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EVALUATION OF IN-VITRO ANTIOXIDANT ACTIVITY OF *SPATHODEA CAMPANULATA* LEAVES

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ABSTRACT

Recently, natural plants have received much attention as sources of biological active substances including antioxidants. In the present study we investigated *In-vitro* antioxidant activity of hexane, ethyl acetate and ethanol extracts of *Spathodea campanulata*. *In-vitro* antioxidant activity was evaluated for extracts by using free radicals, Superoxide (Riboflavin photo reduction method) and DPPH. The selected plant extracts produced concentration dependent percentage inhibition of different free radicals and produced maximum activity at a concentration of 800µg and there after the percentage inhibition was raised gradually to its maximum level with higher concentrations. In the present study we found that the extracts of *Spathodea campanulata* showed good antioxidant activity. Among the three extracts, ethanol extract showed better activity than the other extracts on the tested super oxide free radical, ethyl acetate extract showed better activity than the other extracts on the tested DPPH free radical.

Key Words: *Spathodea campanulata*, leaves, Free radicals, *In-vitro* Antioxidant activity.

INTRODUCTION

Spathodea campanulata is commonly known as the Fountain Tree, African Tulip tree, Pichkari or Nandi Flame belongs to family Bignoniaceae. It is used in traditional herbal medicine for the treatment of ulcers, filaria, gonorrhea, diarrhea and fever (Sy *et al*., 2005). The literature survey revealed that *S. campanulata* contains different phytochemical compounds like Spathodol, Spathoside, Ajugol, Sitosterol, β-sitosterol-3-*O*-β-D-glucopyranoside, Oleanolic acid, Pomolic acid, Ursolic acid, Tomentosolic acid, Caffeic acid, *p*-hydroxybenzoic acid, Phenylethanol esters, Phenolic acids and Flavonoids (Ngouela *et al*., 1991; Rangasamy *et al*., 2008; El-Hela, 2001B; Ngouela *et al*., 1990; Amusan *et al*., 1996; Subramanian *et al*., 1973; Elusiyan *et al*., 2011; Mbosso *et al*., 2008; Ngouela *et al*., 1998) . Since *S. campanulata* have been reported to possess medicinal effects (Niyonzima *et al*., 1999; Makinde *et al*., 1988) the present study was carried out to evaluate the antioxidant activity of *S. campanulata* leaves extracts.

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MATERIALS AND METHODS

Preparation of extract from of Spathodea campanulata

The leaves of *S. campanulata* were collected from Andhra University campus, Visakhapatnam, Andhra Pradesh, India during the month of December 2011 and authenticated by Dr. P. Prayaga Murthy, taxonomist, Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh. Shade dried leaves of *S. campanulata* was powdered and separately extracted in a Soxhlet apparatus for 6 hrs successively with hexane, ethyl acetate, and ethanol were concentrated to dryness under vacuum.

Chemicals and Drugs

All chemicals and solvents were of the analytical grade obtained from S.D. Fine Chemical Pvt. Ltd.,

Mumbai, Sigma Chemical Company, U.S.A., Loba Chemic, Mumbai.

In-vitro anti oxidant activity

For the assessment of free radicals scavenging activity, the hexane, ethyl acetate and ethanol extracts were dissolved in water and 5% dimethyl sulphoxide (DMSO) respectively.

Superoxide radical Scavenging activity (McCord and Day, 1978)

Superoxide scavenging activity of the plant extract was determined by McCord and Fridovich method, 1969, which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium. 0.1 ml of different concentrations of plant extract and 0.1 ml of 6 µM ethylenediamine tetraacetic acid containing NaCN, 0.1 ml of 50 µM nitroblue tetrazolium, 0.05 ml of 2 µM riboflavin were transferred to a test tube, and final volume was made up to 3 ml using phosphate buffer. Then the assay tubes were uniformly illuminated with an incandescent light (40 Watts) for 15 minutes and thereafter the optical densities were measured at 560 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of superoxide production was evaluated by comparing the absorbance values of control and experimental tubes. The percentage inhibition was calculated from the above Formula-1.

