

FREE RADICAL SCAVENGING ACTIVITY OF VARIOUS EXTRACTS OF WHOLE PLANT OF CALYOPTERIS FLORIBUNDA (LAM.): AN IN-VITRO EVALUATIONBHUVANESWARI SANTHARAM^{1*}, GANESH P², SORANAM R³, DIVYA VV⁴, PACKIA LEKSHMI NCJ⁵

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ABSTRACT

Objective: The present research was subjected to screen the free radical scavenging activity of various extracts of whole plant of *Calycopteris floribunda* by different *in-vitro* models.

Methods: The antioxidant activity was evaluated by hydroxyl radical scavenging activity, nitric oxide radical scavenging activity with reference standard ascorbate and total phenol content respectively.

Results: An inhibitory concentration 50% (IC₅₀) value was found ethyl acetate extract of *C. floribunda* is more effective in hydroxyl radical scavenging activity than that of methanolic and petroleum ether extract. The IC₅₀ values of ethyl acetate extract of *C. floribunda* and ascorbate were found to be 530 µg/ml and 410 µg/ml respectively. The ethyl acetate extract of *C. floribunda* was found more effective in the nitric oxide scavenging activity. The IC₅₀ values of ethyl acetate extract of *C. floribunda* and ascorbate were found to be 570 µg/ml and 410 µg/ml respectively. But when compared to all the three extracts with ascorbate (standard), the ethyl acetate extract of *C. floribunda* showed the better result. In addition, the ethyl acetate extract of *C. floribunda* was found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. The results were observed in a concentration dependent manner.

Conclusion: Our findings revealed that ethyl acetate extract of *C. floribunda* possesses interesting antioxidant activity, which may provide protection against free radicals induced damage to biomolecules.

Keywords: *Calycopteris floribunda*, Antioxidant, Ascorbate, Scavenging activity, Inhibitory concentration 50%.

INTRODUCTION

Now a days, the role of free radicals in many ailments and disease including inflammation, rheumatoid arthritis, cancer and cardiovascular diseases has been widely established [1]. Oxygen free radicals are formed in tissue cells by many endogenous and exogenous causes such as metabolism, chemicals, and ionizing radiation [2]. Oxygen free radicals may attack lipids and DNA giving rise to a large number of damaged products [3]. It is commonly recognized that antioxidant radicals can neutralize potentially harmful reactive free radicals in body cells before they cause lipid and protein oxidation and may reduce potential mutation and therefore, help to prevent cancer or heart diseases [4]. Antioxidants may be a great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. Therefore, there is a growing interest day by day in the substances exhibiting antioxidant properties, which are supplied to humans and animals as food components or as specific preventative pharmaceuticals [5]. Recently, there has been an upsurge of finding natural antioxidants from plant materials to replace synthetic antioxidants because the former ones are accepted as green medicine to be safe [6] for health management, whereas the latter ones are quite unsafe and their toxicity is a problem of concern [7]. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant rich foods and the incidence of human diseases [8].

Calycopteris floribunda Lam. (Combretaceae) commonly known as Kokkarai in Hindi, Minnarakoti in Tamil, a scandent woody and climbing shrub which is 5-10 cm long with slender brown streaked branches with vine storing water abundantly. So it is referred as a life saver by the forest dwellers during summer when streams dry up, people quench their thirst by using this plant [9-11]. The leaves have reported to possess anti-diabetic activity [12]. The hepato productive activity of

various stem and leaf extracts have been reported [13,14] and even fruits claimed to treat jaundice. Calycopterone, isocalycopterone and 4-dimethyl-calycopterone showed a wide range activity against solid cell lines [15]. The leaves are reported to have medicinal uses as a laxative and anti-helminthic while the juice derived from the young twigs is used for the treatment of diarrhea, dysentery and malaria [16].

Volatile oil extracted from the leaves of *C. floribunda* was reported to exhibit high antimicrobial activity [17]. Previous phytochemical studies have reported on the isolation of the flavonoids, calycopterin, quercetin and five bi flavonoids [18,19]. An ethnomedicinal survey conducted in Uttara Kannada district, evidence the wound healing activity [20]. The calycopterin is used to synthesize many flavones displaying high anti-proliferative activity [21]. Toxicity studies of *C. floribunda* reported in calf, rabbit and rats [22]. As far as our literature survey could ascertain, this is the first report that envisages the free radical scavenging activity of the whole plant of *C. floribunda* given here.

