Research Article

In Vitro free Radical Scavenging Activity of Various Extracts of Aerial Parts of *Dyschoriste littoralis* Nees

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ABSTRACT

The present study was to investigate *in vitro* antioxidant activities of various extracts of aerial parts of *Dyschoriste littoralis.* The antioxidant activity was evaluated by DPPH (2,2'-diphenyl-β-picrylhydrazyl) radical scavenging activity, Superoxide anion scavenging activity and hydroxyl radical scavenging activity with reference standard Rutin, Quercetin and ascorbate respectively. The ethyl acetate extract of *D.littoralis* and rutin were found to 515µg/ml and 480µg/ml, superoxide anion scavenging activity of ethyl acetate extract *Dyschoriste littoralis* and reference standard Quercetin IC_{50} values were found to be 307 µg/ml and 60 µg/ml and hydroxyl radical scavenging activity of ethyl acetate extract *D. littoralis* and standard ascorbate were found to be 540 µg/ml and 410 µg/ml . An IC_{50} value was found that ethyl acetate extract of *D. littoralis* is more effective in scavenging DPPH radical, superoxide radical and hydroxyl radical scavenging than that of methanol and petroleum ether extract. The above result of possess good an antioxidant activity when compare to the above all standard. These in vitro assays indicate that ethyl acetate extract of *D. littoralis* is a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: *D. littoralis*, Invitro antioxidant, DPPH assay, Superoxide anion, hydroxyl radical.

INTRODUCTION

Natural products from plants are a rich resource used for centuries to cure various ailments. The use of bioactive plant-derived compounds is on the rise, because the main preoccupation with the use of synthetic drugs is the side effects which can be even more dangerous than the diseases they claim to cure. In contrast, plant derived medicines are based upon the premise that they contain natural substances that can promote health and alleviate illness and proved to be safe, better patient tolerance, relatively less expensive and globally competitive. Several substances from natural sources have been shown to contain antioxidants and are under study. 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[1]. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases[2].

Dyschoriste littoralis Nees. belongs to the family Acanthaceae. The Acanthaceae (Acanths) derived from *Acanthus* are made up of 221 genera and 4000 species[3]. 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[4]. *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 diarrhoea and dysentery[5]. The plant has anti microbial activities [5]. The plant having the wound healing activities[6]. 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 examine the free radical scavenging activities of various extract of aerial parts of *Dyschoriste littoralis* Nees. Through various *in vitro* models.

MATERIALS 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[7]. 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[8] 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

DPPH photometric assay[9]

The effect of extract on DPPH radical was assayed using the method of Mensor et al (2001)[9]. A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavenging activity(\%) = \frac{A_{518}\ \text{Control} - A_{518}\ \text{Sample}}{A_{518}\ \text{Control}} \times 100$$

Where A_{518} control is the absorbance of DPPH radical+ methanol; A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard.

Superoxide radical scavenging activity[10]

Superoxide radical (O_2^-) was generated from the photo reduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne et al (1975)[10]. The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Hydroxyl radical scavenging activity[11]

This was assayed as described by Elizabeth and Rao (1990)[11]. The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+} - Ascorbate –EDTA –H_2O_2 system (Fenton reaction). The reaction mixture contained 0.1 ml deoxyribose (2.8mM), 0.1 ml EDTA (0.1 mM), 0.1 ml H_2O_2 (1mM), 0.1 ml Ascorbate (0.1mM), 0.1 ml KH_2PO_4-KOH buffer, P^H 7.4 (20mM) and various concentrations of plant extract in a final volume of 1 ml. The reaction mixture was incubated for 1 hour at 37^0 C. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated.

RESULTS AND DISCUSSION

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation[12]. Phenolic compounds and flavonoids are major constituents of most of the plants reported to possess antioxidant and free radical scavenging activity[13].

DPPH scavenging activity

DPPH is a stable free radical at room temperature often used to evaluate the antioxidant activity of several natural compounds. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants.

The percentage of DPPH radical scavenging activity of petroleum ether extract of *Dyschoriste littoralis* are shown in Table 1. The petroleum ether extract of *Dyschoriste littoralis* exhibited a maximum DPPH scavenging activity of 48.56% at 1000 µg/ml whereas for Rutin (standard) was found to be 69.83% at 1000 µg/ml. The IC_{50} of the petroleum ether extract of *Dyschoriste littoralis* and Rutin were found to be 1080µg/ml and 480µg/ml respectively.

