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In-vitro antioxidant and free radical scavenging potential of stem of *Calycopteris floribunda* lam.

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ABSTRACT

Calycopteris floribunda Lam, (Combretaceae) a scandent woody shrub. The present study was concentrated on the in vitro antioxidant methods like superoxide radical, hydroxyl radical, lipid peroxidation and DPPH radical methods. The chloroform and methanol extracts of *Calycopteris floribunda* stem were subjected for the above methods. The results of antioxidant activity revealed that, the chloroform extract has lower IC_{50} values than the methanolic extract of *Calycopteris floribunda.* The lower IC_{50} value indicates the higher free radical scavenging ability. So, the chloroform extract has better antioxidant activity than methanolic extract. The results were compared with the standard ascorbic acid. The plant contains phytosterols, triterpenoids, alkaloids, saponins, flavonoids, glycosides and tannins. These active constituents alone or in combination may be responsible for the observed antioxidant activity.

Key words: *Calycopteris floribunda*, Antioxidant activity, Ascorbic acid.

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INTRODUCTION

Calycopteris floribunda Lam, a scandent woody shrub with slender brown streaked diffuse branches occasionally twining around supports and storing water abundantly.Commonly known as kokkarai in Hindi, Minnarakoti in Tamil, Adivijama, in Telugu.The plant is also grown in central and southern parts of India [1], where the leaves are reported to have medicinal uses as a laxative and anthelmintic medicine, while the juice derived from the young twigs is used for the treatment of diarrhoea, dysentery and malaria [2]. Fruits are used for jaundice; flowers are reported as anti-tumour agent.Previous phytochemical studies have reported on the isolation of the flavonoids calycoptrin, quercetin and five biflavonoids [3-5] from the leaves and flowers. The present studies were performed to assess the in vitro antioxidant activity by using methods like superoxide radical, hydroxyl radical, lipid peroxidation and DPPH radical methods.

MATERIAL AND METHODS

All the chemicals were used analytical grade obtained from S.D. Fine Chemicals Pvt. Ltd., Mumbai, Sigma chemical company, U.S.A. and Loba chemicals, Mumbai.

Plant material

The stem of *Calycopteris floribunda* Lam. were collected from Bhubaneswar, Orissa state, India, and authenticated by Dr.M.Venkaiah, Associate professor, Dept of Botany, Andhra University. A voucher specimen (TSNDOP08/06) was deposited in the herbarium of our department.

Preparation of extract

Freshly collected plant material was shade dried at room temperature and coarsely powdered in Wiely mill. The powdered stem (1kg) was extracted successively with hexane, chloroform and methanol using soxhlet apparatus. The crude extract was evaporated to dryness in a rotary film evaporator (Roteava,Equitron, Medica instrument, India) and found to be 1.6gm,18.5gm and 28.5gm respectively. Preliminary phytochemical screening of chloroform extract of *C. floribunda* stem revealed the presence of sterols, flavonoids and alkaloids; methanol extract of *C. floribunda* stem revealed the presence of glycosides, saponins, tannins and carbohydrates.

The constituents present in the chloroform and methanolic extracts of *C. floribunda* stem initiated to carry out the anti oxidant activity for the above said extract.

In-vitro anti oxidant study

The chloroform extract and methanolic extract of *C. floribunda* stem tested for its free radical scavenging property using different *in vitro* models. All experiments were performed thrice and the results were averaged.

Superoxide radical scavenging activity

Superoxide radical scavenging activity of the plant extract was measured according to the method of Mc Cord and Fridovich [6], which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium. All the solutions were prepared in phosphate buffer (pH 7.8). The optical density was measured at 560nm. The percentage inhibition was calculated from formula [7].

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured according to the method of Elizabeth and Rao [8] by studying the competition between deoxyribose and test extract for hydroxyl radicals generated by Fenton's reaction. The damage imposed on deoxyribose due to the free radicals was determined calorimetrically by

measuring the thiobarbituric acid reactive substances (TBARS) at 532 nm. Percentage of inhibition was calculated using the formula [7].