DPPH radical Scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca (Nguelefack *et al*., 2011). In DPPH assay method is based on the reduction of alcoholic DPPH solution (dark blue in colour) in the presence of a hydrogen donating antioxidant converted to the non radical form of yellow colored diphenyl-picrylhydrazine. Lower the absorbance higher the free radical scavenging activity (Anita *et al.*, 2011) .An aliquot of 3 ml of 0.004% DPPH solution in ethanol and 0.1 ml of plant extract at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition was calculated from the above Formula-1.

Calculation of percentage inhibition

The percentage inhibition was calculated using the formula:

$$\text{Formula-1: Inhibitory ratio} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where, A_0 is the absorbance of control;
A_1 is the absorbance with addition of plant extract/ ascorbic acid.

Calculation of 50% inhibition concentration

The optical density value obtained with each concentration of the extract/ ascorbic acid was plotted taking concentration on X-axis and percentage inhibition on Y-axis. The graph was extrapolated to find the 50% inhibition concentration of extract/ ascorbic acid.

RESULTS

In-vitro Antioxidant activity Superoxide

In the present study, ethanol, ethyl acetate and hexane extracts of *S.campanulata* leaves were found to possess concentration dependent scavenging activity on superoxide generated by photo reduction of riboflavin and the results are given in Table 1 and Fig 1. The mean IC_{50} values for superoxide radical of, ethanol, ethyl acetate and hexane extracts of *S.campanulata* leaves were found to be 293µg, 373µg and 707µg respectively. The mean IC_{50} value of ascorbic acid was found to be 46.7µg. The results were given in Table 3 and Fig 3

DPPH

The ethanol, ethyl acetate and hexane extracts of *S.campanulata* leaves were found to possess concentration dependent scavenging activity on DPPH radicals and the results are given Table-2 and fig-2. The mean IC_{50} values for DPPH radical of ethanol, ethyl acetate and hexane extracts of *S.campanulata* leaves were found to be 270µg, 63.3µg, and 253µg respectively. The mean IC_{50} value of ascorbic acid was found to be 93.3µg. The results are given in Table 3 and Fig 3.

Table 1. Concentration dependent percent inhibition of Superoxide radical by different extracts of *S.campanulata* and Ascorbic acid in *In-vitro* studies

Name of the extract of *S.campanulata*	Percentage inhibition of Superoxide radical				
	Quantity of extracts/ ascorbic acid in micrograms (µg)				
	50	**100**	**200**	**400**	**800**
EtOH.ext.	11.7	22.6	36.7	61.4	84.1
EA.ext.	6.0	16.0	32.6	51.9	77.6
Hex.ext.	9.4	18	26.5	39.9	52.3
Ascorbic acid	73.5	82.6	90.1	93.4	96.8

Table 2. Concentration dependent percent inhibition of DPPH radical by Different extracts of *S.campanulata* and Ascorbic acid in *In-vitro* studies

Name of the extract of *S.campanulata*	Percentage inhibition of DPPH radical				
	Quantity of extracts/ ascorbic acid in micrograms (µg)				
	50	100	200	400	800
EtOH.ext.	16	29.5	44.8	59.5	73.9
EA.ext.	44	64.6	73.1	78.9	88
Hex.ext.	23.4	37.2	46.4	59.4	83.9
Ascorbic acid	84.6	91.5	93.9	95.1	96.6

Table 3. *In-vitro* 50% inhibition concentration (IC_{50}) of different extracts of *S.campanulata* on DPPH and Superoxide radicals.

Name of the extract of *S.campanulata*	IC_{50} value (µg)	
	Superoxide radical	DPPH radical
EtOH.ext.	293	270
EA.ext.	373	63.3
Hex.ext.	707	253
Ascorbic acid	46.7	93.3

Fig1. Concentration dependent percent inhibition of Superoxide radical by different extracts of *S.campanulata* and Ascorbic acid in *In-vitro* studies