Table 1: Effect of Petroleum ether extract of *Dyschoriste littoralis* Nees on DPPH assay

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Rutin)
1	125	19.46 ± 0.022	18.85 ± 0.076
2	250	28.34 ± 0.014	22.08 ± 0.054
3	500	36.87 ± 0.022	52.21 ± 0.022
4	1000	48.56 ± 0.067	69.83 ± 0.014
		IC_{50} = 1080 µg/ml	**IC_{50} = 480 µg/ml**

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

The percentage of DPPH radical scavenging activity of ethyl acetate extract of *Dyschoriste littoralis* presented in Table 2. The ethyl acetate extract of *Dyschoriste littoralis* exhibited a maximum DPPH scavenging activity of 65.67% at 1000 µg/ml whereas for Rutin (standard) was found to be 69.83% at 1000 µg/ml. The IC_{50} of the ethyl acetate extract of *Dyschoriste littoralis* and Rutin were found to be 515µg/ml and 480µg/ml respectively.

Table 2: Effect of Ethyl acetate extract of *Dyschoriste littoralis* Nees on DPPH assay

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Rutin)
1	125	29.22 ± 0.018	18.85 ± 0.076
2	250	44.33 ± 0.022	22.08 ± 0.054
3	500	49.64 ± 0.012	52.21 ± 0.022
4	1000	65.67 ± 0.054	69.83 ± 0.014
		IC_{50} = 515 µg/ml	**IC_{50} = 480 µg/ml**

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

The percentage of DPPH radical scavenging activity of methanolic extract of *Dyschoriste littoralis* was depicted in Table 3. The methanolic extract of *Dyschoriste littoralis* exhibited a maximum DPPH scavenging activity of 62.38% at 1000 µg/ml whereas for Rutin(standard) was found to be 69.83% at 1000 µg/ml. The IC_{50} of the petroleum ether extract of *Dyschoriste littoralis* and Rutin were found to be 695µg/ml and 480µg/ml respectively.

Table 3: Effect of Methanolic extract of *Dyschoriste littoralis* Nees on DPPH assay

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Rutin)
1	125	16.24±0.032	18.85 ± 0.076
2	250	27.38±0.026	22.08 ± 0.054
3	500	41.98±0.017	52.21 ± 0.022
4	1000	62.38±0.024	69.83 ± 0.014
		IC_{50} = 695 µg/ml	**IC_{50} = 480 µg/ml**

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

The ethyl acetate extract of *Dyschoriste littoralis* was found to more effective than and methanolic and petroleum ether extract. The IC_{50} of the ethyl acetate extract of *Dyschoriste littoralis* and Rutin were found to be 515µg/ml and 480µg/ml respectively.

Superoxide anion scavenging activity

Superoxide is a highly reactive molecule that reacts with various substances produced through metabolic processes. Superoxide dismutase enzymes present in aerobic and anaerobic organisms catalyses the breakdown of superoxide radical.[14]

Percentage scavenging of superoxide anion examined at different concentrations of petroleum ether extract of *Dyschoriste littoralis* (125, 250, 500, 1000 µg/ml) was depicted in table 4. The maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 55.22% and 98.01% respectively. The IC_{50} value of plant extract and Quercetin was recorded as 772µg/ml and 60µg/ml respectively.

Table 4: Effect of Petroleum ether extract of aerial parts of Dyschoriste *littoralis* Nees on Superoxide anion scavenging activity method

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Quercetin)
1	125	22.46 ±0 .034	73.81 ± 0.006
2	250	36.84 ± 0.024	91.31 ± 0.011
3	500	44.16 ± 0.038	92.99 ± 0.024
4	1000	55.22 ± 0.022	98.01 ± 0.012
		IC_{50} = 772 µg/ml	**IC_{50} = 60 µg/ml**

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

Percentage scavenging of superoxide anion examined at various concentrations of ethyl acetate extract of *Dyschoriste littoralis* was depicted in table 5. The percentage scavenging of superoxide radical surged with the enhanced concentration of plant extract. The maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 69.22% and 98.01% respectively. The IC_{50} value of plant extract and Quercetin was recorded as 307µg/ml and 60µg/ml respectively. Superoxide scavenging ability of plant extract might primarily be due to the presence of flavanoids[15].

Table 5: Effect of Ethyl acetate extract of aerial parts of Dyschoriste *littoralis* Nees on Superoxide anion scavenging activity method

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Quercetin)
1	125	38.44 ±0 .045	73.81 ± 0.006
2	250	47.24 ± 0.022	91.31 ± 0.011
3	500	60.16 ± 0.037	92.99 ± 0.024
4	1000	69.22 ± 0.034	98.01 ± 0.012
		IC_{50} = 307 µg/ml	**IC_{50} = 60 µg/ml**

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

Percentage scavenging of superoxide anion examined at different concentrations of methanolic extract of *Dyschoriste littoralis* was depicted in table 6. The maximum scavenging activity of methanolic extract and Quercetin at 1000 µg/ml was found to be 68.21% and 98.01% respectively. The IC_{50} value of plant extract and Quercetin was recorded as 445µg/ml and 60µg/ml respectively.

