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An Investigation of free radical scavenging activity of various extracts of *Olax scandens* (family *Olacaceae)*

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ABSTRACT

Olax scandens Roxb. (family *Olacaceae)* is available in throughout tropical India. In the current study, the aerial parts of different extracts of *Olax scandens* were assessed by various *in-vitro* methods. The antioxidant activity was evaluated by Hydroxyl radical scavenging activity(OH radical), Nitric oxide radical scavenging activity(NO radical), and total antioxidant activity (Phosphomolybdic acid method) with reference standard Ascorbic acid. An IC_{50} value was originated that methanolic extracts of *Olax scandens*is more efficient in Hydroxyl radical scavenging activity, Nitric oxide radical scavenging, total antioxidant activity than that of EA and petroleum ether extract. The methanolic extracts of *Olax scandens* & ascorbate exhibited antioxidant potential possessing IC_{50} 253μg/ml & 135μg/ml (NO radical), 205μg/ml & 57μg/ml (Phosphomolybdic acid method), 265μg/ml & 65μg/ml (OH radical) respectively. But when compared to all the three extracts with ascorbate (standard), the methanolic extract of the *Olax scandens* showed a better result. Moreover, the results were observed in a concentration-dependent manner. Invitro antioxidant studies obviously showed that methanolic extracts of *Olax scandens* have better antioxidant activity. These results indicate that aerial parts of methanolic concentrates *Olax scandals* could serve as a natural antioxidant, which may be useful to prevent free radical-induced diseases.

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INTRODUCTION

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents, and quenchers of singlet oxygen formation (Andlauer and Fürst, 1998). Phenolic and flavonoid compounds are the most important bioactive compounds of the herbal revealed that having antioxidant and free radical scavenging activity (Christensen, 1999). It is generally standard that antioxidants can neutralize potentially dangerous reactive free radicals in body cells before they cause lipid and protein oxidation and may reduce potential mutations and, therefore, help prevent cancer or heart disease. Recently there is a growing interest on the discovery of natural antioxidants, mainly for two reasons, (I) there is epidemical and clinical evidence suggesting that consumption of vegetables and fruits reduces the risk of developing chronic disease, e.g., Cancer; (II) phytochemicals are generally safer than synthetic chemicals (Dast-

malchi *et al.*, 2007). The investigate for new products with antioxidative potential & a smaller number of adverse effects is an active domain of research. Thus, the extension and utilization of huge efficient antioxidants of the natural source was popular (Sakagami *et al.*, 1991).

*Olax scandens*Roxb (family *Olacaceae*) is commonly known as "Parrot Olax, Sprawling olax " in English & locally known as 'Kurpodur' in Telugu. This plant's fruits and leaves have been used for therapeutic & food purposes. *O. Scandensleaves were* used as constipation. *Olax scandens*(Roxb.), is generally known as Badrul in Odiya, used for cooking & different therapeutic purposes (Sinha and Lakra, 2005). *Olax scandens* bark decoction is used treatment fever & cough (Duraipandiyan *et al.*, 2006). *O. scandens*leaves were used for mouth ulcers (Kumar *et al.*, 2010). *O.scandens* boiled leaves were applied in the head for the treatment of headache (Kumar *et al.*, 2010). Still, no literature is available on the antioxidant potency of *O.scandens*. Thus, the present study to assess the antioxidant activities of *Olax scandens.*

MATERIALS AND METHODS

Gathering & Identification of Plant

The aerial parts of *O.scandens*(family *Olacaceae*) were gathered from Medak, Telangana state, India. Plant identification was made from the Botanical investigation of India, Telangana regional center, Hyderabad, (BSI/DRC/2019-20/Tech/174). The *O.scandens* were desiccated under shadowy, segregate, crushed through the grinder (Satheeshkumar *et al.*, 2011).

Preparation of Concentrates

The pulverized materials were progressively concentrated with PE (40-60^{0}C) through hot constant percolation method in Soxhlet equipment (Harbrone, 1984) for twenty-four hours. At that moment, the Marc was subjected to EA (76-78^{0}C) for 24 hrs & then mark was subjected to methanol for 24 hrs. The concentrates were concentrated on the rotational evaporator and subjected to solidify drying in a lyophilizer till dry powder was acquired (Alagumanivasagam *et al.*, 2010).

