our body has developed an efficient antioxidant mechanism comprising of antioxidant enzymes like superoxide dismutase, glutathione peroxidase, and catalase [9]. Antioxidants can also be supplemented exogenously to curb oxidative stress [10]. The major sources of these exogenous antioxidants are fruits, vegetables, and herbs. The antioxidant activity of various plants has been evaluated, and almost all the plants have antioxidant properties on the virtue of a plethora of phytochemicals present in them [11]. Also, new sources of potent antimicrobial agents need to be identified because of toxic effects of currently used drugs and development of resistance in microbes to antibiotics [12]. The excessive and careless use of antibiotics has led to the development of several antibiotic resistant fungus and bacteria. Plants are also an important source of antimicrobial molecules which can be used to prevent various bacterial and fungal infections. Scientists globally are studying the pharmacological properties of plants due to side effects of synthetic drugs. In this study, the phytochemical constituents, antioxidant activity and antimicrobial activity of *Phyllanthus niruri* have been evaluated. *P. niruri* is a tropical shrub belonging to Phyllanthaceae family and grows commonly during winter as a weed in India, Brazil, Malaysia and Indonesia [13, 14]. *P. niruri* is commonly known as 'Chanca Piedra' in Brazil, 'Bhumyamalaki' in South India, 'Dukong Anak' in Malay and 'zhu zi cao' in Chinese [15]. *P. niruri* is used in Ayurvedic, Unani, and Chinese traditional medicine. In Brazil, the preparations of this plant are used to cure intestine infection, and kidney stone [16]. In Chinese medicine, it is used to prevent Hepatitis B infection and prevent liver damage [17]. A

lignin nirtetralin has been isolated from this plant which has anti- hepatitis B virus activity [20]. In different regions of India, the preparations of *P niruri* are used to cure asthma, gonorrhea, bronchitis, and syphili [18, 19]. In Indonesia, the plant is used to improve immune system [13]. Extract of this plant has shown immunomodulatory activity where it increased the proliferation of peripheral blood and phagocytic activity of macrophages [13]. A 35 kDa protein isolated from *P. niruri* has protected against the detrimental effect of indomethacin on hepatocytes [21]. Corilagin and ethyl brevifolincarboxylate found in this plant has shown antitumor activity [14]. This plant has also shown antibacterial, hypoglycaemic, analgesic, anti-inflammatory, cardioprotective, and anti hyperuricemic properties [15]. The antioxidant activity, antimicrobial activity and phytochemical constituents of this plant were evaluated considering the role of this plant in Ayurveda and ethnomedicine across the globe. In the present study, *P. niruri* was collected from Kerala region of India. The hydro-alcoholic extract was taken out and was used for determination of the antioxidant activity using DPPH, total antioxidant activity and reducing power assay. The quantitative phytochemical analysis was done to determine total phenolics and total flavonoids content. The antimicrobial activity of the plant extract was determined by agar well diffusion method against four bacterial strains, two-gram positive bacteria *(Lactobacillus acidophilus, Staphylococcus aureus)* and two gram negative bacteria (*Escherichia coli, Psedumonas* aeruginosa).

MATERIALS AND METHOD

Aluminum chloride, 1, 1 diphenyl 2-picrylydrazyl, Ferric chloride, Folin- Ciocalteau reagent, Gallic acid, Hydrogen chloride, Methanol, Mercuric iodide, Sodium carbonate, Sulphuric acid, Trichloroacetic acid, Quercetin from Hi-Media.

Sample Preparation

Phyllanthus niruri leaves were collected from Kollam district in Kerala in the month of December and January. The leaves were washed with water to remove dust particles. The washed leaves were dried at room temperature and then powdered mechanically. The powdered leaves were extracted with the help of Soxhlet apparatus with methanol (80%) as a solvent. The extract was dried and refrigerated at 4^0C for further usage.

Phytochemical Screening

The phytochemical screenings were performed by following the standard procedures mentioned in Harbone [22]. Screenings for the presence of saponin, alkaloid, tannin, flavonoid, phenol, terpenoid and carbohydrates were performed.

Total Phenolic Content

Total phenolic content was measured according to Folin ciocalteu method [23]. A reaction mixture of 10 ml was made which comprised of 100µg/ml plant extract, 5ml Folin ciocalteu reagent, and 4ml 7% Na_2CO_3 which was mixed, and then incubated at 40^0 C in water bath for 30 minutes. After that, OD was taken at 760nm. Gallic acid of different concentration was taken as standard, and the phenolic content was expressed as Gallic acid equivalents present per milligram of the dried plant extract sample.

