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Study of in-vitro antioxidant activity of various extracts of aerial parts of *Olax scandens*

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ABSTRACT

Olax scandens Roxb. (family *Olacaceae*) Available in throughout tropical India. The current study, aerial parts of different concentrates of *Olax scandens* was evaluated for its *in-vitro* antioxidant potential by Diphenylpicrylhydrazyl activity, superoxide scavenging activity, iron chelating activity taking ascorbate, quercetin & Ethylenediamine tetraacetate as the standard correspondingly. An IC₅₀ value was originated that methanolic concentrates of *Olax scandens* more efficient in Diphenylpicrylhydrazyl radical, superoxide radical activity, Iron chelating capacity compared EA & PE concentrates. The methanolic concentrates of *Olax scandens* & ascorbate exhibited antioxidant potential possessing IC₅₀ 226 μg/ml & 66 μg/ml (DPPH). 185 μg/ml & 60 μg/ml (Superoxide), 287 μg/ml & 65 μg/ml (iron-chelating Activity) respectively. In vitro antioxidant studies obviously show methanolic concentrates of *Olax scandens* have better antioxidant activity. These results indicate that aerial parts of methanolic concentrates *Olax scandens* could serve as a natural antioxidant, which may be useful in preventing free radical-induced diseases.



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INTRODUCTION

A free radical is any species capable of freelance existence that contains a lot of odd unpaired electrons. Electron unpaired being single that is only in an orbital (Halliwell, 1991). The electron unpaired gives convinced characteristic property to the free radical, like as paramagnetism. The chemical reactivity of free radicals is usually more. They may

be + charged, - charged, or neutral charge electrically (Slater, 1984). These RO having a major task in the pathogenesis of many oxidative stress associated disorders like carcinogenesis, cardiovascular diseases, arthritis, diabetics, & neurological disorders (Halliwell and Gutteridge, 1990). The generally used synthetic antioxidants at present are BHA, BHT, PG, & TBHQ. However, these drugs are assumed accountable for liver harm and performing as carcinogens in lab animals (Anagnostopoulou et al., 2006). 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 plants fruits and leaves has been used for therapeutic & food purpose. *O. Scandens* leaves were used as constipation. *Olax scandens* (Roxb.), is gen-

erally known as Badrul in Odiya, used for cooking & different therapeutic purposes (Sinha and Lakra, 2005). *O. lax scandens* bark decoction is used treatment fever & cough. (Duraipandiyam et al., 2006). *O. scandens* leaves were used for mouth ulcers. (Kumar et al., 2015). *O. scandens* boiled leaves were applied in the head for the treatment of headache. (Kumar et al., 2010). Still, no literature are available on the antioxidant potency of *O. lax scandens*. Thus, the present study to assess the antioxidant activities of *O. lax scandens*.

MATERIALS AND METHODS

Gathering & Identification of Plant

The aerial parts of *O. lax scandens* (family *O. lacaceae*) 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. lax 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°C) through hot constant percolation method in Soxhlet equipment (J B Harbrone, 1984) for twenty-four hours. At that moment, the marc was subjected to EA (76-78°C) for 24 hrs & then marc was subjected to methanol for 24 hrs. The concentrates were concentrated through the rotational evaporator and subjected to solidify drying in a lyophilizer till dry powder was acquired. (Vasagam et al., 2010).

Assessment of Antioxidant potential through invitro methods

The variety of concentrates of *O. lax scandens* were used assessment of antioxidant activity by Mentor et al. (2001) method was adopted for Diphenyl picrylhydrazyl radical assay & Winterbourn et al. (1975) method described for Superoxide radical (O_2^-) assay and Benzie and Strain (1996) method was adopted to determine the Iron chelating activity.

RESULTS AND DISCUSSION

DPPH scavenging activity

The DPPH activity of PE concentrates of *O. lax scandens* appeared Table 1. The PE concentrates of *O. lax Scandens* exhibit, a more DPPH activity of 45.08% at 800 $\mu\text{g/ml}$ & ascorbate was recorded 72.82% at 800 $\mu\text{g/ml}$. The IC_{50} of the PE concentrates of *O. lax scandens* & ascorbic acid were recorded 90 $\mu\text{g/ml}$ &

66 $\mu\text{g/ml}$ correspondingly.

DPPH activity of EA concentrates of *O. lax scandens* summarized in Table 2. The EA concentrates of *O. lax Scandens* exhibit more DPPH scavenging potential of 59.12% at 800 $\mu\text{g/ml}$ & ascorbate was recorded 72.82% at 800 $\mu\text{g/ml}$. The IC_{50} of the EA concentrates of *O. lax scandens* & ascorbic acid were recorded 504 $\mu\text{g/ml}$ & 66 $\mu\text{g/ml}$ correspondingly.