METHODS**Collection and identification of plant materials**

The whole plant of *C. floribunda* (Lam) was collected from Pulliyankudi, Nellai District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India, Palayamkottai. The whole plant material of *C. floribunda* (Lam) was dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of extracts

The above powdered materials were successively extracted with petroleum ether (40-60°C) by hot continuous percolation method in

soxhlet apparatus [23] for 24 hrs. Then the marc was subjected to ethyl acetate (76-78°C) for 24 hrs and then marc was subjected to methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Evaluation of antioxidant activity by *in vitro* techniques

Hydroxyl radical scavenging activity

This was assayed as described by Elizabeth and Rao [24]. The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with tetrabutylammonium hexatungstate. Hydroxyl radical was generated by the Fe³⁺-ascorbate - ethylenediaminetetraacetic acid (EDTA) - H₂O₂ system (Fenton reaction). The reaction mixture contained 0.1 ml deoxy ribose (2.8 mm), 0.1 EDTA (0.1 mm), 0.1 ml H₂O₂ (1 mm), 0.1 ml ascorbate (0.1 mm), 0.1 KH₂PO₄ - KOH buffer, pH 7.4 (20 mm) and various concentrations of plant extract in a final volume of 1 ml. The reaction mixture was incubated for 1 hr at 37°C. Deoxyribose degradation was measured thiobarbituric acid reactive substances and the percentage inhibition was calculated.

Nitric oxide radical scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat [25]. The reaction mixture (3 ml) containing 2 ml of sodium nitroprusside (10 mm), 5 ml of phosphate buffer saline (1 m) were incubated at 25°C for 150 minutes. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 minutes for completing diazotization.

Then 1 ml of naphthyl ethylenediamine dihydrochloride (1% EDTA) was added, mixed and allowed to stand for 30 minutes. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess-Illosvery reaction at 40 nm.

Total phenol

The measurement of total phenol is based on Mallick and Singh [26]. To 0.25 g of sample, added 2.5 ml of ethanol and centrifuged at 2°C for 10 minutes. The supernatant was preserved. Then, the sample was re-extracted with 2.5 ml of 80% ethanol and centrifuged. The pooled supernatant was evaporated to dryness. Then, added 3 ml of water to the dried supernatant. To which added 0.5 ml of Folin phenol reagent and 2 ml of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1 minute. The absorbance was measured at 650 nm in a spectrophotometer.

RESULTS

The plants were collected from Nellai District, dried, processed and extracted with petroleum ether, ethyl acetate, and methanol to get the active biomolecules. The crude extracts were subjected for radical scavenging activity and determination of phenols was given in the subsequent subheadings.

Hydroxyl radical scavenging activity

The percentage hydroxyl radical scavenging activity of petroleum ether extract of *C. floribunda* was depicted in Table 1. The petroleum ether extract of *C. floribunda* exhibited a maximum hydroxyl radical scavenging activity of 38.54% at 1000 µg/ml whereas for ascorbate (standard) were found to be 62.00% at 1000 µg/ml. The inhibitory concentration 50% (IC₅₀) values of the petroleum ether extract of *C. floribunda* and ascorbate were found to be 1150 µg/ml and 410 µg/ml respectively.

The percentage of hydroxyl radical scavenging activity of ethyl acetate extract of *C. floribunda* was depicted in Table 2. The ethyl acetate extract of *C. floribunda* exhibited a maximum hydroxyl radical scavenging

activity of 58.34% at 1000 µg/ml, whereas for ascorbate (standard) were found to be 62.00% at 1000 µg/ml. The IC₅₀ values of the ethyl acetate extract of *C. floribunda* and ascorbate were found to be 530 µg/ml and 410 µg/ml, respectively.

The percentage of hydroxyl radical scavenging activity of methanolic extract of *C. floribunda* was depicted in Table 3. The methanolic extract of *C. floribunda* exhibited a maximum hydroxyl radical scavenging activity of 58.77% at 1000 µg/ml whereas for ascorbate (standard) were found to be 62.00% at 1000 µg/ml. The IC₅₀ values of the methanolic extract of *C. floribunda* and ascorbate were found to be 640 µg/ml and 410 µg/ml respectively.

Based on the above result clearly indicate that ethyl acetate of *C. floribunda* was capable of reducing DNA damage at all concentration. The ethyl acetate extract of *C. floribunda* were found to be more effective than petroleum ether and methanolic extract. The IC₅₀ value of ethyl acetate extract of *C. floribunda* and ascorbate was recorded as 530 µg/ml and 410 µg/ml respectively.

Nitric oxide scavenging activity

The reduction of nitric oxide radical scavenging activity of petroleum

Table 1: Hydroxyl radical scavenging activity of petroleum ether extract of *C. floribunda* (Lam.)