Total Flavonoid Content

Aluminum chloride method was used for the determination of flavonoid content [24]. In 1 mg plant extract (0.5ml), 1.5 ml methanol was added. Then, 0.1 ml of the aluminum chloride (10%), 0.1 ml of the 1M potassium acetate, and 2.8ml the distilled water were added in the mentioned order and left at room temperature for 30 min. The absorbance of the mixture was taken at 415nm after incubation. Quercetin was used as standard, and the total flavonoid content of 1 mg plant extract was determined and expressed in terms of quercetin equivalent.

DPPH Scavenging Activity

The antioxidant activity of plant extract was measured by 2, 2 diphenyl 1-picrylhydrazyl (DPPH) radical scavenging activity as mentioned by Liyana - Pathiana and Shahidi [25]. One ml of 0.135 mM DPPH was prepared in methanol and mixed with 1.0 ml of plant extract ranging from 0, 3, 6, 9, 12, 15, 18, 21 and 24 µg/ml followed by incubation at room temperature for 30 minutes. The OD of the reaction mixture was taken at 517nm. The scavenging ability was calculated as:-

DPPH scavenging activity (%) = [(Abs control- Abs sample)/Abs control] × 100

Where,

Abs control is the absorbance of DPPH + methanol;

Abs sample is the absorbance of DPPH + sample (Extract/Ascorbic acid)

Determination of Total Antioxidant Activity

The total antioxidant activity of the extracts of *P. niruri* was evaluated by using the phosphor-molybdenum method according to the procedure of Prieto *et al [26].* To 3ml of plant extract, 3ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The OD was measured at 695nm after incubation of the mixture at 90^0 C for 90 minutes. The antioxidant activity was expressed as the equivalents of ascorbic acid for 1 milligram of the plant extract.

Determination of Reducing Power Assay

The reducing power of plant extract was evaluated using the method of Oyaizu [27]. Plant extract (1mg) was taken in distilled water (1ml) and phosphate buffer (2.5m, pH 0.6, 0.2M) with 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50^0 C for 20 minutes followed by addition of 10 % trichloroacetic acid and centrifugation at 3000 rpm for 10 min. To the supernatant, 5ml distilled water, and 0.5 ml ferric chloride was added, and absorbance was taken at 700 nm. The reducing power was expressed as the equivalents of ascorbic acid for 1 milligram of plant extract.

Bacterial Strains and Culture Conditions

Strains of *Lactobacillus acidophilus, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli*

were obtained from Department of Microbiology and Biochemistry, Lovely Professional University, Punjab, India. These four bacterial strains were inoculated in Nutrient agar media and incubated at 37^0C for 24 hours.

Antibacterial Assay

The antimicrobial activity of these bacterial strains was assessed by the agar well diffusion assay. Nutrient Agar media was prepared and poured in Petri-dishes. After the solidification of agar media, 0.1 ml of each bacterial strain was spread over the media. Wells (7 mm) were made in the Petri-dishes, and 50µg/ml (100µl) extract of *P. niruri* in DMSO was deposited into respective wells. The plates were incubated for 24 hours at 37^0C. DMSO and Vancomycin were used as controls. After incubation, the zone of inhibition around the wells was detected, and the diameter of these inhibition zones was measured and recorded.

RESULTS AND DISCUSSIONS

Herbal medicine has been used for centuries for the treatment of various diseases. It is an important part of Ayurveda, Siddha and Yunani medicine. Different parts of various plants are used by indigenous people across the world to cure wounds, snake bites, abdominal pain, skin infections and several other diseases. In a study by WHO, it was estimated that 40% of the world population still depend on herbs and plants as medicine [28]. Several phytochemicals such as vincristine, artemisin, quinine, digoxin have been isolated from plants which have shown a broad range of pharmacological activities [29, 30]. The modern sedentary lifestyle, stress, pollution, junk food, and alcohol have exacerbated the harms caused by free radicals. The free radicals are associated with diseases such as diabetes, arthritis, cancer, Parkinson's disease, and Alzheimer's disease [4]. The phytochemicals like phenol, flavonoid, saponin, alkaloid, and terpenoid can scavenge free radicals. These compounds have previously shown strong anticancer, antidiabetic, anti-inflammatory and antimicrobial activity [31]. The emergence of antibiotic-resistant bacteria is another major health concern globally. The search for novel antioxidant and antimicrobial compounds is carried out throughout the globe by scientists meticulously.

This study aimed at phytochemical screening, determining antioxidant activity and antibacterial activity of *Phyllanthus niruri*. It showed the presence of phenol, flavonoid, saponin, alkaloid, and terpenoid. These compounds are responsible for the antioxidant activity and antibacterial activity.