Table 6: Effect of Methanolic extracts aerial parts of *Dyschoriste littoralis* Nees on Superoxide anion scavenging activity method

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Quercetin)
1	125	25.67 ± 0.023	73.81 ± 0.006
2	250	43.86 ± 0.067	91.31 ± 0.011
3	500	52.76 ± 0.082	92.99 ± 0.024
4	1000	68.21±0.034	98.01 ± 0.012
		IC_{50} = 445 µg/ml	**IC_{50} = 60 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 extract of *Dyschoriste littoralis* is more effective in scavenging superoxide radical than that of methanol and petroleum ether extract. But when compare to the all the three extracts with Quercetin (standard), the ethyl acetate extract of the *Dyschoriste littoralis* showed the better result.

Hydroxyl radical scavenging activity

The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins[16]. The percentage of Hydroxyl radical scavenging activity of petroleum ether extract of *Dyschoriste littoralis* was presented in Table 7. The petroleum ether extract of *Dyschoriste littoralis* was exhibited a maximum Hydroxyl radical scavenging activity of 50.34 % at 1000 µg/ml whereas for ascorbate (standard) were found to be 62.00 % at 1000 µg/ml. The IC_{50} values of the petroleum ether extract of *Dyschoriste littoralis* and ascorbate were found to be 1010µg/ml and 410µg/ml respectively.

Table 7: Effect of Petroleum ether extract of aerial parts of *Dyschoriste littoralis* Nees on Hydroxyl radical scavenging activity

S. No	Concentration(µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	18.23 ± 0.045	27.63±0.076
2	250	29.65 ± 0.026	49.53 ±0.054
3	500	37.45 ± 0.028	55.12±0.022
4	1000	50.34 ± 0.012	62.00±0.014
		IC_{50} = 1010 µg/ml	**IC_{50} = 410 µg/ml**

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

The percentage of hydroxyl radical scavenging activity of ethyl acetate extract of *Dyschoriste littoralis* was presented in Table 8. The ethyl acetate extract of *Dyschoriste littoralis* was exhibited a maximum hydroxyl radical scavenging activity of 59.87 % at 1000 µg/ml whereas for ascorbate (standard) were found to be 62.00 % at 1000 µg/ml. The IC_{50} values of the ethyl acetate extract of *Dyschoriste littoralis* and ascorbate were found to be 540µg/ml and 410µg/ml respectively

Table 8: Effect of Ethyl acetate extract of *Dyschoriste littoralis* Nees on Hydroxyl radical scavenging activity

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	32.23 ± 0.022	27.63±0.076
2	250	39.65 ± 0.046	49.53 ±0.054
3	500	48.92 ± 0.034	55.12±0.022
4	1000	59.87 ± 0.021	62.00±0.014
		IC_{50} = 540 µg/ml	**IC_{50} = 410 µg/ml**

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

The percentage of hydroxyl radical scavenging activity of methanolic extract of *Dyschoriste littoralis* was presented in Table 9. The methanolic extract of *Dyschoriste littoralis* was exhibited a maximum hydroxyl radical scavenging activity of 62.56 % at 1000 µg/ml whereas for ascorbate (standard) were found to be 62.00 % at 1000 µg/ml. The IC_{50} of the methanolic extract of *Dyschoriste littoralis* and ascorbate were found to be 650µg/ml and 410µg/ml respectively.

Table 9: Effect of methanolic extract of *Dyschoriste littoralis* Nees *on* Hydroxyl radical scavenging activity

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	18.87 ± 0.034	27.63±0.076
2	250	32.34 ± 0.054	49.53 ±0.054
3	500	45.65 ± 0.062	55.12±0.022
4	1000	62.56 ± 0.069	62.00±0.014
		IC_{50} = 650 µg/ml	**IC_{50} = 410 µg/ml**

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

The ethyl acetate extract of *Dyschoriste littoralis* were found to more effective than and methanolic and pet. ether extract. The IC_{50} of the ethyl acetate extract of *Dyschoriste littoralis* and Ascorbate were found to be 540µg/ml and 410µg/ml respectively.

CONCLUSION

The present study was clearly indicated the ethyl acetate extract of *Dyschoriste littoralis* showed strong antioxidant activity by inhibiting DPPH radical scavenging, superoxide scavenging and Hydroxyl radical scavenging when compared with respective standard. The ethyl acetate extract of *Dyschoriste littoralis* was found to more effective free radical scavenging activities than that of methanolic and petroleum ether extract. This effect may be due to the presence of poly phenolic and flavonoids compounds in the ethyl acetate extract of *Dyschoriste littoralis.* Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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