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Estimation of total Phenolic and flavonoids content and Studies on antioxidant activity of different extracts of *Olax scandens by FRAP assay*

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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 FRAP assay taking ascorbic acid as the standard and estimation of total phenolic content and flavonoids content. The IC_{50} value was originated that methanolic concentrates of *Olax scandens* are more efficient in antioxidant activity by FRAP methods compared EA & PE concentrates. The methanolic concentrates of *Olax scandens* & ascorbate exhibited antioxidant potential possessing IC_{50} 207μg/ml & 50μg/ml by Ferric reducing ability Power assay. The methanolic and EA concentrates of *Olax scandens* showed the total phenolic content (14.426 $\pm$ 0.032, 4.128 $\pm$ 0.025) respectively, *and flavonoids* content (11.526 $\pm$ 0.054, 3.682 $\pm$ 0.042) respectively. Invitro antioxidant studies show methanolic concentrates of *Olax scandens* have better antioxidant activity as well as a higher content of total phenolic and flavonoids content. 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

Free radicals and reactive oxygen and nitrogen species (ROS, RNS) may trigger the development of a variety of malignant diseases and are considered as a mutagenic compound. One puff Cigarette smoke contains approximately $10^{14-16.}$ Free radicals are causing lung cancer, the prominent malignant disease worldwide (Church *et al.*, 1985). Iron plays a vital role in the normal functioning of the body, but an excess amount leads to cell injury. These reduced metals may form reactive hydroxyl radicals and thereby resulting in oxidative stress during Fenton reaction (Hippeli and Elstner, 1999). There is substantial evidence of the role of oxidative stress in the majority of degenerative diseases. Flavonoids are major secondary metabolites responsible for the intense antioxidant activity and were correlated with the number and position of phenolic hydroxyl groups located in their molecule. Medicinal herbs are commonly used traditionally than that of allopathic drugs. The new investigation of herbal products with free radical scavenging activity and a minimal number of adverse effects is an active domain of research. Naturals antioxidants mainly originated from herbal in the form of phenolic compounds (flavonoids, phenolic acid and alcohols, stilbenes,

tocopherols,) ascorbate and carotenoids. The practice of natural antioxidants present in food and other biological materials has concerned extensive attention due to their presumed safety, dietary and beneficial value (Ajila *et al.*, 2007).

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 have been used for therapeutic & food purpose. *O. Scandens* leaves were used as constipation. *Olax scandens* (Roxb.), is generally known as Badrul in Odiya, used for cooking & different therapeutic purposes (Rekha and Valeria, 2005). *Olax scandens* bark decoction is used in treatment fever & cough (Duraipandiyan *et al.*, 2006). *O. scandens*leaves were used for mouth ulcer (Owk *et al.*, 2015). *O.scandens* boiled leaves were applied on head for the treatment of headache (Kumar *et al.*, 2010b). Still, no literature is available on the antioxidant potency and estimation of phenolic and flavonoids content of *O.scandens*. Thus, the present study to assess antioxidant activities and evaluation of phenolic and flavonoids content of *Olax scandens.*

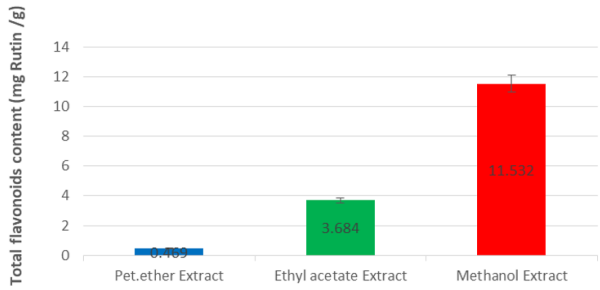

Figure 1: Total Flavonoids content of various concentratess of aerial parts of Olax scandens

