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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND COMPARATIVE IN-VITRO ANTIOXIDANT STUDIES OF DIFFERENT EXTRACTS OF *DIPTERACANTHUS PROSTRATES* NEES.

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ABSTRACT

The present study was conducted with the objective of preliminary phytochemical screening and evaluation of antioxidant activity of the toluene and methanolic extract of whole plant of Dipteracanthus prostatus Nees. by using DPPH radical scavenging assay and nitric oxide scavenging assay method. The different concentration (50,100, 200, 400 and 500 µg/ml) of standard and test sample (i.e. toluene and methanolic extract of whole plant of Dipteracanthus prostatus Nees.) was prepared and evaluation of anti-oxidant activity was done by DPPH radical scavenging assay and nitric oxide scavenging assay method. The anti-oxidant activity is exhibited in percentage inhibition. Toluene and methanolic extract of Dipteracanthus prostrates Nees showed gradual increasing percentage inhibition with increasing concentration of standard and test sample in both assay method. In DPPH assay method toluene extract showed more percentage inhibition (58.84%) as compared to methanolic extract (55.77%) at 500 µg /ml while in Nitric oxide Free radical Scavenging assay method toluene extract showed more percentage inhibition (57.33%) as compared to methanolic extract (52.24%) at 500 µg /ml. So, the toluene extract showed more percentage inhibition in DPPH assay method as compared to Nitric oxide Free radical Scavenging assay method.

Keywords: Antioxidant activity, Dipteracanthus prostatus Nees, DPPH assay, Nitric oxide radical scavenging assay.

INTRODUCTION

Free radicals are a class of highly reactive, unstable molecules containing one or more unpaired electrons, derived from the metabolism of oxygen. Free radicals and reactive species are constantly formed in the human body by exogenous chemicals or normal endogenous metabolic processes. These species are capable of causing cellular damage by oxidizing different bio-molecules of the body, such as proteins, membrane lipids, enzymes and nucleic acid. Excessive production of free radicals and reactive oxygen species may lead to oxidative damage and can result in different diseases like degenerative disorders, diabetes, cancer, cirrhosis, neurological disorders, inflammation, arthritis and ageing [1, 2]. The human body has an own antioxidant system which acts in response with these free radicals and reactive oxygen species and neutralizes them. This natural antioxidant system includes several types of enzymes like superoxide, catalase, dismutase and glutathione and antioxidant compounds such as tocopherol, ascorbic acid, polyphenols, phenolic acids, and flavonoids, which shield the body from the toxic effects of the free radical species and prevent oxidative stress, but when an imbalance between reactive oxygen species and antioxidant system occur these free radicals generates in the body [3, 4].

Antioxidants are the compounds, able to slowing or preventing the oxidation of other molecules. They play a key role in the prevention of body from diseases by reducing the oxidative damage to cellular component caused by ROS [5]. There is lots of synthetic antioxidant (butylated hydroxyl toluene, butylated hydroxyl anisole, propylgallate and tertiary butyl-hydroquinone) are available, but their use is limited due to their toxic and carcinogenic effect [6]. Alternatively, use of herbal plant extracts as anti-oxidant, due to their proved activity and lesser side effect is a promising approach. Recent studies suggested that the plant derived antioxidants holds enormous therapeutic importance in free radical mediated diseases [7-9].

Dipteracanthus prostrates Nees (Family: Acanthaceae) is an erect hoary pubescent, up to 50 cm tall, basally woody and much branched shrub locally known as Haadjud by tribal peoples. It is widely distributed plant in Africa, Arab, Srilanka, Pakistan and India. In India it is generally found in Tamil Nadu, Western Ghat and Andhra Pradesh [10, 11]. The stem of the plant is greenish and rounded, becoming angular with age. Leaves are 4-10 mm long, lamella elliptic, ovate and densely pubescent on both sides. Flowering period is July- November. Flowers are sessile, 3-4 cm long, pale white in color, usually solitary, axillary, and 2-3 in cymes. Fruit capsule is elliptic clavate, glabrous, 1.4-1.8 cm in length, 8-10 seeded. Seeds are flat and orbicular [12]. Previous phytochemical studies confirmed that *Dipteracanthus prostrates* contains a rich amount of bioactive compounds, including flavonoids, saponins, steroids, phenols, tannins, and lignin [13]. In traditional medicinal system of India different parts of the plant have been used in the treatment of a variety of diseases. It is used as cardiotonic, antiulcer, antioxidant, paranychia, venereal diseases, rheumatic complaints, eye diseases, insect bite and healing of wounds [14, 15].

