Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6 Issue 2, 2014

Research Article

IN VITRO ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS OF WHOLE PLANT OF *CALYCOPTERIS FLORIBUNDA* (LAM.)

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Received: 04 Feb 2014, Revised and Accepted: 14 Mar 2014

ABSTRACT

Objective: The aim of the present study was to investigate the in vitro antioxidant potential of various extracts of whole plant of *Calycopteris floribunda* (Lam.) as well as to establish the best procedure to obtain extracts containing active principles.

Methods: In this context, we conducted a bioassay of the extracts using DPPH (α, α -Diphenyl- B -Picryl Hydrazyl) radical scavenging activity, superoxide anion scavenging activity and iron chelating activity. The results were compared with reference standard antioxidants rutin, quercetin and EDTA respectively.

Results: The ethyl acetate extract of *Calycopteri floribunda* exerted effective DPPH radical scavenging activity. The IC_{50} Values of ethyl acetate of *C. floribunda* and Rutin were found to be 510µg/ml and 480µg/ml respectively. The lower the IC_{50} Value indicates the higher free radical scavenging ability. An IC_{50} value was found that ethyl acetate extract of *C. floribunda* is more effective in scavenging superoxide radical when compared to methanol and petroleum ether extracts. But when compared to all the three extracts with Quercetin (standard) the ethyl acetate extract of *C. floribunda* showed similar result. The Iron Chelating activity of the ethyl acetate extracts of the whole plant of *C. floribunda* was found more superior than the other two plant extracts.

Conclusion: It is concluded that a whole plant of ethyl acetate and methanolic extracts of *C. floribunda* possessed pharmacologically important phyto constituents like phenolic compounds and flavonoids which impart strong antioxidant and free radical scavenging activities. The result of the current study indicated potential of the plant in modulation of oxidative stress.

Keywords: *Calycopteris floribunda,* antioxidant activity, DPPH assays, Superoxide anion, Iron chelating activity.

INTRODUCTION

Reactive oxygen species (ROS), which include free radicals such as superoxide anion radicals (O_2^-) and hydroxyl radicals (OH^-), singlet ($^1O_2$) as well as non-free radicals species (H_2O_2) are various forms of activated oxygen and often generated by oxidation of biological reactions [1-3]. Free radicals which have one or more unpaired electrons are produced during normal and pathological cell metabolites. Damage induced by ROS includes DNA mutation, protein oxidation and lipid peroxidation contributing to the \development of degenerative diseases like cancer, diabetes, atherosclerosis, inflammation and premature aging [4]. Antioxidants help organisms deal with oxidative stress caused by free radical damage. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal material to replace synthetic oxidants which are being restricted due to its side effects such as carcinogenicity [5].

Several substances from natural sources have been shown to contain antioxidants and are under study. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [6]. Many researchers have focused on the antioxidant activity of plant extracts or isolated substances from plants due to fact that free radicals have been related to some diseases as well as to aging process [7]. Recent reports indicate that there is an inverse relationship between the dietary intake antioxidant-rich foods and the incidence of human diseases [8]. There is a plethora of plants that have been found to possess strong antioxidant activity [9].

Calycopteris floribunda Lam. (Combertaceae) commonly known as Kokkarai in Hindi, Minnarakoti in Tamil, a scadent woody and climbing shrub which is 5-10cm 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 [10-12]. The leaves have reported to posses anti-diabetic activity [13]. The hepato protective activity of various stem and leaf extracts have been reported [14-15] and even fruits claimed to treat jaundice. Calycopterone, Isocalycopterone and 4- dimethyl-calycopterone showed a wide range activity against solid cell lines [16].

The leaves are reported to have medicinal uses as a laxative and anti-helmintic while the juice derived from the young twigs is used for the treatment of diarrhoea, dysentery and malaria [17].

Volatile oil extracted from the leaves of *C. floribunda* and reported it to exhibit high antimicrobial activity [18]. Previous phytochemical studies have reported on the isolation of the flavonoids, calycopterin, quercetin and five bi flavonoids [19-20]. An Ethnomedicinal survey conducted in Uttara Kannada district, evidence the wound healing activity [21]. The calycopterin is used to synthesize many flavones displaying high antiproliferative activity [22]. Toxicity studies of *C.floribunda* reported in Calf, rabbit and rats [23].