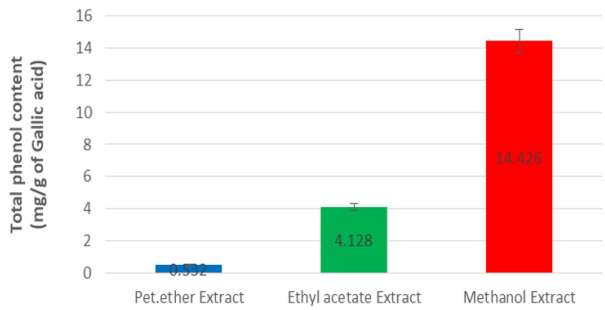

Figure 2: Total Phenolic content of various concentratess of aerial parts of Olax scandens

Methodology

Gathering and Identification of Plant

The aerial parts of *O.scandens* (family *Olacaceae*) were gathered from Medak, Telangana state, India. Plant identification was made from Botanical investigation of India, Telangana regional centre, Hyderabad,(BSI/DRC/2019-20/Tech/174). The *O.scandens* were desiccated under shadowy, segregate, crushed through a grinder (Borse *et al.*, 2012).

Preparation of Concentrates

The pulverized materials were packed in a muslin cloth and concentrated with pet.ether, ethyl acetate and methanol as solvents respectively according to the increasing order of polarity (Shajiselvin and KottaiMuthu, 2010) through hot constant percolation method in Soxhlet equipment (Ahirrao *et al.*, 2008) for twenty-four hours. The concentrates were concentrated through the rotational evaporator and subjected to solidify drying in a lyophilizer till dry powder was acquired (Kumar *et al.*, 2010a; Alagumanivasagam.G *et al.*, 2012).

Assessment of Antioxidant potential through invitro methods

The variety of concentrates of *O.scandens* were used assessment of antioxidant activity by (Benzie and Strain, 1996) was adopted for the FRAP assay, and (Cameron *et al.*, 1943) method described for total flavonoids content and determination of whole phenolic compound was estimated by the methods of (Malik and Singh, 1980).

RESULTS AND DISCUSSION

FRAP assay

The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe3+-TPTZ) complex and produces a coloured ferrous tripyridyltriazine (Fe2+-TPTZ). Generally, the reducing properties are linked with the presence of compounds which exert their action by breaking free radical chain by donating a hydrogen atom (Duh *et al.*, 1999). The ferric reducing ability of PE concentrates *Olax scandens* and ascorbate have appeared in Table 1. Reducing ability were expressed in terms of % inhibition of generated free radicals, respectively, with respect to various concentrations. The more Reducing ability of PE concentrates, and ascorbate 800 μg/ml was recorded at 51.67% and 98.07%. The IC_{50} of PE concentrates of *Olax scandens* and ascorbate were found as 965μg/ml and 50μg/ml correspondingly.

Ferric reducing ability of EA concentrates of *Olax scandens* and ascorbate were presented in Table 2. The Ferric reducing ability of EA concentrates and ascorbate 800 μg/ml was recorded at 69.02% and 98.07%. The IC_{50} value of ethyl acetate concentrates of *Olax scandens* and ascorbate was found 556μg/ml and 50μg/ml correspondingly.

Table 1: Reducing ability of PE concentrates of Olax scandens (Roxb.) on FRAP assay

S.No	Concentration (μg/ml)	% of activity($\pm$SEM)* (PE concentrates)	(Ascorbate)
1	100	22.43 $\pm$ 0.022	72.04 $\pm$ 0.014
2	200	26.78 $\pm$ 0.035	82.05 $\pm$ 0.034
3	400	35.43 $\pm$ 0.012	86.04 $\pm$ 0.026
4	800	51.67 $\pm$ 0.047	98.07 $\pm$ 0.041
		IC50 = 965 μg/ml	IC50 = 50 μg/ml

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

Table 2: Reducing ability of EA concentrates of Olax scandens (Roxb.) on FRAP assay