DPPH potential of methanolic concentrates of *O. lax scandens* appeared in Table 3. The methanolic concentrates of *O. lax scandens* having more DPPH scavenging potential of 66.24% at 800 $\mu\text{g/ml}$ & ascorbate was recorded 72.82% at 800 $\mu\text{g/ml}$. The IC_{50} of the methanolic concentrates of *O. lax scandens* & ascorbic acid were recorded 226 $\mu\text{g/ml}$ & 66 $\mu\text{g/ml}$ correspondingly.

The methanolic concentrates of *O. lax scandens* was recorded to more activity than PE & EA concentrates. The IC_{50} of the methanolic concentrates of *O. lax scandens* & ascorbic acid were found to be 226 $\mu\text{g/ml}$ & 66 $\mu\text{g/ml}$ correspondingly.

Superoxide activity

Superoxide radical potential of PE concentrates of *O. lax scandens* appeared Table 4. The more Superoxide radical potential of PE concentrates & standard at 1000 $\mu\text{g/ml}$ was recorded at 48.65% & 98.01%. IC_{50} of PE concentrates & standard was recorded as 1070 $\mu\text{g/ml}$ & 60 $\mu\text{g/ml}$ correspondingly.

Superoxide radical potential of EA concentrates of *O. lax scandens* appeared in Table 5. The more SO scavenging potential of EA concentrates & standard 1000 $\mu\text{g/ml}$ was recorded 64.12% & 99.12% correspondingly. EA concentrates & Quercetin IC_{50} was recorded as 495 $\mu\text{g/ml}$ & 60 $\mu\text{g/ml}$ correspondingly.

Superoxide radical scavenging potential of methanolic concentrates of *O. lax scandens* appeared in Table 6. Superoxide radical scavenging potential was more in methanolic concentrates & Quercetin (standard) at 1000 $\mu\text{g/ml}$ was recorded 70.28% & 99.12%. Methanolic concentrates & standard IC_{50} was recorded as 185 $\mu\text{g/ml}$ & 60 $\mu\text{g/ml}$ correspondingly.

IC_{50} values & Superoxide radical potential revealed that methanol concentrates of *O. lax scandens* is better activity in scavenging superoxide radical when compared EA & PE extracts.

Iron chelating potential

The iron complex potential of PE concentrates *O. lax scandens* & Ethylenediamine tetraacetate were appeared in Table 7. The more iron-binding potential of PE concentrates & Ethylenediamine tetraac-

Table 1: DPPH radical activity of *Olax scandens* PE extract

| S.no | Extract ($\mu\text{g/ml}$) | % of activity($\pm\text{SEM}$)* | |
|------|------------------------------|-----------------------------------|----------------------------|
| | | PE concentrates | Ascorbate |
| 1 | 100 | 10.29 \pm 0.023 | 54.19 \pm 0.024 |
| 2 | 200 | 19.74 \pm 0.045 | 59.24 \pm 0.032 |
| 3 | 400 | 34.56 \pm 0.063 | 65.32 \pm 0.054 |
| 4 | 800 | 45.08 \pm 0.043 | 72.82 \pm 0.062 |
| | | IC50 = 990 $\mu\text{g/ml}$ | IC50 = 66 $\mu\text{g/ml}$ |

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

Table 2: DPPH radical activity of *Olax scandens* EA extract

| S.no | Extract ($\mu\text{g/ml}$) | % of activity($\pm\text{SEM}$)* | |
|------|------------------------------|-----------------------------------|----------------------------|
| | | (EA concentrates) | (Ascorbate) |
| 1 | 100 | 28.33 \pm 0.024 | 54.19 \pm 0.024 |
| 2 | 200 | 39.75 \pm 0.034 | 59.24 \pm 0.032 |
| 3 | 400 | 47.12 \pm 0.023 | 65.32 \pm 0.054 |
| 4 | 800 | 59.12 \pm 0.015 | 72.82 \pm 0.062 |
| | | IC50 = 504 $\mu\text{g/ml}$ | IC50 = 66 $\mu\text{g/ml}$ |