Assessment of Antioxidant potential through invitro methods

The variety of concentrates of *O.scandens* were used assessment of antioxidant activity by (Dc, 1964) method was adopted for NO radical assay & (Prieto *et al.*, 1999) method described for total antioxidant activity and (Kunchandy and Rao, 1990) method was adopted to determine the OH radical assay.

RESULTS AND DISCUSSION

Nitric oxide scavenging activity

Nitric oxide scavenging activity of PE concentrates of *O.scandens* appeared in Table 1. The PE concentrates of *O.scandens* exhibit a more Nitric oxide scavenging activity of 52.67% at 1000 μg/ml & ascorbate was recorded 76.34% at 1000 μg/ml. The IC_{50} of the PE concentrates of *O.scandens* & ascorbic acid were recorded 822μg/ml & 135μg/ml correspondingly.

Nitric oxide scavenging activity of EA concentrates on *O.scandens* appeared in Table 2. The EA concentrates on *O.scandens* exhibit a more Nitric oxide scavenging activity of 64.79% at 1000 μg/ml & ascorbic acid was recorded 76.34% at 1000 μg/ml. The IC_{50} of the EA concentrates of *O.scandens* & ascorbic acid were recorded 505μg/ml & 135μg/ml correspondingly.

Nitric oxide scavenging activity of methanol concentrates of *O.scandens* appeared in Table 3. The methanol concentrates of *O.scandens* exhibit a more Nitric oxide scavenging activity of 69.8722% at 1000 μg/ml & ascorbic acid was recorded at 76.34% at 1000 μg/ml. The IC_{50} of methanol concentrates of *O.scandens* & ascorbic acid were recorded 253μg/ml & 135μg/ml correspondingly.

IC_{50} values & Nitric oxide scavenging potential revealed that methanol concentrates of *O.scandens* are better activity in scavenging Nitric oxide scavenging activity when compared ethyl acetate & PE extracts.

Phosphomolybdic acid method

The total antioxidant activity of PE concentrates of *O.scandens* appeared in Table 4. The PE concentrates of *O.scandens* exhibit a total antioxidant activity of 35.67% at 300 μg/ml & ascorbic acid was recorded 98.12% at 300 μg/ml. The IC_{50} of the PE concentrates of *O.scandens* & ascorbic acid were recorded 790μg/ml & 57μg/ml correspondingly.

The total antioxidant activity of EA concentrates on *O.scandens* appeared in Table 5. The EA concentrates on *O.scandens* exhibit a total antioxidant activity of 40.66% at 300 μg/ml & ascorbic acid was recorded 98.12% at 300 μg/ml. The IC_{50} of the EA concentrates of *O.scandens* & ascorbic acid were recorded 495μg/ml & 57μg/ml correspondingly.

The total antioxidant activity of methanol concentrates on *O.scandens* appeared inTable 6. The methanol concentrates of *O.scandens* exhibit a total antioxidant activity of 65.42% at 300 μg/ml & ascorbic acid was recorded 98.12% at 300 μg/ml. The IC_{50} of the methanol concentrates of *O.scandens* &

Table 1: Nitric oxide scavenging activity of *O.scandens* PE Extract

S.no	Extract(μg/ml)	% of activity($\pm$SEM)*	
		PE concentrates	Ascorbic acid
1	125	32.32$\pm$0.012	48.54 $\pm$0.046
2	250	39.56$\pm$0.018	57.31 $\pm$0.034
3	500	45.23$\pm$0.024	68.24 $\pm$0.045
4	1000	52.67$\pm$0.048	76.34 $\pm$0.012
		IC_{50} = 822 μg/ml	IC_{50} = 135 μg/ml

* Every value was articulated as mean $\pm$ SEM for 3 experimentation

Table 2: Nitric oxide scavenging activity of *O.scandens* EA Extract

S.no	Extract(μg/ml)	% of activity($\pm$SEM)*	
		EA concentrates	Ascorbic acid
1	125	33.12 $\pm$0.032	48.54 $\pm$0.046
2	250	44.23 $\pm$0.024	57.31 $\pm$0.034
3	500	50.35 $\pm$0.031	68.24 $\pm$0.045
4	1000	64.79 $\pm$0.042	76.34 $\pm$0.024
		IC_{50} = 505 μg/ml	IC_{50} = 135 μg/ml