Lipid peroxidation inhibition activity

The inhibition of lipid peroxidation was performed as per the method described by Ohkawa et al.,[9]. Rat liver homogenate was used as the source of polyunsaturated fatty acids for determining the extent of lipid peroxidation. The absorbance was measured at 532 nm. Percentage of inhibition was calculated using the formula [7].

DPPH radical scavenging activity

DPPH radical scavenging activity was measured according to the method of Braca et al., [10]. An aliquot of 3ml of 0.004% DPPH solution in ethanol and 0.1ml of plant extract at various concentrations were mixed and incubated at 37˚c for 30 min. and absorbance of the test mixture was read at 517nm.The percentage of inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the formula [7].

$$\text{Percentage of inhibition} = \frac{A_o - A_1}{A_o} \times 100$$

Where A_o = Absorbance of the control

A_1 = Absorbance of the plant extract/ standard

Statistical analysis

Linear regression analysis was used to calculate IC_{50} values [11].

RESULTS AND DISCUSSION

Superoxides are produced from molecular oxygen due to oxidative enzymes [11] of body as well as via non enzymatic reactions such as auto oxidation by catecholamines [12]. In the present study chloroform extract and methanol extract of *C.floribunda* stem was found to scavenge the superoxides generated by photo reduction of riboflavin. The chloroform extract and methanol extract of *C.floribunda* stem produced dose dependent inhibition of superoxide radicals. The IC_{50} values for superoxide radical with chloroform extract and methanol extract of *C.floribunda* stem were found to be 193.50 µg, 273.89 µg; with ascorbic acid were found to be 140.76 µg respectively. The chloroform extract of *C.floribunda* stem was found to have better superoxide radical scavenging activity when compared to methanol extract of *C.floribunda*, *as* shown in Table-1, Fig-1.

A single hydroxyl radical can result in formation of many molecules of lipid hydroperoxides in the cell membrane, which may severely disrupt its function and lead to cell death. The chloroform extract and methanol extract of *C.floribunda* stem showed concentration dependent activity and the ascorbic acid at various concentrations produced dose dependent inhibition of hydroxyl radicals. The IC_{50} values for hydroxyl radical with chloroform extract and methanol extract of *C.floribunda* stem were found to be 290.69 µg, 343.37 µg, with ascorbic acid was found to be 231.96 µg respectively. The chloroform extract of *C.floribunda* stem was found to have better hydroxyl radical scavenging activity when compared to methanol extract of *C.floribunda*, *as* shown in Table-2, Fig-2.

Free radicals induce lipid peroxidation in polyunsaturated lipid rich areas like brain and liver [13]. In this study, *in vitro* lipid peroxidation was induced in rat liver by using ammonium ferrous sulphate and ascorbic acid. The extract showed concentration dependent prevention towards generation of lipid peroxides. The IC_{50} values for the lipid peroxidation inhibiting activity with chloroform extract and methanol extract of *C.floribunda* stem were found to be 324.33 µg, 376.87 µg; with ascorbic acid was found to be 183.51 µg respectively. The chloroform extract of *C.floribunda* stem was found to have higher lipid peroxidation inhibition than the methanol extract of *C.floribunda as* shown in Table-3, Fig-3.

DPPH assay has been extensively used for screening antioxidant activity because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentration [14]. When DPPH radicals encounter a proton donating substance such as an antioxidant, it would be scavenged and the absorbance is reduced. Thus, the DPPH radicals were widely used to investigate the scavenging activity of some natural compounds. The mean IC_{50} values for DPPH radical with chloroform extract of *C.floribunda* stem and methanol extract of *C.floribunda* stem were found to be 90.71 µg, 117.68 µg; with ascorbic acid were found to be 75.22 µg respectively. The chloroform extract of *C.floribunda* stem was found to have better DPPH radical scavenging activity when compared to methanol extract of *C.floribunda*, *as* shown in Table-4, Fig-4.