S. no	Concentration (µg/ml)	Percentage of activity (±SEM)*	
		Sample (petroleum ether extract)	Standard (ascorbate)
1	125	16.34±0.033	27.63±0.076
2	250	26.15±0.045	49.53±0.054
3	500	33.12±0.048	55.12±0.022
4	1000	38.54±0.076	62.00±0.014
		IC ₅₀ =1150 µg/ml	IC ₅₀ =410 µg/ml

All values are expressed as mean±SEM for three determinations. SEM: Standard error mean, IC₅₀: Inhibitory concentration 50%, *C. floribunda*: *Calycopteris floribunda*

Table 2: Hydroxyl radical scavenging activity of ethyl acetate extract of *C. floribunda* (Lam.)

S. no	Concentration (µg/ml)	Percentage of activity (±SEM)	
		Sample (ethyl acetate extract)	Standard (ascorbate)
1	125	34.12±0.042	27.63±0.076
2	250	40.43±0.023	49.53±0.054
3	500	49.38±0.058	55.12±0.022
4	1000	58.34±0.043	62.00±0.014
		IC ₅₀ =530 µg/ml	IC ₅₀ =410 µg/ml

All values are expressed as mean±SEM for three determinations. SEM: Standard error mean, IC₅₀: Inhibitory concentration 50%, *C. floribunda*: *Calycopteris floribunda*

Table 3: Hydroxyl radical scavenging activity of methanolic extract of *C. floribunda* (Lam.)

S. no	Concentration (µg/ml)	Percentage of activity (±SEM)	
		Sample (methanolic extract)	Standard (ascorbate)
1	125	20.48±0.032	27.63±0.076
2	250	34.69±0.054	49.53±0.054
3	500	47.78±0.048	55.12±0.022
4	1000	58.77±0.028	62.00±0.014
		IC ₅₀ =640 µg/ml	IC ₅₀ =410 µg/ml

All values are expressed as mean±SEM for three determinations. SEM: Standard error mean, IC₅₀: Inhibitory concentration 50%, *C. floribunda*: *Calycopteris floribunda*

ether extract of *C. floribunda* and ascorbate was illustrated in Table 4. The maximum scavenging activity of petroleum ether and ascorbate at 1000 µg/ml were found to be 52.08% and 62.00% respectively. The IC₅₀ values of the petroleum ether extract and ascorbate were recorded as 930 µg/ml and 410 µg/ml respectively.

The reduction of nitric oxide radical by the ethyl acetate extract of *C. floribunda* and ascorbate was illustrated in Table 5. The maximum scavenging activity of ethyl acetate extract of *C. floribunda* and ascorbate at 1000 µg/ml were found 66.32% and 62.00% respectively. The IC₅₀ values of the ethyl acetate extract and ascorbate were recorded as 570 µg/ml and 410 µg/ml respectively.

The reduction of nitric oxide radical by the methanolic extract of *C. floribunda* and ascorbate was illustrated in Table 6. The maximum scavenging activity methanolic extract and ascorbate at 1000 µg/ml were found 51.98% and 62.00% respectively. The IC₅₀ values of the methanolic extract and ascorbate were recorded as 860 µg/ml and 410 µg/ml respectively.

Based on the above results the IC₅₀ values and percentage of scavenging capacity, it was found that ethyl acetate extract of *C. floribunda* is more effective in scavenging nitric oxid radical than petroleum ether and methanolic extracts. But when compared to all the three extracts with ascorbate (standard), the ethyl acetate extract of the *C. floribunda* showed a similar result.

Total phenol

The total amount of phenolic content of various extract of whole plant of *C. floribunda* was illustrated in Table 7. Total phenolic content was found high in ethyl acetate extract (6.47 mg/g) followed by methanolic extract (3.29 mg/g). The lowest content of total phenol was observed in petroleum ether extract of the plant (1.56 mg/g).

Based on the result the ethyl acetate extract of *C. floribunda* was found to have higher content of phenolic compounds than that of petroleum ether and methanolic extract of *C. floribunda*.

DISCUSSION

Free radical induced oxidative stress, which involve preventive mechanisms, repair mechanism, physical defenses and antioxidant defenses [27]. Antioxidant compounds scavenge free radicals and thus inhibit the oxidative mechanisms that lead to degenerative diseases [28]. Antioxidant compounds may function of free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation [29].

Phenolic compounds such as phenolic acids, polyphenols and flavonoids are commonly found in plants and have been reported to have multiple biological effects, including antioxidant activity [30]. A strong correlation exists between phenolic content and anti-oxidative activity of the plant extract, a property attributed to the free radical terminating potential of phenolic compounds [31].