* Every value was articulated as mean$\pm$ SEM for 3 experimentation

Table 3: Nitric oxide scavenging activity of *O.scandens* methanol Extract

S.no	Extract (μg/ml)	% of activity($\pm$SEM)*	
		(Methanolic concentrates)	Ascorbic acid
1	125	39.14 $\pm$0.032	48.54 $\pm$0.046
2	250	50.22 $\pm$0.024	57.31 $\pm$0.034
3	500	62.34 $\pm$0.012	68.24 $\pm$0.045
4	1000	69.87 $\pm$0.017	76.34 $\pm$0.024
		IC_{50}= 253 mg/ml	IC_{50}= 135 mg/ml

*Every value was articulated as mean $\pm$ SEM for 3 experimentation

Table 4: Total antioxidant activity of *O.scandens* PE Extract

S.no	Extract(μg/ml)	% inhibition($\pm$SEM)*	
		PE concentrates	Ascorbate
1	50	16.65 $\pm$0.010	50.76 $\pm$0.024
2	100	23.46 $\pm$0.018	61.68 $\pm$0.035
3	200	30.12 $\pm$0.022	74.64 $\pm$0.048
4	300	35.67 $\pm$0.018	98.12 $\pm$0.021
		IC_{50} = 790μg/ml	IC_{50} = 57 μg/ml

* Every value was articulated as mean$\pm$ SEM for 3 experimentation

Table 5: Total antioxidant activity of *O.scandens* EA Extract

S.no	Extract(μg/ml)	% inhibition($\pm$SEM)*	
		(EA concentrates)	(EA concentrates)
1	50	11.76 $\pm$0.034	50.76 $\pm$0.024
2	100	20.44 $\pm$0.022	61.68 $\pm$0.035
3	200	28.54 $\pm$0.065	74.64 $\pm$0.048
4	300	40.66 $\pm$0.024	98.12 $\pm$0.021
		IC_{50} = 495 μg/ml	IC_{50} = 57 μg/ml

* Every value was articulated as mean$\pm$ SEM for 3 experimentation

Table 6: Total antioxidant activity of *O.scandens* methanol Extract

S.no	Extract(μg/ml)	% inhibition($\pm$SEM)*	
		Methanolic concentrates	Ascorbate
1	50	36.12 $\pm$0.020	50.76 $\pm$0.024
2	100	42.65 $\pm$0.028	61.68 $\pm$0.035
3	200	49.56 $\pm$0.027	74.64 $\pm$0.048
4	300	65.42$\pm$0.012	98.12 $\pm$0.021
		IC_{50} = 205μg/ml	IC_{50} = 57 μg/ml

* Every value was articulated as mean$\pm$ SEM for 3 experimentation

Table 7: Hydroxyl radical scavenging activity of *O.scandens* PE concentrates

S.no	Extract(μg/ml)	% inhibition ($\pm$SEM)*	
		PE concentrates	PE concentrates
1	125	15.76 $\pm$ 0.012	70.34 $\pm$ 0.038
2	250	24.45 $\pm$ 0.043	85.54 $\pm$ 0.015
3	500	33.89 $\pm$ 0.047	90.23 $\pm$ 0.024
4	1000	50.37 $\pm$ 0.032	96.50 $\pm$ 0.016
		IC_{50} = 990 μg/ml	IC_{50} = 65 μg/ml

* Every value was articulated as mean $\pm$ SEM for 3 experimentation

Table 8: Hydroxyl radical scavenging activity of *O.scandens* of EA concentrates

S.no	Extract(μg/ml)	% inhibition ($\pm$SEM)*	
		EA concentrates	Ascorbic acid
1	125	32.78 $\pm$ 0.011	70.34 $\pm$ 0.038
2	250	40.68 $\pm$ 0.034	85.54 $\pm$ 0.015
3	500	49.46 $\pm$ 0.027	90.23 $\pm$ 0.024
4	1000	59.54 $\pm$ 0.032	96.50 $\pm$ 0.016
		IC_{50} = 510 μg/ml	IC_{50} = 65μg/ml

* Every value was articulated asmean $\pm$ SEM for 3 experimentation

Table 9: Hydroxyl radical scavenging activity of *O.scandens* Methanolic concentrates

S.no	Extract (μg/ml)	% inhibition($\pm$SEM)*	
		Methanol concentrates	Ascorbic acid
1	125	34.22 $\pm$ 0.022	70.34 $\pm$ 0.038
2	250	48.81 $\pm$ 0.034	85.54 $\pm$ 0.015
3	500	59.62 $\pm$ 0.030	90.23 $\pm$ 0.024
4	1000	65.34 $\pm$ 0.022	96.50 $\pm$ 0.016
		IC_{50} = 265 μg/ml	IC_{50} = 65 μg/ml

* Every value was articulated as mean $\pm$ SEM for 3 experimentation

ascorbic acid were recorded 205μg/ml & 57μg/ml correspondingly.

