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EVALUATION OF *IN VITRO* ANTIOXIDANT ACTIVITIES OF DIFFERENT EXTRACTS OF AERIAL PARTS OF *Dyschoriste littoralis* Nees

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ABSTRACT

The present study was to investigate invitro antioxidant activities of various extracts of aerial parts of Dyschoriste littoralis. The antioxidant activity was evaluated by Nitric oxide radical scavenging activity, Iron chelating activity with reference standard Ascorbate and total phenol content respectively. An IC_{50} value was found that ethyl acetate extract of Dyschoriste littoralis is more effective in Nitric oxide radical scavenging activity, Iron chelating activity than that of methanolic and petroleum ether extract. The IC_{50} values of the ethyl acetate extract of Dyschoriste littoralis and ascorbate were found to be 360µg/ml,260 µg/ml and 410µg/ml, 65 µg/ml in the Nitric oxide radical scavenging activity and Iron chelating activity respectively. But when compare to the all the three extracts with ascorbate (standard), the ethyl acetate extract of Dyschoriste littoralis showed the better result. In addition, ethyl acetate extract of Dyschoriste littoralis was found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. All the above invitro studies clearly indicate that the ethyl acetate extract of Dyschoriste littoralis has a significant antioxidant activity. These invitro assays indicate that this plant extracts are a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

KEY WORDS

D. littoralis, Invitro antioxidant, Nitric oxide scavenging, Iron chelating, total phenol.

INTRODUCTION

Oxygen free radicals are formed in tissue cells by many endogenous and exogenous causes such as metabolism, chemicals, and ionizing radiation[1]. Oxygen free radicals may attack lipids and DNA giving rise to a large number of damaged products[2]. Iron is known to be involved in the generation of reactive oxygen species (ROS) and in the formation of highly toxic hydroxyl radicals from other active oxygen species such as hydrogen peroxide[3]. The enhanced generation of ROS *in vivo* could be quite deleterious, since they are involved in mutagenesis, apoptosis, ageing, and carcinogenesis[4]. Antioxidant compounds like Phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases.

Dyschoriste littoralis Nees. belongs to the family Acanthaceae. The Acanthaceae (Acanths) derived from *Acanthus* are made up of 221 genera and 4000 species[5]. Traditionally the most important part use in *Acanthaceae* is the leaves and they are used externally for wounds. *Acanthaceae* possess antifungal, cytotoxic, anti-inflammatory, anti-pyretic, anti-oxidant, insecticidal, hepatoprotective, immunomodulatory, Anti-platelet aggregation, anti-viral potential and many members of the family are used as medication for asthma[6]. *Dyschoriste* is a genus of

worldwide distribution in warm regions. Members of the genus are commonly known as snake herb.

Dyschoriste littoralis could be used as potential drug for the treatment of pain, fever and inflammation. *Dyschoriste littoralis* Nees. are considered a very efficacious remedy for all sorts of coughs being administrated along with ginger. The leaves are used for rheumatism. The leaves were dried made into cigarettes and smoked in asthma and their juice is used treatment of diarrhea and dysentery[7]. The plant has anti-microbial activities[7]. The plant having the wound healing activities[8]. However, no data are available in the literature on the antioxidant activity of aerial parts of *Dyschoriste littoralis* Nees. Therefore, we undertook the present investigation to investigate free radical scavenging activities of different extract of aerial parts of Dyschoriste *littoralis* Nees. through various *in vitro* models.

MATERIAL AND METHODS

Collection and Identification of Plant materials

The aerial parts of *Dyschoriste littoralis* Nees. (Acanthaceae) were collected during January to April from Tirunelveli District of Tamil Nadu, India. The identified plant species was confirmed with Voucher specimen available in the Survey of Medicinal Plant Unit (SMP), Govt. Siddha Medical College, Palayamkottai, Tirunelveli, Tamil Nadu (voucher no: 25834). The taxonomic features of the plant confirmed with the Flora of Presidency of Madras[9]. The aerial parts of *Dyschoriste littoralis* were dried under shade, segregated, pulverized by a mechanical grinder.

Preparation of Extracts

The above powered materials were successively extracted with Petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus[10] for 24 hrs. Then the marc was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then mark was subjected to Methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Evaluation of Antioxidant activity by *in vitro* Techniques:

Nitric oxide radical scavenging activity[11]

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat (1964)[11]. The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M) were incubated at 25^{0}C for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological P^H spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Illosvery reaction at 540 nm.