Natural antioxidants such as phenolic acids, flavonoids and tannins possess potent antioxidant activity [15]. Sterols like β-sitosterol have been reported for antioxidant activity [16]. Terpenoids are also reported to possess antioxidant activity [17].

Phytochemical analysis reveal that *Calycopteris floribunda* contains phytosterols, triterpenoids, alkaloids, saponins, flavonoids, glycosides and tannins; Hence, the observed activity may be due to the presence of any of these constituents. The extracts merits further experiments *in vivo.*

TABLE-1: Percentage inhibition and IC_{50} values of superoxide radical scavenging activity *in vitro* by chloroform extract and methanol extract of *Calycopteris floribunda* stem.

Extract/ Compound	Quantity in micrograms (µg)					IC_{50} values
	10	50	100	200	300	
AA	15.56±1.76	31.93±2.91	47.63±4.79	69.7±1.93	76.1±1.38	140.76
MECF	7.16±3.14	16.0±2.66	25.06±1.88	40.13±1.18	52.4±1.7	273.89
CECF	9.70±1.81	19.46±1.85	33.6±1.80	52.2±1.39	70.8±0.95	193.50

AA – Ascorbic acid, MECF-Methanol extract of *Calycopteris floribunda*, CECF- Chloroform extract of *Calycopteris floribunda*,

TABLE-2: Percentage inhibition and IC_{50} values of hydroxyl radical scavenging activity *in vitro* by chloroform extract and methanol extract of *Calycopteris floribunda* stem.

Extract/ Compound	Quantity in micrograms (µg)						IC_{50} values
	10	50	100	200	300	400	
AA	2.53±1.51	17.9±3.52	28.83±2.22	52.9±1.63	63.13±0.37	74.83±2.25	231.96
MECF	2.56±1.58	11.8±0.97	20.73±0.92	36.8±0.86	44.9±1.02	53.83±1.29	343.37
CECF	4.3±0.87	14.66±0.81	24.5±0.86	40.8±0.78	54.86±0.44	60.9±0.50	290.69

TABLE-3: Percentage inhibition and IC_{50} values of Lipid peroxidation *in vitro* by chloroform extract and methanol extract of *Calycopteris floribunda* stem.

Extract/ Compound	Quantity in micrograms (µg)						IC_{50} values
	10	50	100	200	300	400	
AA	7.63±1.90	25.5±3.17	42.73±5.24	66.83±2.10	71.06±1.45	79.0±0.90	183.51
MECF	2.73±0.89	13.83±1.52	27.50±1.66	37.00±1.85	45.03±1.54	58.06±0.63	376.87
CECF	1.30±1.57	10.3±1.55	19.93±1.50	28.46±1.00	39.90±1.68	52.20±1.04	324.33

TABLE-4: Percentage inhibition and IC_{50} values of DPPH radical scavenging activity *in vitro* by chloroform extract and methanol extract of *Calycopteris floribunda* stem.

Extract/ Compound	Quantity in micrograms (µg)				IC_{50} values
	10	50	100	200	
AA	27.23±1.41	46.3±0.81	63.16±3.35	78.7±1.37	75.22
MECF	19.53±0.83	40.9±1.89	49.66±1.47	65.33±1.38	117.68
CECF	24.4±0.60	44.2±0.87	58.2±1.95	72.5±1.17	90.71

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Fig.1. *In vitro* concentration dependent percentage inhibition of superoxide radical scavenging activity by chloroform extract and methanol extracts of *Calycopteris floribunda* stem and Ascorbic acid.