In the light of above mentioned facts about plant, present study was designed to preliminary identification of different phytoconstituents present in methanolic and toluene extract of whole plant of *Dipteracanthus prostrates* and to investigate the comparative antioxidant activities of the both extracts using DPPH free radical scavenging assay and nitric oxide radical scavenging assay.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Dipteracanthus prostrates (whole plant) was collected in the month of December from the ABS Botanical Conservation, Research & Training Centre, Kaaripati, Salem, Tamil Nadu, India. The herbarium of the plant was prepared and authenticated by Dr. A. Balasubramanian (Executive Director) Former Siddha Research Consultant (Ayush), Ministry of Health & Family Welfare, New Delhi, India. The specimen voucher number (AUT/JNU/029) was deposited with the herbarium in the Department of Pharmacognosy, ABS Botanical Conservation, Research and Training Centre, Kaaripati, Salem, Tamil Nadu, India for future reference.

Extraction

Whole plant of *Dipteracanthus prostrates* was shade dried for four weeks, pulverized to the coarse powder, passed through sieve no. 20 to maintain uniformity and coarsely dried powder was first defatted with petroleum ether (60-80°C) to remove fatty materials and then successively extracted with toluene and then finally extracted with methanol using soxhlet apparatus. After complete extraction extracts were collected, and concentrated in vacuum under reduced pressure using a rotary flash evaporator and the dried crude extracts were stored separately in air tight containers at 4°C for further study..

Preliminary Phytochemical Screening

Both the crude extracts (toluene and methanolic) of whole plant of *Dipteracanthus prostrates* were subjected to qualitative phytochemical investigation for the identification of the different phytoconstituents using standard tests and procedures [16].

Chemicals and Reagents

1, 1-diphenyl-2- picryl - hydrazyl (DPPH) and Griess reagent were purchased from Aldrich Sigma, St. Louis, USA. Ascorbic acid, sodium nitroprusside, phosphate buffer was purchased from Himedia Laboratories, Mumbai, India. All the other reagents, solvents and chemicals used in the study were of analytical grade and procured from S.D. Fine Chemicals, Mumbai, India.

EVALUATION OF ANTIOXIDANT ACTIVITY

The antioxidant activity of both the extract was determined by different *in-vitro* methods such as free radical scavenging activity (FRSA) using 1,1-diphenyl-2- picryl - hydrazyl

(DPPH) and Nitric oxide scavenging activity. All the assays were carried out in triplicate, and average values were considered.

In-vitro Free Radical Scavenging Activity (FRSA) Using DPPH

DPPH, commonly known as 1,1-diphenyl-2-picrylhydrazyl, is a cell permeable, stable free radical used to assess the ability of compounds to act as free radical scavengers to measure the antioxidant activity. The reaction of DPPH with an antioxidant or reducing compound produces the corresponding hydrazine DPPH2, which can be followed by a color change from purple to pale yellow [17]. The free radical scavenging activity of both the extracts was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) [18]. 0.1 mM solution of DPPH was prepared in methanol, and 1 ml of it was added to different concentrations of toluene and methanolic extract (50, 100, 200, 400 and 500 g/ml) in the test tube and final volume of 3 ml was made with methanol. The mixture were shaken vigorously and allowed to stand at room temperature for 30 min. Absorbance of the resulting mixture was measured at 517 nm against methanol as blank, by using a UV-visible spectrophotometer (Systronics, 2203, Japan). Each sample was then measured in triplicate and results were represented as mean. The ascorbic acid was used as a standard antioxidant in this method. Percentage of DPPH free radical scavenging activity (FRSA) was determined as follows:

$$\%\,(FRSA) = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{\text{Absorbance of control}} \times 100$$

……. (Equation 1)