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

Table 3: DPPH radical activity of *Olax scandens* methanolic extract

| S.no | Extract ($\mu\text{g/ml}$) | % of activity($\pm\text{SEM}$)* | |
|------|------------------------------|-----------------------------------|----------------------------|
| | | (Methanolic concentrates) | Ascorbate |
| 1 | 100 | 40.23 \pm 0.022 | 54.19 \pm 0.024 |
| 2 | 200 | 49.34 \pm 0.043 | 59.24 \pm 0.032 |
| 3 | 400 | 57.55 \pm 0.048 | 65.32 \pm 0.054 |
| 4 | 800 | 66.24 \pm 0.023 | 72.82 \pm 0.062 |
| | | IC50 = 226 $\mu\text{g/ml}$ | IC50 = 66 $\mu\text{g/ml}$ |

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

Table 4: Activity of PE concentrates of *Olax scandens* on Superoxide radical method

| S.no | Extract ($\mu\text{g/ml}$) | % inhibition ($\pm\text{SEM}$)* | |
|------|------------------------------|-----------------------------------|----------------------------|
| | | (PE concentrates) | (Quercetin) |
| 1 | 125 | 19.15 \pm 0.012 | 72.76 \pm 0.012 |
| 2 | 250 | 27.09 \pm 0.014 | 90.29 \pm 0.014 |
| 3 | 500 | 35.33 \pm 0.022 | 96.89 \pm 0.010 |
| 4 | 1000 | 48.65 \pm 0.026 | 99.12 \pm 0.018 |
| | | IC50 = 1070 $\mu\text{g/ml}$ | IC50 = 60 $\mu\text{g/ml}$ |

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

Table 5: Activity of EA concentrates of *Olox scandens* on Superoxide radical method

| S.no | Extract ($\mu\text{g/ml}$) | % of inhibition ($\pm\text{SEM}$)* | |
|------|------------------------------|---|--|
| | | (Ethyl acetate concentrates) | (Quercetin) |
| 1 | 125 | 20.12 \pm 0.014 | 72.76 \pm 0.012 |
| 2 | 250 | 44.49 \pm 0.025 | 90.29 \pm 0.014 |
| 3 | 500 | 50.23 \pm 0.033 | 96.89 \pm 0.010 |
| 4 | 1000 | 64.12 \pm 0.024 | 99.12 \pm 0.018 |
| | | IC ₅₀ = 495 $\mu\text{g/ml}$ | IC ₅₀ = 60 $\mu\text{g/ml}$ |

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

Table 6: Activity of Methanolic concentrates *Olox scandens* on Superoxide radical method

| S.no | Extract ($\mu\text{g/ml}$) | % inhibition ($\pm\text{SEM}$)* | |
|------|------------------------------|---|--|
| | | Methanolic extract | Quercetin |
| 1 | 125 | 35.18 \pm 0.071 | 72.76 \pm 0.012 |
| 2 | 250 | 54.23 \pm 0.024 | 90.29 \pm 0.014 |
| 3 | 500 | 62.34 \pm 0.044 | 96.89 \pm 0.010 |
| 4 | 1000 | 70.28 \pm 0.017 | 99.12 \pm 0.018 |
| | | IC ₅₀ = 185 $\mu\text{g/ml}$ | IC ₅₀ = 60 $\mu\text{g/ml}$ |

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

Table 7: Iron-binding potential of *O.scandens* PE concentrates

| S.no | Extract ($\mu\text{g/ml}$) | % inhibition ($\pm\text{SEM}$)* | |
|------|------------------------------|---|--|
| | | PE concentrates | Ethylenediamine tetraacetate |
| 1 | 125 | 26.90 \pm 0.012 | 57.52 \pm 0.014 |
| 2 | 250 | 34.56 \pm 0.032 | 64.76 \pm 0.022 |
| 3 | 500 | 42.65 \pm 0.010 | 82.12 \pm 0.045 |
| 4 | 1000 | 51.76 \pm 0.016 | 96.34 \pm 0.034 |
| | | IC ₅₀ = 980 $\mu\text{g/ml}$ | IC ₅₀ = 65 $\mu\text{g/ml}$ |

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

Table 8: Iron-binding potential of *O.scandens* of EA concentrates

| S.no | Extract ($\mu\text{g/ml}$) | % inhibition ($\pm\text{SEM}$)* | |
|------|------------------------------|---|--|
| | | EA concentrates | Ethylenediamine tetraacetate |
| 1 | 125 | 32.30 \pm 0.024 | 57.52 \pm 0.014 |
| 2 | 250 | 43.38 \pm 0.013 | 64.76 \pm 0.022 |
| 3 | 500 | 50.28 \pm 0.023 | 82.12 \pm 0.045 |
| 4 | 1000 | 64.70 \pm 0.026 | 96.34 \pm 0.034 |
| | | IC ₅₀ = 495 $\mu\text{g/ml}$ | IC ₅₀ = 65 $\mu\text{g/ml}$ |