S.No	Concentration (μg/ml)	% of activity($\pm$SEM)* (EA concentrates)	(Ascorbate)
1	100	23.34 $\pm$ 0.032	72.04 $\pm$ 0.014
2	200	36.43 $\pm$ 0.024	82.05 $\pm$ 0.034
3	400	51.98 $\pm$ 0.045	86.04 $\pm$ 0.026
4	800	69.02 $\pm$ 0.076	98.07 $\pm$ 0.041
		IC50 = 556 μg/ml	IC50 = 50 μg/ml

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

Table 3: Reducing ability of Methanolic concentrates of Olax scandens (Roxb.) on FRAP assay

S.No	Concentration (μg/ml)	% of activity($\pm$SEM)* (Methanolic concentrates)	(Ascorbate)
1	100	37.14 $\pm$ 0.012	72.04 $\pm$ 0.014
2	200	53.12 $\pm$ 0.018	82.05 $\pm$ 0.034
3	400	63.36 $\pm$ 0.022	86.04 $\pm$ 0.026
4	800	70.42 $\pm$ 0.026	98.07 $\pm$ 0.041
		IC50 = 207 μg/ml	IC50 = 50 μg/ml

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

Table 4: The total flavonoids content of various concentrates of aerial parts of Olax scandens

S.No	Concentratess	Total flavonoids content (mg Rutin /g) ($\pm$SEM)*
1	Petroleum ether concentrates of Olax scandens	0.469 $\pm$ 0.012
2	Ethyl acetate concentrates of Olax scandens	3.684 $\pm$ 0.042
3	Methanolic concentrates of of Olax scandens	11.532 $\pm$ 0.054

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

The ferric reducing ability of methanolic concentrates of *Olax scandens* and ascorbate were presented in Table 3. The more Ferric reducing ability of methanolic concentrates and ascorbate 800 μg/ml were recorded 70.42% and 98.07%. The IC_{50} value of methanol concentrates of *Olax scandens* and ascorbate was recorded as 207μg/ml and 50μg/ml correspondingly.

IC_{50} values and Ferric reducing ability revealed that methanol concentrates of *Olax scandens* are a huge activity in Ferric reducing ability when compared ethyl acetate and petroleum ether concentrates. But when compared to the all the three concentrates, the methanol concentrates of the *Olax scandens* showed better results.

Total Flavonoids

The antioxidant activity of phenolics and flavonoids is mainly due to their redox properties which make

Table 5: The total Phenolic content of various concentrates of aerial parts of Olax scandens

S.No	Concentratess	Total phenol content (mg/g of Gallic acid) (±SEM)*
1	Petroleum ether concentrates of Olax scandens	0.532 ± 0.012
2	Ethyl acetate concentrates of Olax scandens	4.128 ± 0.025
3	Methanolic concentrates of Olax scandens	14.426 ± 0.032

* Every value was articulated asmean ± SEM for 3 experimentation

them act as reducing agents, hydrogen donors, singlet oxygen quenchers and as well as potential metal chelators (Pietta, 2000). The total amount of flavonoid content of various concentrates of the aerial plant of *Olax scandens* was present in Table 4 and Figure 1 .

Based on the result the methanolic concentrates of *Olax scandens* was found higher content of flavonoids contents (11.53 ± 0.054) than that of petroleum ether and ethyl acetate concentrates of *Olax scandens.*

Total phenol

Phenolic compounds are famous as powerful chain-breaking antioxidants. The phenolic compounds might contribute directly to antioxidative action. The total amount of phenolic content of various concentrates of aerial parts of *Olax scandens* was present in Table 5 and Figure 2 .

Based on the result, the methanolic concentrates of *Olax scandens* was found higher content of phenolic components (14.426 ± 0.032) than that of petroleum ether and ethyl acetate concentrates of *Olax scandens.* Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups (Hatano *et al.*, 1989).

CONCLUSIONS

Based on the above results, among the three different extracts, methanolic concentrates of *Olax scandens* exhibited higher potency of antioxidant activity by FRAP method due to the presence of total phenolic and flavonoids compounds. Poly phenolic and flavonoids compounds are known to act as an antioxidant. This result indicates 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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Conflict of interest statement

There are no conflicts of interest.

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