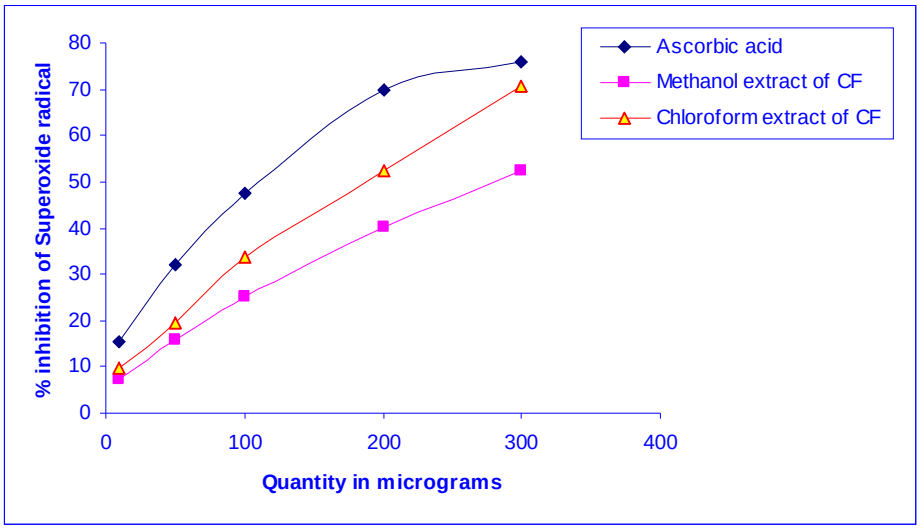

Fig.2. In *vitro* concentration dependent percentage inhibition of hydroxyl radical scavenging activity by ethyl acetate extract and methanol extract of *Calycopteris floribunda* stem and Ascorbic acid.

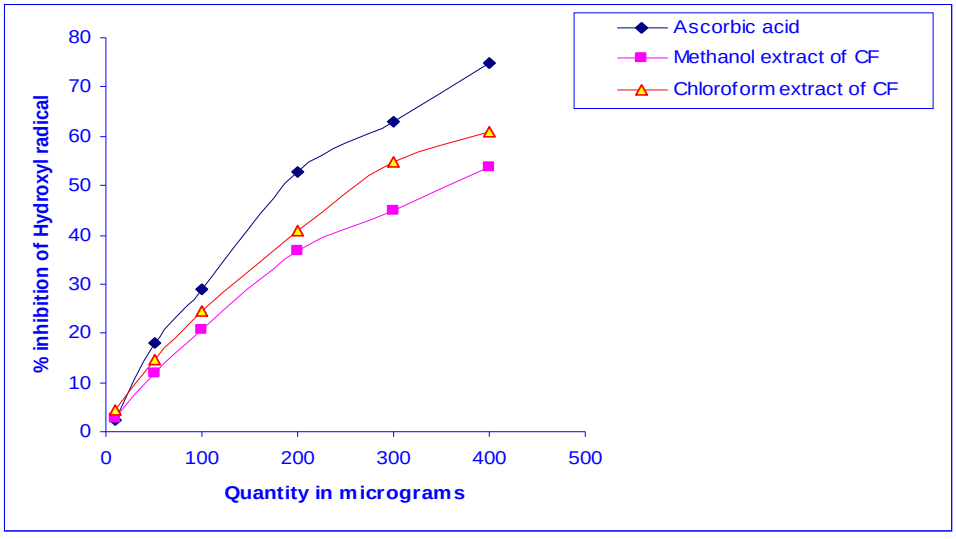

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Fig.3. *In vitro* concentration dependent percentage inhibition of lipid peroxidation by chloroform extract and methanol extract of *Calycopteris floribunda* stem *and Ascorbic acid*