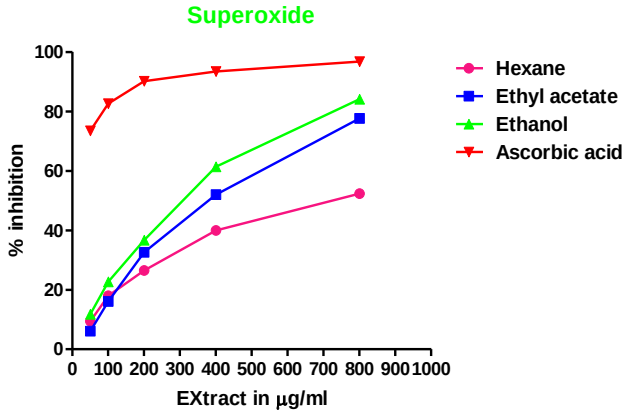


Fig 2. Concentration dependent percent inhibition of DPPH radical by different extracts of *S.campanulata* and Ascorbic acid in In-vitro studies

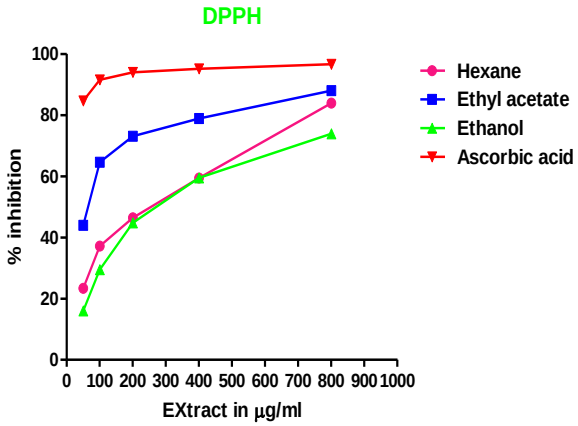
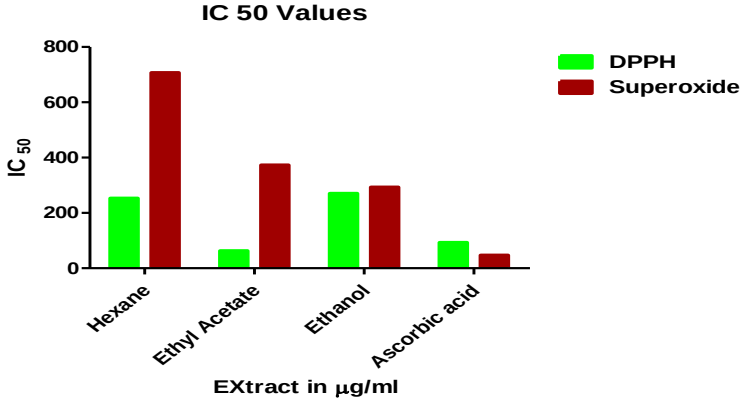


Fig 3. *In vitro* 50% inhibition concentration (IC_{50}) of different extracts of *S.campanulata* on DPPH and Superoxide radicals



DISCUSSION

Preliminary Phytochemical screening of the extracts of *S.campanulata* leaves showed the presence of sterols, triterpenes and flavonoids. Natural antioxidants such as plant-phenols, flavonoids and tannins possess potent antioxidant activity (Sanchez-Moreno, 2002; Maryam Z *et al.,* 2009). Sterols like β-sitosterol have been reported for antioxidant activity (Cai *et al.,* 2004). Terpenoids are also reported to possess antioxidant activity (Dragland *et al.,* 2003).The presence of phenolic compounds in this plant may contribute to its antioxidative properties and thus the usefulness of these plants in herbal medicament. Phenols have been found to be useful in the preparation of some antimicrobial and antioxidant compounds (Brewer, 2011; Jin Dai and Russell, 2010).

The result of scavenging activity assay in this study indicates that the plant was potently active. This suggests that the plant extract contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. The plant extracts were capable of scavenging super oxide and DPPH in a concentration dependent manner.

CONCLUSION

Among the three extracts of *S.campanulata*, the ethanolic extract showed better activity than other extracts on the tested super oxide free radical. The order of activity is the following manner: Ascorbic acid >ethanol extract > ethyl acetate extract> hexane extract.

Among the three extracts of *S.campanulata*, the ethyl acetate extract showed better activity than other extracts on the tested DPPH free radical. The order of activity is the following manner: Ascorbic acid > ethyl acetate extract > hexane extract > ethanol extract.

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