In-vitro Nitric Oxide Radical Scavenging Activity

Nitric oxide is generated in biological tissues by specific nitric oxide synthases, which metabolizes arginine to citrulline with the formation of NO via a five electron oxidative reaction [19]. The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO. Under aerobic conditions, NO reacts with oxygen to produce stable products (nitrate and nitrite), the quantities of which can be determined using Griess reagent [20, 21]. The scavenging capacity for nitric oxide radicals was measured according to the method of Marcocci *et al.*, [22]. 1ml of 10 mM sodium nitroprusside in phosphate buffer saline (PBS, pH 7.4) was mixed with 1 ml of test solution of different concentrations of toluene and methanolic extracts and the mixture was incubated at 25°C for 150 min. From the incubated mixture, 1 ml was taken out and 1 ml of Griess' reagent (1% sulphanilamide, 2% o-phosphoric acid 0.1% naphthyl ethylene diamine dihydrochloride) was added to it. Absorbance of the developed chromophore formed by the diazotization of nitrite with sulfanilamide subsequent coupling with naphthyl ethylene diamine dihydrochloride was read at 546 nm. Each sample was then measured in triplicate and the results were represented as men. The ascorbic acid was used as a standard antioxidant in this method. The percentage NO radical scavenging activity was determined using equation 1:

Statistical Analysis

Results were expressed as the mean ± standard error of mean (S.E.M.). The IC50 value was obtained by interpolation from linear regression analysis

Results

Extraction and Phytochemical Screening

After the extraction, percentage yield, color, odour and consistency of both extracts were recorded (Table 1). The phytochemical screening revealed that toluene and methanolic extract of *Dipteracanthus prostrates* whole plant contain a rich amount of flavonoids, alkaloids, glycosides, tannins, carbohydrates and phenolic compounds. The result of the phytochemical screening is shown in table 2.

In-vitro free radical scavenging activity of toluene and methanolic extract of Dipteracanthus prostrates (FRSA) using the DPPH method

Several methods are reported for evaluation of antioxidant activities of plant extract but due to the chemical complexity of different compounds present in extract could lead to variable results, depending on the method employed. Therefore, an approach with multiple assays for evaluating the antioxidant potential of extracts would be more revealing and even essential. In this study, free radical scavenging activity of toluene and methanolic extract of whole plant of *Dipteracanthus prostrates* was measured by DPPH free radical scavenging assay and results were compared with standard antioxidant ascorbic acid. The results of the free radical scavenging activity of different extracts tested by the DPPH method are depicted in Table-3. Toluene and methanolic extract of *Dipteracanthus prostrates*

Table 1: Percentage yield, color, odour and consistency of different extracts

S. No.	Extract	Color	Odour	Consistency	% Yield
1	Toluene	Green	Characteristic	Solid	8.37%
2	Methanolic	Green	Characteristic	Semi Solid	12.4%

Table 2: Phytochemical screening of toluene and methanolic extract of *Dipteracanthus prostrates*

S. No.	Chemical Test	Toluene extract	Methanolic extract
1	Carbohydrates	(+)	(+)
2	Tannins	(+)	(+)
3	Alkaloids	(+)	(+)
4	Glycosides	(+)	(+)
5	Flavonoids	(+)	(+)
6	Steroids and sterols	(+)	(+)
7	Proteins and amino acids	(+)	(+)
8	Saponins	(–)	(+)

showed gradual increasing percentage inhibition with increasing concentration at 517 nm as anti oxidative agent by DPPH assay but toluene extract showed more percentage inhibition (58.84%) as compared to methanolic extract (55.77%) at 500 g/ml. Ascorbic acid as a standard antioxidant showed a gradual increase in percentage inhibition with increasing concentration at 517 nm by DPPH assay (Table 3).

In-vitro free radical scavenging activity of toluene and methanolic extract of Dipteracanthus prostrates (FRSA) using nitric oxide radical scavenging method

Scavenging of nitric oxide radicals was based on the generation of nitric oxide from sodium nitroprusside in buffered saline solution, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent. Toluene and methanolic extract of *Dipteracanthus prostrates* showed gradual increasing percentage inhibition with increasing concentration at 546 nm in spectrophotometer as anti oxidative agent by nitric oxide free radical scavenging method assay but toluene extract showed more percentage inhibition (57.33%) as compared to methanolic extract (55.24%) at 500 g/ml. Ascorbic acid showed a gradual increase in percentage inhibition with increasing concentration at 546 nm as standard antioxidative agent by nitric oxide free radical scavenging assay method. The results are shown in Table 4.