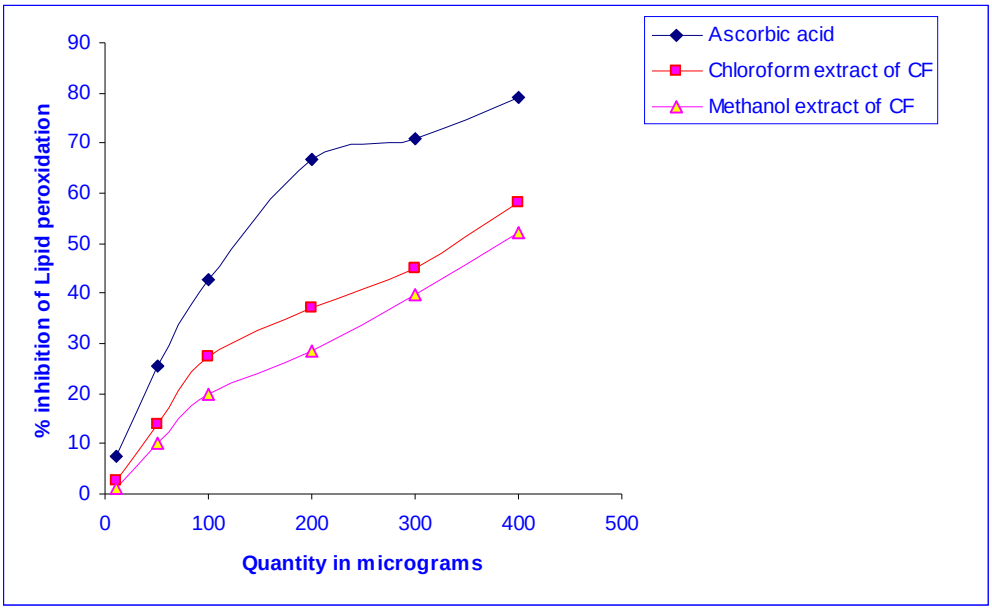
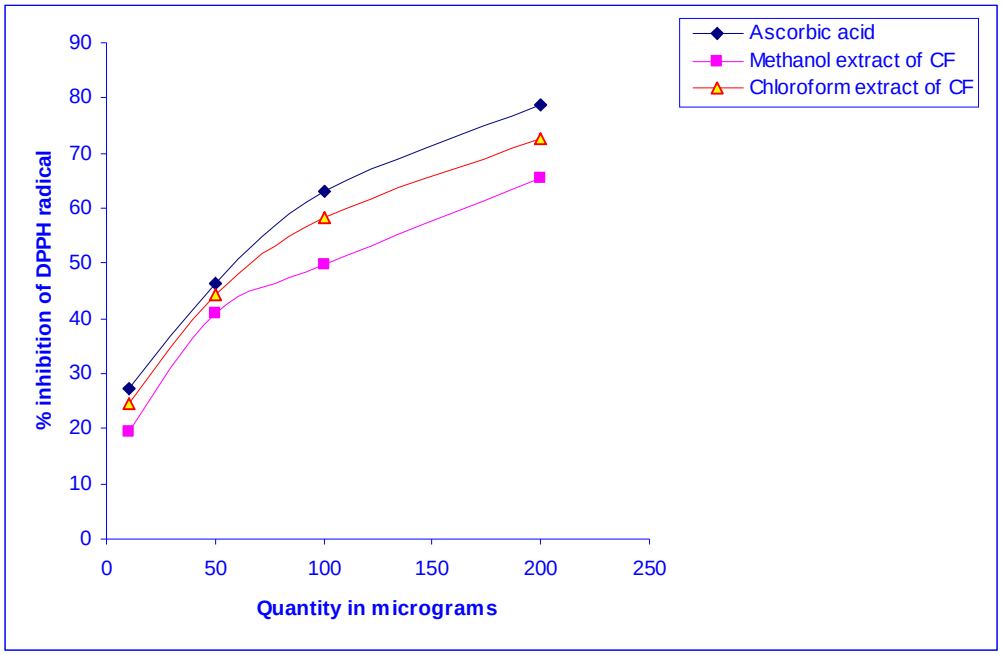


Fig.4. *In vitro* concentration dependent percentage inhibition of DPPH radical scavenging activity by chloroform extract and methanol extract of *Calycopteris floribunda* stem and Ascorbic acid.



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