As far as our literature survey could ascertain, no reports concerning the in vitro anti-oxidative activities of the whole plant of *C.floribunda* given here. Therefore we undertook the present study to investigate the antioxidant activities of various extracts of the whole plant of *C. floribunda* through various in vitro models.

MATERIALS AND METHODS

Collection and Identification of Plant materials

The whole plant of *C. floribunda* (Lam.) was collected form 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 by hot continuous percolation method in Soxhlet apparatus [24] for 24 hrs

with Petroleum ether (40-60^{0}C) followed by Ethyl acetate (76-78^{0}C) and Methanol. 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

DPPH photometric assay

The effect of extract on DPPH radical was assayed using the method of Mensor *et al* (2001) [25]. A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbances of the samples were measured at 518 nm and the percentage radical scavenging activity was calculated as follows.

$$Scavengingactivity\,(\%) = \frac{A_{518}\,\text{Control} - A_{518}\,\text{Sample}}{A_{518}\,\text{Control}} \times 100$$

Where A_{518} control is the absorbance of DPPH radical+ methanol, A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard.

Superoxide radical scavenging activity

Superoxide radical (O_2^-) was generated from the photo reduction of riboflavin and was detected by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne *et al* (1975) [26].

The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample.

The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in triplicate and the percentage inhibition was calculated by comparing the results of control and test samples.

Iron chelating activity

The principle is based on the formation of *O*-Phenanthroline-Fe^{2+} complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride (200µM) and 2 ml of various concentrations ranging from 10 to 1000µg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates [27].

RESULTS AND DISCUSSION

Free radical scavenging potentials of three different extracts of C. *floribunda* were tested by DPPH method. Among the three extracts tested, highest rate of DPPH scavenging was exerted by ethyl acetate extract (62.62%) at 1000 µg/ml concentration followed by methanolic extract (50.38%) and petroleum ether extract, whereas the standard Rutin exhibited 69.83% activity. The IC_{50} values and the percentage of DPPH scavenging activities for different extracts and the standard were tabulated in the table1.

The IC_{50} value found were 1120µg/ml, 510µg/ml, 1070µg/ml and 480µg/ml for petroleum ether extract, ethyl acetate extract, methanolic extract and standard Rutin respectively. A dose dependant DPPH radical scavenging activity was found, where the activity increased with the increase in the concentration of all plant extracts. Among the three extracts tested from *C. floribunda,* ethyl acetate showed strong activity compared to standard Rutin, petroleum ether showed moderate activity and methanolic extract exerted a weak activity.

Among the various natural antioxidants, phenolic compounds are reported to have the character of quenching oxygen-derived free radicals by donating a hydrogen atom or an electron to the free radical [28].Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases [29].

They are also involved in autoimmune disorders like rheumatoid arthritis etc [30]. Phenolic compounds and flavonoids are major constituents of most of the plants reported to possess antioxidant and free radical scavenging activity [31].

Therefore, the importance of search for natural antioxidants has increased in the recent years so many researchers focused the same [32]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [33] often used to evaluate the antioxidant activity of several natural compounds. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Literature survey revealed high level of phenolic content showed fast decrease in absorbance of DPPH radical [34].

Table 1: DPPH radical scavenging activity of different extracts of *C. floribunda* (Lam.)

S. No.	Concentration (µg/ml)	Radical scavenging activity*			
		Ethyl acetate extract	Petroleum ether extract	Methanolic extract	Rutin
1	125	35.45 ± 0.048	20.22 ± 0.048	13.24±0.042	18.85 ± 0.076
2	250	46.68 ± 0.034	29.45 ± 0.016	16.34±0.036	22.08 ± 0.054
3	500	55.34 ± 0.062	38.12 ± 0.034	21.98±0.014	52.21 ± 0.022
4	1000	62.62 ± 0.023	41.94 ± 0.082	50.38±0.065	69.83 ± 0.014
IC_{50} (µg/ml)		510	1120	1070	480

*All values are expressed as mean ± SEM for three determinations.