Hydroxyl radical is highly reactive oxygen centered, radical formed from the reaction of various hydroperoxides transition of various hydroperoxides transition metal ion. It attacks protein, DNA, polyunsaturated fatty acids in membranes and most biological molecule it contacts and is known to be capable of abstracting hydrogen atoms from membrane lipids and beings about peroxidic reaction of lipids [32].

Nitric oxide is regarded as an important mediator of acute and chronic inflammation, which can easily react with superoxide anion to form peroxynitrite (O⁻NOO⁻) a potent oxidizing molecule capable of eliciting lipid peroxidation and cellular damage [33-35]. Hence, it is worthwhile to investigate the NO scavenging potential of the plant extract.

The antioxidant potential of phenolic compounds has been shown in a number of *in-vitro* studies. Phenolic compounds are capable

Table 4: Nitric oxide scavenging activity of petroleum ether extract of *C. floribunda* (Lam.)

S. no	Concentration (µg/ml)	Percentage of activity (±SEM)*	
		Sample (petroleum ether extract)	Standard (ascorbate)
1	125	21.65±0.065	27.63±0.076
2	250	35.50±0.039	49.53±0.054
3	500	41.57±0.045	55.12±0.022
4	1000	52.08±0.034	62.00±0.014
		IC ₅₀ =930 µg/ml	IC ₅₀ =410 µg/ml

All values are expressed as mean±SEM for three determinations. SEM: Standard error mean, IC₅₀: Inhibitory concentration 50%, *C. floribunda*: *Calycopteris floribunda*

Table 5: Nitric oxide scavenging activity of ethyl acetate extract of *C. floribunda* (Lam)

S. no	Concentration (µg/ml)	Percentage of activity (±SEM)	
		Sample (ethyl acetate extract)	Standard (ascorbate)
1	125	31.75±0.041	27.63±0.076
2	250	46.48±0.048	49.53±0.054
3	500	57.15±0.042	55.12±0.022
4	1000	66.34±0.022	62.00±0.014
		IC ₅₀ =570 µg/ml	IC ₅₀ =410 µg/ml

All values are expressed as mean±SEM for three determinations. SEM: Standard error mean, IC₅₀: Inhibitory concentration 50%, *C. floribunda*: *Calycopteris floribunda*

Table 6: Nitric oxide scavenging activity of methanolic extract of *C. floribunda* (Lam)

S. No	Concentration (µg/ml)	Percentage of activity(±SEM)*	
		Sample (methanolic extract)	Standard (ascorbate)
1	125	37.43±0.25	27.63±0.076
2	250	39.28±0.18	49.53±0.054
3	500	43.67±0.74	55.12±0.022
4	1000	51.98±0.64	62.00±0.014
		IC ₅₀ =860 mg/ml	IC ₅₀ =410 mg/ml

All values are expressed as mean±SEM for three determinations. SEM: Standard error mean, IC₅₀: Inhibitory concentration 50%, *C. floribunda*: *Calycopteris floribunda*

Table 7: The total phenolic content of various extracts of whole plant of *C. floribunda* (Lam)

S. no	Extracts of <i>C. floribunda</i>	Total phenol content (mg/g of catechol) (±SEM)*
1	Petroleum ether extract	1.56±0.022
2	Ethyl acetate extract	6.47±0.038
3	Methanolic extract	3.29±0.012

All values are expressed as mean±SEM for three determinations. SEM: Standard error mean, IC₅₀: Inhibitory concentration 50%, *C. floribunda*: *Calycopteris floribunda*

of direct chain breaking antioxidant action by radical scavenging. In addition to having potential for independent antioxidant action, polyphenols have been suggested to spare essential oxidants [36]. Phenolic compounds and flavonoids are major constituents of most of the plants reported possessing antioxidant and free radical scavenging activity [37]. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups [38].

CONCLUSION

The present study was clearly indicated that the high scavenging property of whole plant extracts of *C. floribunda* may be due to hydroxyl groups existing in the phenolic compounds that can provide the necessary component as a radical scavenger. The ethyl acetate extract of *C. floribunda* showed strong antioxidant activity by initiating hydroxyl radical scavenging. Nitric oxidizeradical scavenging activities were compared with standard ascorbate. However, the ethyl acetate extract of *C. floribunda* showed moderate activity when compared with standard ascorbate.

In addition, the ethyl acetate extract of *C. floribunda* was found to contain a noticeable amount of phenols, which play a major role in controlling antioxidants. Further studies are materialized for the isolation and identification of individual phenolic compounds and also *in vivo* studies are needed for better understanding their mechanism of action as antioxidant. In conclusion, results of this study showed in the whole plant extracts of *C. floribunda* to encompass significant amount of antioxidant compounds, with the extracts exhibiting rich antioxidant activities.

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