DISCUSSION

As we know that the free radical and reactive species become an important etiological factor in the pathogenesis of several diseases. Although the numbers of antioxidants are available to reduce the risk associated with free radicals, efficacy and safety of synthetic antioxidants have become a concern among scientist and important current issue in discovery of natural antioxidants [23]. Studies suggested that plant derived bioactive constituents having antioxidant activity such as vitamins, alkaloids, tannins, terpenoids, phenolic compounds, flavonoids and coumarins play a key role in the management of several diseases like neurodegenerative disorders, diabetes and cardiovascular disorders [24, 25]. Plant derived herbal drugs becomes a promising alternative to the available synthetic antioxidnats.

In present study the phytochemical screening revealed that toluene and methanolic extract of *Dipteracanthus prostrates* whole plant contain a rich amount of flavonoids, alkaloids, glycosides, tannins, carbohydrates, proteins & amino acids, saponins, and phenolic compounds.

Free radical scavenging activity using DPPH is an extensively used, comparatively quick method. DPPH is a stable free radical; antioxidants exert their radical scavenging activity

Table 3: Antioxidant activity of toluene and methanolic extract of *Dipteracanthus prostrates* by DPPH method

Sample	Concentration (g/ml)	Absorbance at 517 nm (Mean ± S.E.M.)	% Inhibition
Ascorbic acid (Standard)	50	0.347±0.002	37.36
	100	0.232±0.005	58.12
	200	0.179±0.004	67.68
	400	0.14±0.001	75.27
	500	0.09±0.002	83.75
TEDP	50	0.528±0.004	4.69
	100	0.453±0.005	18.23
	200	0.364±0.005	34.29
	400	0.318±0.005	43.68
	500	0.228±0.008	58.84
MEDP	50	0.548±0.002	1.08
	100	0.453±0.003	18.23
	200	0.406±0.002	26.71
	400	0.328±0.002	40.79
	500	0.245±0.006	55.77

Results are represented as Mean ± S.E.M., and Control OD at 517 nm 0.554.

Table 4: Antioxidant activity of toluene and methanolic extract of *Dipteracanthus prostrates* by Nitric oxide Free radical scavenging method

Sample	Concentration (g/ml)	Absorbance at 546 nm (Mean ± S.E.M.)	% Inhibition
Ascorbic acid (Standard)	50	0.476±0.001	41.30
	100	0.373±0.001	54.00
	200	0.310±0.001	61.77
	400	0.233±0.001	71.27
	500	0.173±0.001	78.66
TEDP	50	0.619±0.001	24.53
	100	0.571±0.001	29.59
	200	0.523±0.002	35.51
	400	0.410±0.001	49.44
	500	0.346±0.001	57.33
MEDP	50	0.629±0.001	22.44
	100	0.581±0.001	28.36
	200	0.546±0.001	32.67
	400	0.421±0.002	48.08
	500	0.363±0.002	55.24

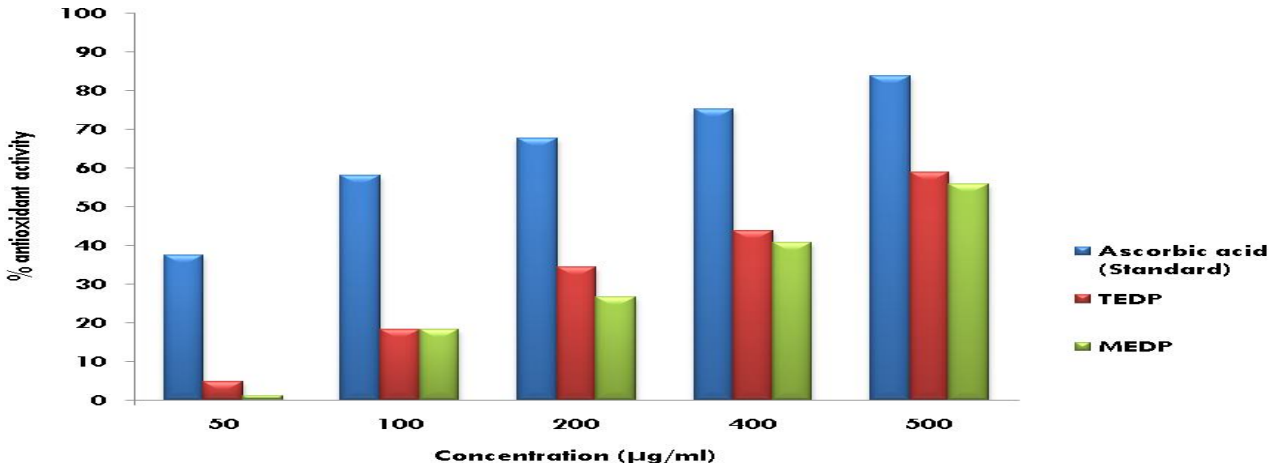

Figure 1: % **a**ntioxidant activity of toluene and methanolic extract of *Dipteracanthus prostrates* by DPPH free radical scavenging activity