Superoxide radical is known to be a very harmful species to cellular components as a precursor of more reactive oxygen species [35]. Superoxide dismutase enzymes present in aerobic and anaerobic organisms catalyses the breakdown of superoxide radical [36].

Among the three extracts tested, highest rate of superoxide radical scavenging was exerted by methanolic extract (81.21%) at 1000 µg/ml concentration followed by ethyl acetate extract (76.54%) and petroleum ether extract(57.65%), whereas the standard Quercetin exhibited 98.01% activity. The IC_{50} values and the percentage of superoxide radical scavenging activities for different extracts and the standard were tabulated in the table2. The IC_{50} value found were for 460 µg/ml, 238µg/ml, 430µg/ml and 60µg/ml for petroleum ether extract, ethyl acetate extract, methanolic extract and standard Quercetin respectively. Superoxide scavenging ability of plant extract might primarily be due to the presence of flavanoids [37].

It was found that ethyl acetate extract of *C. floribunda* is more effective in scavenging superoxide radical than that of methanol and petroleum ether extract.

Table 2: Superoxide anion scavenging activity of different extracts of *C. floribunda* (Lam.)

S. No.	Concentration (µg/ml)	Superoxide anion scavenging activity*			
		Ethyl acetate extract	**Petroleum ether extract**	**Methanolic extract**	**Quercetin**
1	125	45.340 ± 0.042	26.34 ±0.018	26.48 ± 0.032	73.81 ± 0.006
2	250	57.56 ± 0.076	40.42 ± 0.034	43.48 ± 0.056	91.31 ± 0.011
3	500	70.32 ± 0.043	52.126 ± 0.045	69.76 ± 0.078	92.99 ± 0.024
4	1000	76.54 ± 0.058	57.65 ± 0.086	81.21±0.016	98.01 ± 0.012
IC_{50} (µg/ml)		238	460	430	60

*All values are expressed as mean ± SEM for three determinations

Metal chelating capacity was significant as they reduced the concentration of the catalyzing transition metal in lipid peroxidation [38]. Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, iron is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins and other cellular components [39-40]. The above results from various free radicals scavenging systems reveal that ethyl acetate of *C. floribunda* are more effective than that of petroleum ether and methanolic extract.

The results indicted the plant extract possess iron binding capacity which might be due to the presence of polyphenols that averts the cell from free radical damage by reducing of transition metal ions[41].

Various plant extracts were proved to be good chelators [42] and correlation exists between phenols, flavanoids and chelating activity. A highest iron binding capacity was noted with ethyl acetate extract (83.76%) followed by methanolic (54.66%) and petroleum ether extracts (83.76%) (Table 3).

Table 3: Iron chelating activity of different extracts of *C. floribunda* (Lam.)

Sl.No	Concentration (µg/ml)	Iron chelating activity *			
		Ethyl acetate extract	Petroleum ether extract	Methanolic extract	EDTA
1	125	36.34 ± 0.029	16.18 ± 0.026	29.42 ± 0.042	58.68 ± 0.007
2	250	47.65 ± 0.076	27.38 ± 0.029	36.65 ± 0.038	65.87 ± 0.018
3	500	68.17 ± 0.024	30.68 ± 0.012	41.49 ± 0.016	83.83 ± 0.012
4	1000	83.76 ± 0.043	47.64 ± 0.045	54.66 ± 0.042	97.90 ± 0.019
IC_{50} (µg/ml)		378	1140	1020	65

*All values are expressed as mean ± SEM for three determinations

CONCLUSION

In conclusion, our results suggest that ethyl acetate extracts of whole plant of *C. floribunda* investigated contain large amounts of phenolics compounds, which exhibit significant antioxidant and free radical scavenging activities. So this plant extract is a potent source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Further studies are warranted for isolation and identification of individual phenolic compounds and in vivo studies are needed for understanding their mechanism of action as an antioxidant prior to clinical use.

ACKNOWLEDGMENTS

The authors are grateful to the authorities of KR College of Arts & Science, Kovilpatti and SB College of Pharmacy Sivakasi, Tamilnadu, India for providing required facilities.

