In vitro & In vivo analysis of the antioxidant Activity of medicinal winter weed Phyllanthus niruri L.

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Abstract:

Phyllanthus niruri Linn. is a widespread tropical plant commonly found in coastal areas, best known by the common names stonebreaker or seed-under-leaf belongs to family Euphorbiaceae. It is known for a variety of uses like hepatoprotective action, lipid lowering action, antidiabetic action, and antifungal action. Current investigation is directed to detect the antioxidant potential of *P. niruri* L. Methanolic fractions were subjected to DPPH radical scavenging assay, FRAP assay alongwith their total phenolic content as a surrogate marker for antioxidant capacity. Methanol extracts of leaves and seed showed significance scavenging activity among the entire extracts with high phenolic content. Results of present study confirmed the antioxidant potential of *P. niruri* L.

Keywords: Antioxidant activity; total phenolic content; DPPH assay, FRAP assay

Introduction

Utilization of supplementary antioxidants through herbs, foods or supplements (phytochemicals) are beneficial to defense against the harmful reactive oxygen species (ROS) such as superoxide radical anion, hydroperoxyl radical are generated in cells from (Shi *et al*., 2001), which are byproducts of metabolism and accountable for the development of a extensive number of degenerative diseases such as cardiovascular disease (CVD), diabetic, cirrhosis and several cancers (Halliwell, 1996). Phytochemicals produced by the plant kingdom for defense, protection, cell to cell signaling and as attractants for pollinators (Kartal *et al*., 2007) are bioactive non-nutrient plant compounds that have been linked to the reduction of risk related to chronic diseases. Primary metabolites are indispensable need for the growth of plants. Many primary metabolites are precursors for pharmacologically active secondary metabolites used as pharmaceutical compounds such as antipsychotic drugs (Sanchez and Demain, 2008).

Phytochemicals are structurally assorted and diverse natural compounds, based on their biosynthetic origins they can be classified into fundamentally four classes with medicinal and nutritional value exerting constructive effects on human health like phenolics terpenoids, and polyphenolics and nitrogen-containing alkaloids and sulphur-containing compounds (Crozier *et al*., 2006). Plant polyphenols with antioxidant capacity could scavenge and quench reactive

chemical species thereby minimising oxidative damage resulting from excessive light exposure. From histort plant polyphenols are vital components for both human beings and animal diets as well, which are proven safe to be consumed and commercialized as herbal products (Glucin et al., 2005).

Food antioxidants such as α-tocopherol, ascorbic acid, carotenoids, amino acids, peptides, proteins, flavonoids and other phenolic compounds might also play a significant role as physiological and dietary antioxidants (Shahidi , 2005).Natural antioxidants are known to exhibit a wide range of biological effects including antibacterial, antiviral, antiinflammatory, antiallergic, antithrombotic and vasodilatory activities (Cook and Samman ,1996).

Therefore, investigation and research on plant extracts elucidates beneficial positive effects on human health to cure ailments. Antioxidant property analysis (Pa'ee *et al*., 2024) of plant extract might confirm to be a potential substitute compared to those medicines which have severe side effects on human health in long run. One of such versatile genus that have been used for in folk medicine for decades and known to have immense variety of secondary metabolites is the genus *Phyllanthus*, species viz., *P. niruri*, *P. urinaria*, *P. amarus* (Masturah *et al*.,2006) has been used traditionally for treatment of kidney and gallbladder stones, liver related diseases, viral infection as well. Research on *Phyllanthus* sp. has been widely conducted in India. Antioxidant activity (Siddiqui *et al*., 2023) and hepatoprotective potential found in *P. niruri* has been reported by (Harish and Shivanandappa, 2006). Research studies confirmed the anti-diabetic, anti-cancer and anti-inflammation properties of *P. amarus*, (Mahajan *et al*., 2023) also shown to have anti-mutagenic and anti-carcinogenic effects (Sripanidkulchai *et al*., 2002). Antioxidant activities of methanolic extracts of five *Phyllanthus* species in India have been reported by Kumaran and Karunankaran (2005). Therefore, present study was undertaken to evaluate the antioxidant potential of *Phyllanthus niruri* L extracts in terms of its potential antioxidant activity by using DPPH and FRAP assay along with its polyphenol content.

Material and methods

Chemicals 2,2-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6- tripyridyl-s-triazine (TPTZ), potassium ferricyanide, potassium persulfate, trichloroacetic acid, gallic acid and quercetin were purchased from Sigma Chemical Co. (St. Louis, MO, USA)., $FeCl_3$ and ascorbic acid from HIMEDIA Laboratories Pvt. Ltd. (Germany); Folin-Ciocalteu phenol reagent, anhydrous sodium carbonate (Na_2CO_3), hydrochloric acid (HCl), glacial acetic acid, potassium chloride, sodium acetate trihydrate and solvent methanol were obtained from Merck Chemical Supplies (Damstadt, Germany). All the chemicals used, were of analytical grade.

Determination of antioxidant activity

Collection of Plant Material: The plants upto 70 centimeters elevated having rising herbaceous branches and most important having seed under leaf were identified and collected from Jhalana and nearby area around University Campus.

Preparation of Plant Extracts:

Plant parts were assorted manually followed by air drying of leaves, stem, roots, seeds and callus under shade at room temperature (25 -26°C) to invariable weights. The dried plant materials were ground separately to powder form, 10 grams specified weight of each ground plant materials were mixed separately in ethanol with regular shaking for 72 hrs on an orbital shaker at room temperature. Filtration of extracts was carried out using a Buckner funnel and Whatman No 1 filter paper. After filtration each filtrate was concentrated to dryness under reduced pressures at 40ºC using a rotary evaporator. The concentrated dried extracts were resuspended in ethanol to make volume 10 mg/ml stock solution.