The presence of phytochemicals in the extract of *P. niruri* was screened qualitatively. The result showed the presence of phenols, flavonoids, alkaloids, terpenoid and saponins (Table 1). Phenols and flavonoids have significant antioxidant properties. Phenols are also associated with the ability to inhibit the growth of bacteria [32]. Furthermore, these compounds have shown anti-inflammatory, anticancer and antidiabetic activity. The presence of these compounds formed the basis of further evaluation of the antioxidant and antimicrobial properties of the *P. niruri* extract.

As, phenols and flavonoids are the secondary metabolites, and have antimicrobial and antioxidant activity, their presence was quantitatively determined. The extract of *P. niruri* showed the presence of phenols and flavonoids both qualitatively and quantitatively. The analysis of the total phenol content showed the presence of total phenol content equivalent to 28.05 µg of gallic acid in 1mg of the plant extract. Flavonoids which are responsible for pigmentation in plants was also evaluated quantitatively. By the standard graph formed using quercetin, the flavonoid content in 1 mg of the plant extract was found to be equivalent to 61.41 µg of quercetin.

Table 1: Phytochemical screening of *Phyllanthus niruri* plant extract

Serial Number	Phytochemicals	Method	Observation	*Phyllanthus niruri*
1	Saponins	Emulsion formation test	Formation of emulsion	Positive
2	Alkaloids	Mayer's reagent test	No characteristic changes	Positive
3	Phenols	Ferric chloride test	Blue color appeared	Positive
4	Terpenoids	Sulphuric acid test	Reddish brown color	Positive
5	Flavonoids	Ammonia test	Yellow color disappeared on standing	Positive

Table 2: Antibacterial activity of *Phyllanthus niruri*

Bacterial strain	Diameter of zone of inhibition formed by *Phyllanthus niruri* (mm)	Diameter of zone of inhibition formed by vancomycin
Gram-positive		
1. Lactobacillus acidophilus	10	13
2. Staphylococcus aureus	13	20
Gram-negative		
1. Escherichia coli	10	13
2. Pseudomonas aeruginosa	12	19