Iron chelating activity[12]

The method of Benzie and strain (1996)[12] was adopted for the assay. The principle is based on the formation of *O*-Phenanthroline-Fe^{2+} complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride (200µM) and 2 ml of various concentrations ranging from 10 to 1000µg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

Total phenol[13]

The measurement of total phenol is based on Mallick and Singh (1980)[13]. To 0.25g of sample, added 2.5 ml of ethanol and centrifuged at 2^{o}C for 10 mins. The supernatant was preserved. Then, the sample was re-extracted with 2.5 ml of 80% ethanol and centrifuged. The pooled supernatant was evaporated to dryness. Then, added 3 ml of water to the dried supernatant. To which added 0.5 ml of Folins phenol reagent and 2 ml of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1 min. the absorbance was measured at 650 nm in a spectrophotometer.

RESULTS AND DISCUSSION

Nitric oxide scavenging activity

Nitric oxide is a diffusible free radical which is an important effect or molecule in diverse biological systems[14].The reduction of nitric oxide radical by the petroleum ether extract of *Dyschoriste littoralis* and ascorbate was illustrated in Table 1. The maximum scavenging activity of petroleum ether extract and ascorbate at 1000 µg/ml were found to be 50.18 % and 60.64% respectively. The IC_{50} value of petroleum ether

extract and ascorbate were recorded as 1010µg/ml and 410µg/ml respectively.

Table 1: Nitric oxide scavenging activity of Pet.ether extract of *Dyschoriste littoralis*

S.No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	26.98 ±0 .034	26.87 ± 0.076
2	250	35.12 ± 0.068	30.30 ± 0.054
3	500	46.77 ± 0.072	55.23 ± 0.022
4	1000	50.12 ± 0.084	60.64 ± 0.014
		IC_{50} = 1010 µg/ml	**IC_{50} = 410 µg/ml**

***All values are expressed as mean ± SEM for three determinations**

The reduction of nitric oxide radical by the ethyl acetate extract of *Dyschoriste littoralis* and ascorbate was illustrated in Table 2. The maximum scavenging activity of ethyl acetate extract and ascorbate at 1000 µg/ml were found to be 62.67% and 60.64% respectively. The IC_{50} value of ethyl acetate extract and ascorbate were recorded as 360µg/ml and 410µg/ml respectively.

Table 2: Nitric oxide scavenging activity of Ethyl acetate extract of *Dyschoriste littoralis*

S.No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	39.16 ± 0.014	26.87 ± 0.076
2	250	46.42 ± 0.062	30.30 ± 0.054
3	500	56.54 ± 0.048	55.23 ± 0.022
4	1000	62.67 ± 0.070	60.64 ± 0.014
		IC_{50} =360 µg/ml	**IC_{50} = 410 µg/ml**

***All values are expressed as mean ± SEM for three determinations**

The reduction of nitric oxide radical by the methanolic extract of *Dyschoriste littoralis* and ascorbate was noted to be concentration dependent and was illustrated in Table 3. The maximum scavenging activity of methanolic extract and ascorbate at 1000 µg/ml were found to be 55.86% and 60.64% respectively. The IC_{50} value of methanolic extract and ascorbate were recorded as 772µg/ml and 410µg/ml respectively.

Table 3: Nitric oxide scavenging activity of Methanolic extract of *Dyschoriste littoralis*

S.No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	24.67 ± 0.064	26.87 ± 0.076
2	250	33.66 ± 0.034	30.30 ± 0.054
3	500	44.78 ± 0.078	55.23 ± 0.022
4	1000	55.86 ± 0.045	60.64 ± 0.014
		IC_{50} = 772 µg/ml	**IC_{50} = 410 µg/ml**

***All values are expressed as mean ± SEM for three determinations**

Based on the above results the IC_{50} values and percentage scavenging capacity, it was found that ethyl acetate of *Dyschoriste littoralis* is more effective in scavenging nitric oxide radical than that of methanolic and petroleum ether extract. But when compare to the all the three extracts with Ascorbate (standard), the ethyl acetate extract of the *Dyschoriste littoralis* showed the similar result.