IC_{50} values & total antioxidant potential revealed that methanol concentrates of *O.scandens* are better activity in scavenging total antioxidant potential when compared ethyl acetate & PE extracts.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of PE concentrates *O.scandens* & ascorbic acid appeared in Table 7. The more Hydroxyl radical scavenging activity of PE concentrates & Ascorbic acid1000 μg/ml were recorded, 50.37% & 96.50 %. The IC_{50} of PE concentrates of *O.scandens* & ascorbic acid were found as 990μg/ml & 65μg/ml correspondingly.

Hydroxyl radical scavenging activity of EA concentrates of *O.scandens* & ascorbic acid were presented inTable 8. The more Hydroxyl radical scavenging activity of EA concentrates & ascorbic acid 1000 μg/ml was recorded at 59.54% & 96.50 %. The IC_{50} value of ethyl acetate concentrates of *O.scandens*& ascorbic acid was found 510μg/ml & 65μg/ml correspondingly.

Hydroxyl radical scavenging activity of methanolic concentrates of *O.scandens*& ascorbic acid were presented inTable 9. The more Hydroxyl radical scavenging activity of methanolic concentrates & ascorbic acid 1000 μg/ml were recorded, 65.34% & 96.50 %. The IC_{50} value of methanol concentrates of *O.scandens* & Ascorbic acid was recorded as 265μg/ml & 65μg/ml correspondingly.

IC_{50} values & Hydroxyl radical scavenging activity revealed that methanol concentrates on *O.scandens* is a huge activity in Hydroxyl radical scavenging activity when compared ethyl acetate & petroleum ether concentrates. But when compared to the all the three concentrates, the methanol concentrates of the *O.scandens* showed a better result.

Assessment of antioxidant activity, so many *in vitro* methods have been used a variety of concentrates of *O.scandens*. The results of antioxidant activity by NO radical activity, total antioxidant activity & OH radical potential were expressed in terms of % inhibition of generated free radicals, respectively, with respect to various concentrations. (Sivakrishnan *et al.*, 2012) Hydroxyl radical is the most ROS and causes severe injury to the adjacent biomolecule. Hydroxyl radical scavenging activity was estimated by generating hydroxyl radicals using ascorbic acid–iron EDTA. The hydroxyl radicals were produced by the oxidation reaction with the DMSO to give in HCHO, which provides a suitable method to identify hydroxyl radicals by treatment with Nash reagent (Pavithra and Vadivukkarasi, 2015). The methanolic concentrates of *O.scandens* exhibited better hydroxyl radical scavenging activity compared to other extracts. The scavenging of the hydroxyl radicals, possibly due to the presence of hydrogen donating ability phenolic compounds in the ethyl acetate extracts.

NO is produced from L-arginine through vascular endothelial cells, phagocytes, and certain cells of the brain. NO is a free radical since its unpaired electron and displays significant reactivity with confident types of proteins and other free radicals. The toxicity of Nitric oxide becomes a side effect when it reacts with superoxide radical, forming a highly reactive peroxynitrite anion ($ONOO-$) (Nagmoti *et al.*, 2012). NO produced from sodium nitroprusside reacts with oxygen to form nitrite. The nitrite ions diazotize with sulphanilamide acid and couple with naphthyl ethylenediamine, producing pink colored, which absorbs at 546 nm (Balakrishnan *et al.*, 2009). Among the three different plant concentrates tested, interestingly, in the NO radical activity of the methanol extract of *O.scandens* exhibited more NO radical potential comparable with that of ascorbic acid.

CONCLUSIONS

The current trends, antioxidative activity of the herbs having more interest due to their possible use as natural additives to substitute synthetic ones.

Among the three various extract methanol extracts of *O.scandens* exhibited higher potency of antioxidant activity. These results indicate that methanol extract of *O.scandens* might serve as a natural antioxidant, which may be useful to prevent free radical-induced diseases.

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