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Research Article

IN VITRO ANTIOXIDANT EVALUATION OF *PSEUDARTHRIA VISCIDA* LINN

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ABSTRACT

The study of free radicals and antioxidants in biology is producing medical revolution that promises a new age of health and disease management. The present study was performed to evaluate the *in vitro* antioxidant effect of the ethanolic extract of *Pseudarthria viscida* Linn. against free radical damage by different standard methods such as DPPH (1,1-diphenyl-2-picrylhydrazyl), Nitric Oxide (NO), ABTS(2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) and Hydrogen peroxide free radical model. The test extract exhibited significant inhibition in Nitric oxide and DPPH free radical formation with IC_{50} values of 78.1±1.75 and 35.00±1.15 µg/ml respectively. Whereas in the cases of ABTS and Hydrogen peroxide free radicals IC_{50} values of 81.00±3.85 and 449.60±2.55 µg/ml. Out of these four free radicals the extract showed potent activity on Nitric oxide and DPPH, which is compared to that of ascorbic acid and rutin taken as standards.

Keywords: *Pseudarthria viscida;* DPPH; ABTS; Nitric oxide; hydrogen peroxide.

INTRODUCTION

Oxygen is essential for survival however, its univalent reduction generates several harmful reactive oxygen species (ROS), inevitable to living cells and highly associated with the wide range of pathogenesis such as diabetes, liver damage, inflammation, aging, neurological disorders and cancer. In spite of comprehensive network of cellular defensive antioxidants, many ROS still escape this surveillance inflicting serious anomalies favouring such diseases states[1-3]. Though synthetic antioxidants, BHT, BHA and radioprotector, Warfarin are being used widely,however, due to their potential health hazards, they are under strict regulation [4-5]. Antioxidant principles from natural resources are multifaceted in their multitude/magnitude of activities and provide enormous scope in correcting the imbalance through regular intake of proper diet. Therefore, in the recent years, the interest is centered on antioxidants derived from herbal medicine in view of their medicinal benefits [6-8] .Phytoantioxidants, commonly available, less toxic, serving food and medicinal components have been suggested to reduce threat of wide range of ROS [8-10]

The plant *Pseudarthria viscida* (Family:Fabaceae) is semierect diffuse undershrub, distributed throughout all districts of south India, also reported from Srilanka and Timor. Traditionally, the plant used in treatment of intermittent fever, urinary diseases, tumors, oedema, burning sensation,difficult breathing and toxic conditions[11]. Herbal medicine is frequently a part of a larger therapeutic system such as traditional and folk medicine. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of many diseases a literature survey indicated only antifungal work[12] and antihypertensive work[13]. Phytochemical screening of *Pseudarthria viscida* indicated the presence of flavonoids, tannins and proteins[14].

MATERIALS AND METHODS

Reagents

DPPH (Sigma – Aldrich, USA), Methanol (HPLC grade, Merck, India), Ascorbic acid (Analytical grade, Merck, India), Rutin, Potassium persulphate, ABTS (2, 2'–azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), Phosphate buffered saline, Dimethyl sulfoxide (Merck, India), Hydrogen peroxide, sodium nitroprusside, Sulphanilic acid (0.3% w/v), Naphthyl ethylene diamine dihydrochloride (NEDD, 0.1%).

Plant material

The plant *Pseudarthria viscida* Linn. (Family: Fabaceae) was collected from Centre for Indian Medical Heritage, Kanjikode, Palakkad District, Kerala, India. The plant material was taxonomically identified by the Botanical Survey of India, Southern Circle, TNAU campus, Coimbatore. (No. BSI/SC/5/23/06-07/Tech-116).

Extraction

The whole plant of *Pseudarthria viscida* Linn was dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve No. 42 and stored in an airtight container for further use.

The dried powder material of whole plant (500gm) was defatted with petroleum ether (60-80°C) by hot continuous extraction method in a soxhlet apparatus for 48-72 hrs. The defatted powder material was further extracted with ethanol (95%v/v) for 72 hrs in soxhlet apparatus.

The extract was made solvent free by distillation process and the resulting semisolid mass was vacuum dried to yield a solid residue i.e. Pet. ether extract (1%w/w) ethanolic extract (5% w/w).

In vitro antioxidant activity

DPPH Assay[15]

The assay was carried out in a 96 well microtitre plate. To 200 µl of DPPH solution, 10 µl of various concentrations of the extract or the standard solution was added separately in wells of the microtitre plate. The plates were incubated at 37 ºC for 30 min. Absorbance was measured at 490 nm using ELISA reader. IC_{50} value is the concentration of the sample required to scavenge, 50 % DPPH free radical.

ABTS radical cation decolourisation assay[16]

ABTS (54.8 mg) was dissolved in 50 ml of distilled water to 2 mM concentration and potassium persulphate (17 mM, 0.3 ml) was added. The reaction mixture was left to stand at room temperature overnight in dark before use. To 0.2 ml of various concentrations of the extracts or standards, 1.0 ml of distilled DMSO and 0.16 ml of ABTS solution was added to make a final volume of 1.36 ml. Absorbance was measured spectrophotometrically, after 20 min at 734 nm. The assay was performed in triplicate.