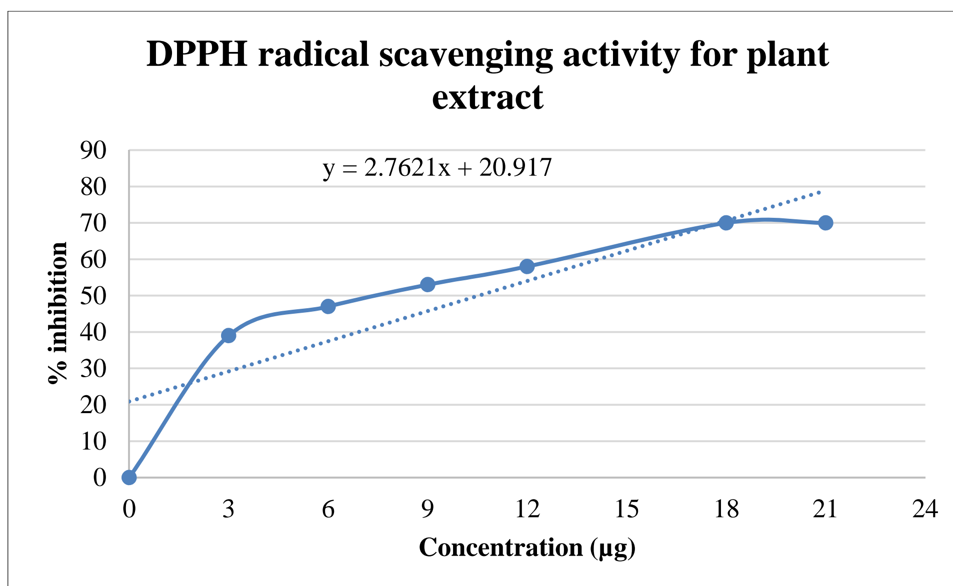

Figure 1: DPPH scavenging activity of *Phyllanthus niruri* extract

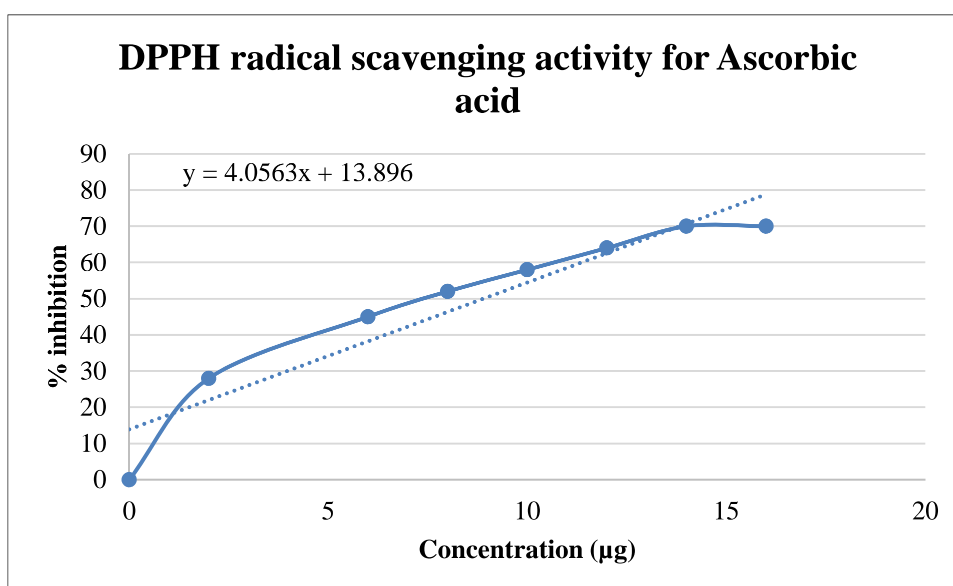

Figure 2: DPPH scavenging activity of ascorbic acid

The antioxidant activity of the plant extract was determined by total antioxidant activity assay, ferric reducing power assay and DPPH free radical scavenging assay. The extract showed significant antioxidant activity. The 1 mg/ml of plant extract showed the presence of total antioxidant activity equivalent to 216.28 µg of ascorbic acid which was further confirmed by DPPH radical scavenging assay. The extract was found to scavenge the DPPH free radical successfully. The antioxidants present in the plant extract were able to reduce DPPH$^{\cdot}$ to DPPH-H, thus changing the color from purple to yellow. The plant extract was found to have an IC_{50} value of 10.53 µg, and for ascorbic acid, the IC_{50} value was 8.90µg for DPPH radical scavenging activity (Figure 1 and 2). By comparing with the standard graph using ascorbic acid for the evaluation of total reducing power, it was found that 1 mg of *P. niruri* was equivalent to 152 µg of ascorbic acid. The plant extract showed marvelous antioxidant activity and ability to reduce ferric chloride and scavenge DPPH radical. These abilities of the plant extract are due to the presence of the various phytochemicals in the plant extract including phenols and flavonoids. It has been shown that higher the amount of phenol and flavonoids, higher will be the antioxidant activity and reducing power. By comparing with the previous data, the plant used in our study was having higher antioxidant activity (IC_{50} value of 9.1 µg) than the methanolic extract of *P. niruri* obtained from Mysore, India by Harish and Shivanandappa.[33] Whereas, the IC_{50} value of our plant for DPPH scavenging activity was lower than that of the sample from Indonesia in the study by Da'I et al [34]. These studies suggest that the phytochemical constituents are affected by the environmental factors thus affecting their antioxidant activity and other pharmacological activities.

The plant extract of *P. niruri* has already shown the presence of phenol and flavonoids quantitatively and qualitatively in previous assays. Phenol, flavonoids, and tannins are a major class of phytochemicals that have antimicrobial activity [35]. The plant extract inhibited the growth of all the bacterial strains under study. The diameter of the zone of inhibition formed by the plant extract is 10mm, 10mm, 12mm, and 13mm, respectively for *Escherichia. coli, Lactobacillus acidophilus, Psuedomonas aeruginosa and Staphylococcus aureus* (Table 2). *P. niruri* showed the maximum activity against *Staphylococcus aureus.* It showed the antibacterial activity against both Gram positive and Gram negative bacteria. The diameter of the zone of inhibition formed by vancomycin ranged between 13 to 20mm. Here, the zone of inhibition of the plant extract had a smaller diameter than the zone of inhibition of vancomycin. This may be because that the plant extract was in crude form. The result supports the role of phenol and flavonoid in antibacterial activity and suggests that *P. niruri* can be a potential source of antibacterial compounds.

CONCLUSION

The present study showed the antioxidant and antimicrobial activity of plant extract of *Phyllanthus niruri.* The antioxidant and antimicrobial activity of the plant extract may be due to the presence of different phytochemicals such as phenol, flavonoid, terpenoid, and saponin. The study supports the use of *P. niruri* in Ayurveda and traditional medicine throughout the world. It can be used as a potential source of antioxidant which can be used to cure various ailments and an antibacterial drug that can be used to inhibit the growth of various pathogenic and antibiotic resistant bacterial strains.

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