DPPH Radical Scavenging Assay:

Radical scavenging activity of ethanolic extracts of specific plant part, against stable DPPH (2,2'-diphenyl-2-picrylhydrazyl hydrate, Germany) was determined spectrophotometrically. DPPH reduction occurs when it reacts with an antioxidant compound present in extracts (Brand-Williams *et al.,*1995), which can donate hydrogen, so it is reduced. Reduction reaction is qualtitatively analysed by change in color from deep-violet to light-yellow, measured spectrophotometrically at 517 nm on a UV/visible light spectrophotometer. Radical scavenging activity was modified and standardized according to lab conditions, measured by method of Brand-Williams, Cuvelier, and Berset. For measurement freshly prepared solution of DPPH in ethanol (6x10-5M) was used (Kaur, C and Kapoor, 2002; Suksorn and Sangsricha, 2008), before UV measurements. Each herbal samples 100 µL was added to 300 µL of 6x10-5 mol/L ethanolic solution of DPPH. The absorbance (Szabo *et al.,* 2007) at 517 nm was recorded after dark incubation for 30 minutes at room temperature using an ultraviolet-visible spectrophotometer. Lower optical density or absorbances of the reaction mixture specify higher free radical scavenging activity. Absorption of blank sample containing the same amount of ethanol and DPPH solution was prepared and measured for avoiding any reagents related absorbance. Scavenging capacity of the samples were compared to that of control set (ethanol, 0% radical scavenging). Radical scavenging activity was calculated by the following equation:

%DPPH radical scavenging activity $= 100\left(\frac{A_{control} - A_{sample}}{A_{control}}\right)$

Here, A control = absorption of blank sample (t=0 min);

A sample = absorption of tested solution (t=15 min).

Reducing ability by FRAP Assay

Modified method of Benzie and Strain (Van Acke *et al.,* 1996) was adopted for the determination of the total antioxidant activity by FRAP assay of the plant extracts. The stock solutions were prepared, included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM FeCl3·6H2O solution. From the stock solution, the fresh working solution were prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3 \cdot 6H_2O$. The temperature of the solution was increased to 37 °C before use. Plant extracts (100 µL) were allowed to react with 2000 µl of the FRAP solution for 30 min in the dark incubation. Readings of the colored product (ferrous tripyridyltriazine complex) were recorded at 593 nm. The standard curve was plotted between 200 and 1000 µM FeSO4 and linear pattern was achieved. Results are expressed in µM Fe (II)/g dry mass and compared with that of ascorbic acid. Increased absorbance of the reaction mixture specifies increased reducing power.

Determination of Total Phenolics:

The total phenolic content of the herbal samples was estimated according to Folin-Ciocalteu spectrophotometric method (Bocco *et al.,*1991 and Singleton *et al.,* 1999). 100 µL of the extraction was mixed with 250 µl of 10% (v/v) Folin-Ciocalteu's phenol reagent and allowed to react for 10 min. Then, 100 µl of 7.5% (w/v) Na_2CO_3 solution was added. After incubation for 15 min at 50°C then the absorbance at 760 nm was determined. The measured absorbance was compared to a standard curve of gallic acid (GA) solution and the total phenolic content was expressed as milligrams of gallic acid equivalents (mg GAE/ml). Each sample reading was done in triplicate to minimize the error and making test reliable.

Results and Discussion

The extracts were evaluated for their antioxidant potential against selected which showed good activity. % DPPH radical scavenging activity of seeds (98.07%) and leaves (92.14%) were found to be highest and the weakest antioxidant activity was found in root extract (60.33%) as shown in Table 1. Callus extract also showed significant antioxidant activity (78.11%). Ferric acid reducing ability was found to be highest in stem (175.8 µM Fe (II)/g) and roots (148.62 µM Fe (II)/g) and lowest in callus (116.61µM Fe (II)/g) as shown in Table 2. Phenolic content of seeds and leaves extract (21.56, 20.10 mg GAE/ml) was found to be highest. A satisfactory amount of phenolics was also found in callus (15.89 mg GAE/ml) as shown in Table 3.

All the parts of plants showed significant antioxidant activity. Medicinal plants thus can be considered as promising sources of natural antioxidants for medicinal and commercial uses. Plant extracts are quite safe and their toxicity is a not a problem of concern unlike those of synthetic antioxidants, they could be exploited as antioxidant additives or as nutritional supplements. In conclusion, this study has indicated that results of the DPPH, FRAP and TPC assays provide essentially identical information in regard to the antioxidant capability of extracts of plants.

Table 1: Antioxidant activity of ethanolic extracts of *P.niruri* via DPPH assay

S.No	Extracts	% DPPH radical scavenging activity
1	Leaves	**92.14%**
2	Roots	60.33%
3	Stem	72.89%
4	Seeds	**98.07%**
5	Callus	78.11%
6	Ascorbic acid	100%

Table 2. Total antioxidant (FRAP) activity of the ethanolic extracts (leaf, stem, root, seed and callus) of *P. niruri* L.

S.No	**Extracts**	**[e]FRAP**
1.	Leaves	127.4
2.	Stem	**175.8**
3.	Root	**148.62**
4	Seeds	130.45
5	Callus	116.61
6	Ascorbic acid	230.96

[e]Expressed in units of μmol Fe (II)/g.

Table 3: Total phenolic content of ethanolic extracts of different plant parts of *P. niruri*

S.No	**Plant Parts**	**Total Phenolic Content (mg GAE/ml)**
1.	Leaves	**20.10**
2.	Stem	19.60
3.	Root	9.06
4.	Seeds	**21.56**
5.	Callus	15.89

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