Scavenging of hydrogen peroxide[17]

A solution of hydrogen peroxide (20mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Various concentrations of 1ml of the extracts or standards in methanol were added to 2 ml of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm, after 10 min against a blank solution that contained extracts in PBS without hydrogen peroxide.

Scavenging of Nitric Oxide radical[18]

In the present investigation, Griess Ilosvay reagent is modified by using Naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). Nitrite ions react with Griess reagent, which forms a purple azo dye. In presence of test components, likely to be scavengers, the amount of nitrites will decrease. The degree of decrease in the formation of purple azo dye will reflect the extent of scavenging. The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (PBS, pH 7.4, 1 ml) and extract in DMSO at various concentrations or standard was incubated at 25° C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 min for completion of diazotisation. Then, 1 ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A pink coloured chromophore was formed. The absorbance of these solutions was measured at 540 nm.

RESULTS AND DISCUSSION

In the present study, ethanolic extract of *Pseudarthria viscida* Linn were studied for *in vitro* antioxidant activity by four radical scavenging methods which is summarized in Table 1. The extract showed potent scavenging activity with IC_{50} values of 78.1±1.75 and 35.00±1.15µg/ml, respectively in the cases of nitric oxide and DPPH free radicals. The other methods shown moderate activity which is all compared with standards. The variations in activity may be due to the fact that diversity in the basic chemical structure of phytoconstituents possesses different degree of antioxidant activity against different free radicals. The preliminary phytochemical investigation revealed the presence of phenolic compounds in the ethanol extract of the plant. Plant phenolics are known to exhibit potent antioxidant activity[19]. Hence, the observed antioxidant activity of the extracts of *Pseudarthria viscida* Linn may be due to the presence of these constituents. DPPH has been used to evaluate the free radical- scavenging activity of natural antioxidants. DPPH which is a radical itself with a purple color, changes into a stable compound with a yellow color by reacting with an antioxidant, and the extent of the reaction depends on the hydrogen donating ability of the antioxidant[20]. The ability of ethanolic extract of PV scavenge DPPH radicals suggests that it is an electron donor and can react with free radicals to convert them to more stable products and terminate radical chain reactions. The technique in ABTS assay involves the reaction between ABTS and potassium persulphate to produce the ABTS radical cation, a blue green chromogen. In the presence of the antioxidant reductant, the colored radical cation is converted back to colorless. It is applicable for the study of both water-soluble and lipid-soluble antioxidants.

Hydrogen peroxide itself not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells[21]. Thus removing of H_2O_2 is very important for antioxidant defense in cell or food systems.

Nitric oxide is produced by several different types of cells, including endothelial cells and macrophages. The early release of nitric oxide through the activity of constitutive nitric-oxide synthase is important in maintaining the dilation of blood vessels the much higher concentrations of nitric oxide produced by inducible nitric-oxide synthase in macrophages can result in oxidative damage. Nitric oxide reacts with free radicals, thereby producing the highly damaging peroxynitrite. Nitric oxide injury takes place for the most part through the peroxynitrite route because peroxynitrite can directly oxidize LDLs, resulting in irreversible damage to the cell membrane.

Table 1 *: In vitro* antioxidant activity of ethanol extract of *Pseudarthria viscida* Linn

Extracts / Standards	IC_{50} values ± SE µg/ml* by methods							
	DPPH		ABTS		H_2O_2		Nitric oxide	
	Concentration µg/ml	% Inhibition	Concentration µg/ml	% Inhibition	Concentration µg/ml	% Inhibition	Concentration µg/ml	% Inhibition
Ethanol extract of PV	125	89.50±0.75	500	79.50±1.15	500	52.65±0.25	500	72.36±0.80
	62.5	82.80±1.65	250	71.36±0.80	250	41.50±0.27	250	61.90±1.50
	31.25	45.90±0.50	125	62.18±0.50	125	28.65±0.80	125	56.25±0.50
	15.6	15.70±1.00	62.5	44.50±0.35	62.5	16.90±0.28	62.5	47.80±0.65
IC_{50} µg/ml	35.00±1.15		81.00±3.85		449.60±1.30		78.10±1.75	
Standards								
Ascorbic acid	2.69 ± 0.05		11.25 ± 0.49		187.33 ± 1.93		---	
Rutin	---		0.51± 0.01		36.66 ± 0.22		65.44±2.56	

*Average of four determinations, values were mean ± S.E.M *P<0.001(Turkey-Kramer equation)

CONCLUSION

Based on the results of the present study, we conclude that the plant extract possesses antioxidant potential. However, further studies are necessary to examine underlying mechanisms of antioxidant effects and to isolate the active compound (s) responsible for these pharmacological activities.

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