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

Table 9: Iron-binding potential of *O.scandens* Methanolic concentrates

| S.no | Extract ($\mu\text{g/ml}$) | % inhibition($\pm\text{SEM}$)* | |
|------|------------------------------|---|--|
| | | Methanol concentrates | EthyleneDiamine tetra acetate |
| 1 | 125 | 36.22 \pm 0.010 | 57.52 \pm 0.014 |
| 2 | 250 | 47.42 \pm 0.014 | 64.76 \pm 0.022 |
| 3 | 500 | 60.56 \pm 0.016 | 82.12 \pm 0.045 |
| 4 | 1000 | 68.89 \pm 0.021 | 96.34 \pm 0.034 |
| | | IC ₅₀ = 287 $\mu\text{g/ml}$ | IC ₅₀ = 65 $\mu\text{g/ml}$ |

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

etate 1000 $\mu\text{g/ml}$ were recorded, 51.76% & 96.34%. The IC₅₀ of PE concentrates of *O.scandens* & Ethylenediamine tetraacetate were found as 980 $\mu\text{g/ml}$ & 65 $\mu\text{g/ml}$ correspondingly.

The iron complex potential of EA concentrates of *O lax scandens* & Ethylenediamine tetraacetate were presented in Table 8. The more iron-binding capacity of EA concentrates & Ethylenediamine tetraacetate 1000 $\mu\text{g/ml}$ was recorded at 64.70% & 96.34%. The IC₅₀ value of ethyl acetate concentrates of *O lax scandens* & Ethylenediamine tetraacetate were found 495 $\mu\text{g/ml}$ & 65 $\mu\text{g/ml}$ correspondingly.

The iron complex potential of methanolic concentrates of *O lax scandens* & Ethylenediamine tetraacetate were presented in Table 9. The more iron-binding potential of methanolic concentrates & Ethylenediamine tetraacetate 1000 $\mu\text{g/ml}$ were recorded, 68.89% & 96.34%. The IC₅₀ value of methanol concentrates of *O lax scandens* & Ethylenediamine tetraacetate was recorded as 287 $\mu\text{g/ml}$ & 65 $\mu\text{g/ml}$ correspondingly.

IC₅₀ values & iron binding potential revealed that methanol concentrates of *O lax scandens* is huge activity in iron chelating potential when compared ethyl acetate & petroleum ether concentrates. But when compared to the all the three concentrates, the methanol concentrates of the *O lax scandens* showed the better result.

Assessment of antioxidant activity, so many *in vitro* methods have been used a variety of concentrates of *O lax scandens*. Diphenylpicrylhydrazyl is a stable N₂-centered free radical generally utilized for testing the antioxidant potential of herbal concentrates. When the stable Diphenylpicrylhydrazyl radical accepts an electron from the antioxidant compound, the violet colour of the Diphenylpicrylhydrazyl as reduced to yellow colored diphenylpicrylhydrazine radical which was measured colorimetrically. Substances which are able to perform this reaction can be considered as antioxidants & therefore radical scavengers (Dehpour *et al.*, 2009). The results of antioxidant activity by Diphenylpicrylhy-

drazyl radical activity, superoxide radical activity & iron-chelating potential were expressed in terms of % inhibition of generated free radicals respectively with respect to various concentrations. Among the three different plant concentrates tested, interestingly, in the DPPH radical activity of the methanolic of *O lax Scandens* exhibited more Diphenylpicrylhydrazyl radical potential comparable with that of ascorbic acid.

Superoxides could be produced in huge amounts by various biological processes. It is known to be more injurious to cellular components as an originator of the most ROS, contributing to tissue damage & many disorders (G B J M Halliwell, 1999). The methanolic concentrates of *O lax scandens* exhibited higher ability in scavenging superoxide anion radical when compared to the strand quercetin.

The iron-chelating potential of all the concentrates was measured by Fe-ferrozine complex formation. Ferrozine-Fe complex is producing red-colored, which absorbs at 562nm (Yamaguchi *et al.*, 2000). It was revealed that Ethylenediamine tetraacetate, which forms σ bond with iron, are efficient as secondary antioxidants, for the basis that they decrease the redox potential, thereby stabilizing the oxidized form of the iron ion (Duh *et al.*, 1999). Iron chelating potential of methanolic concentrates of *O lax Scandens* exhibited higher ability in scavenging compared to standard Ethylenediamine tetraacetate.

CONCLUSION

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 concentrates methanolic concentrates of *O lax scandens* exhibited higher potency of antioxidant activity. These results indicate that aerial parts of methanolic concentrates *O lax Scandens* could serve as a natural antioxidant, which may be useful in preventing free radical-induced diseases.

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