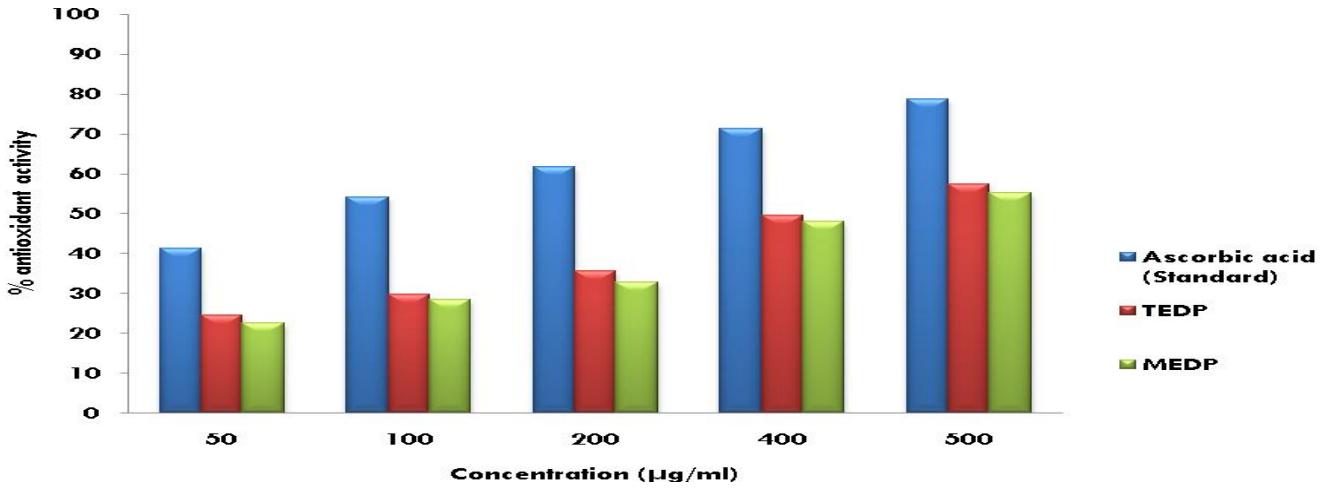

Figure 2: % antioxidant activity of toluene and methanolic extract of *Dipteracanthus prostrates* by nitric oxide free radical scavenging activity

due to their hydrogen-donating ability. To be converted into a diamagnetic molecule DPPH accepts an electron or hydrogen radical [26].

Nitric oxide (NO) is a vital bioregulatory molecule, having a number of functional and physiological effects, including neural signal transduction, control of blood pressure, platelet function, antimicrobial and antitumor activity. Since small concentrations of NO is beneficial for normal physiological function of the body, but at the time of inflammation and infection, elevated level of NO due to increased production may lead to several undesirable side effects such as mutagenesis and carcinogenesis [21, 27].

Toluene and methanolic extract of *Dipteracanthus prostrates* showed gradual increasing percentage inhibition with increasing concentration of standard and test sample in both assay methods. In DPPH assay method toluene extract showed more percentage inhibition (58.84%) as compared to methanolic extract (55.77%) at 500 g/ml (Table 3) while in Nitric oxide free radical scavenging assay method toluene extract showed more percentage inhibition (57.33%) as compared to methanolic extract (55.24%) at 500 g/ml (Table 4). The antioxidant potential of *Dipteracanthus prostrates* may be a result of the presence of phenolic compounds in the plant.

CONCLUSION

On the basis of findings of the present study, it can be concluded that we can say that *Dipteracanthus prostrates*, contains rich amounts of phytoconstituents which demonstrates high scavenging activity in both the *in-vitro* methods i.e., DPPH free radical scavenging activity and nitric oxide radical scavenging activity. Result of the *in-vitro* antioxidant activities indicate that *Dipteracanthus prostrates* is a significant source of natural antioxidant, which might be supportive in the prevention of several diseases. Further studies are required to elucidate the isolated phytoconstituents of the plant to understand their mechanism of action.

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