Iron chelating activity:

Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, iron is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins and other cellular components[15]. Iron binding capacity of the petroleum ether extract of *Dyschoriste littoralis* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in table 4. The maximum chelating of metal ions at 1000µg/ml for petroleum ether extract and EDTA was found to be 49.15% and 97.90% respectively. The IC_{50} value of plant extract and EDTA was recorded as 970µg/ml and 65µg/ml respectively.

Table 4: Effect of Pet.ether extract of *Dyschoriste littoralis* on Iron-chelating method

S.No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (Petroleum ether extract)	Standard (EDTA)
1	125	29.11 ± 0.036	58.68 ± 0.007
2	250	30.80 ± 0.018	65.87 ± 0.018
3	500	38.17 ± 0.045	83.83 ± 0.012
4	1000	49.15 ± 0.068	97.90 ± 0.019
		IC_{50} = 970 µg/ml	**IC_{50} = 65 µg/ml**

***All values are expressed as mean ± SEM for three determinations**

Iron binding capacity of the ethyl acetate extract of *Dyschoriste littoralis* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in table 5. The maximum chelating of metal ions at 1000µg/ml for ethyl acetate extract and EDTA was found to be 76.24% and 97.90% respectively. The IC_{50} value of plant extract and EDTA was recorded as 260µg/ml and 65µg/ml respectively.

Table 5: Effect of Ethyl acetate extract of *Dyschoriste littoralis* onIron-chelating method

S.No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (Ethylacetate extract)	Standard (EDTA)
1	125	36.56 ± 0.052	58.68 ± 0.007
2	250	50.79 ± 0.064	65.87 ± 0.018
3	500	69.86 ± 0.046	83.83 ± 0.012
4	1000	76.24 ± 0.042	97.90 ± 0.019
		IC_{50} = 260 µg/ml	**IC_{50} = 65 µg/ml**

***All values are expressed as mean ± SEM for three determinations**

Iron binding capacity of the methanolic extract of *Dyschoriste littoralis* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in table 6. The maximum chelating of metal ions at 1000µg/ml for plant extract and EDTA was found to be 66.87% and 97.90% respectively. The IC_{50} value of plant extract and EDTA was recorded as 485µg/ml and 65µg/ml respectively.

Table 6: Effect of Methanolic extract of *Dyschoriste littoralis* on Iron-chelating method

S.No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (Methanolic extract)	Standard (EDTA)
1	125	27.64 ± 0.042	58.68 ± 0.007
2	250	39.45 ± 0.063	65.87 ± 0.018
3	500	50.71 ± 0.025	83.83 ± 0.012
4	1000	66.87 ± 0.062	97.90 ± 0.019
		IC_{50} = 485 µg/ml	**IC_{50} = 65 µg/ml**

***All values are expressed as mean ± SEM for three determinations**

Based on the above results indicated, the ethyl acetate extract of *Dyschoriste littoralis* was found to most effective than that of methanolic and petroleum ether. The results indicted the plant extract possess iron binding capacity which might be due to the presence of polyphenols that averts the cell from free radical damage by reducing of transition metal ions[31].

Total phenol

Phenolic compounds are known as powerful chain breaking antioxidants[16]. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups[17]. The poly phenolic compounds may be involved in directly to antioxidant action[18]. The total amount of phenolic content of various extract of aerial parts of *Dyschoriste littoralis* was present in Table 7.

Table 7: The total Phenolic content of various extracts of *Dyschoriste littoralis*

S.No	Extracts	Total phenol content (mg/g of Catechol) (±SEM) *
1	Pet.ether extract of *Dyschoriste littoralis*	1.342± 0.054
2	Ethyl acetate extract of *Dyschoriste littoralis*	7.78 ± 0.063
3	Methanolic extract of *Dyschoriste littoralis*	4.23 ± 0.042

***All values are expressed as mean ± SEM for three determinations**

Based on the result the ethyl acetate and methanolic extract of *Dyschoriste littoralis* was found higher content of phenolic components (7.78 ± 0.063 and 4.23 ± 0.042 respectively) than that of petroleum ether extract of *Dyschoriste littoralis.*

CONCLUSION

The present study was clearly indicated the ethyl acetate extract of *Dyschoriste littoralis* showed strong antioxidant activity by inhibiting Nitric oxide radical scavenging activities and iron chelating when compared with standard Ascorbate. In addition, the ethyl acetate extract and methanolic of *Dyschoriste littoralis* was found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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