REFERENCES

1. Ceruitti PA. Oxidant Stress and Carcinogenesis. Eur J Clin Invest 1991; 21:1-11.
2. Yildirim A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of Rumex crispus L. extracts. J Agr Food Chem 2001; 49:4083-9.
3. Gulcin I, Oktay M, Kufrevioglu OI, Aslan A. Determination of antioxidant of Lichen Cetrariaislandica (L) Ach. J Ethnopharmacol 2002b; *79* (3):325-329.
4. Finkel T, Holbrook NJ. Oxidants, Oxidative stress and the biology of ageing. Nat *2000; 408:*239-47.
5. Ito N, Fukushima S, Hasegawa A, Shibata M, Ogiso T. Carcinogenicity of butylated hydroxyanisole in F344 rats. J Nat Canc Inst 1983; *70:343*-7.
6. Kumaraswamy MV Satish S. Antioxidant and Anti-Lipoxygenase activity of Thespesia lampas Dalz & Gibs Advan Biol Res 2008; *2(3)*: 56-9.
7. Willcox JK, Ash SL, Catignani GL. Antioxidants and Prevention of Chronic disease. Crit Rev Food Sci Nut 2004; 44:*275*-95.
8. Halliwell B, Advances in pharmacology, Academic Press; 1997.
9. Badami S, Gupta MK, Suresh B. Antioxidant activity of ethanolic extract of Striga orobanchioides. J Ethnopharmacol 2003; 85: 227-30.
10. Chopra RN, Nayar SL, Chopra I, Glossary of Indian Medicinal plants. New Delhi: 1956.
11. Assessment of risk of hepatotoxicity with kava products. WHO; 2001.
12. Indian Medicinal Plants: A Compendium of 500 Species. Chennai: Orient Longman 1995.
13. Sreenu T, Jyothibasu T, Delhiraj N, Suresh Kumar K. Hypoglycemic activity of Hydro-alcoholic extract of Calycopteris floribunda induced by Streptozotocin in Rats. Int J Pharmaceut sci Drug Res 2012; 4(4):250-2.
14. Chinna EM, Satyanarayana T. Hepatoprotective activity of extracts from Stem of Calycopteris floribunda Lam against Carbon tetrachloride induced toxicity in Rats. Int J Pharmacog Phytochem Res 2010;2: 53-7.
15. Thalla S, Pentela B. Hepatoprotective effect of hydro-alcoholic extract of Calycopteris floribunda leaves on rifampicin-isoniazid induced rats, Int J chem pharm sci 2011; 2 :15-21.
16. Wall MC, Wani MC, Fullas F, Oswald JB, Brown M, Santisuk T, Reutrakul V, McPhaiL AT, Farnsworth NR, Pezzuto JM, Kinghorn AD, Besterman JM. The Calycopterones, a new class of Biflavonoids with novel cytotoxicity in diverse panel of Human Tumour Cell lines. J. Med. Chem 1994; 37:1465-70.

17. Chopra RN, Indigenous Drugs of India.3rd ed. Kolkata: Academic publishers; 2006.
18. Liu JJ, Yang DL, Zhang Y, Yuan Y, Cao FX, Zhao JM, Peng XB. Chemical Component and Antimicrobial activity of Volatile oil of Calycopteris floribunda. J Cent SouUnivof Tech 2009; *16:* 931-5.
19. Rodrigue ZE, Vander Velde G, Mabry TJ, Subramanian SS, Nair AGR. The Structure of Calycopterin[JJ]. Phytochem 1972; 11*:*2311-12.
20. Mayer R. Five biflavonoids from Calycopteris floribunda (Combretaceae). Phytochem *2004;* 65*:* 593-601.
21. Bhat P, Hegde G, Hegde GR, Ethnomedicinal practices in different Communities of Uttara Kannada district of Karnataka for treatment of Wounds. J Ethnopharmacol 2012; 143: 501-14.
22. Lewin G, Shridhar NB, Aubert G, Thoret S, Dubois J, Cresteil T. Synthesis of Antiproliferative flavones from Calycopterin, major flavonoid of Calycopteris floribunda Lamk. Bio Med Chem 2011; 19(1): 186-96.
23. Sreekanth P, Narayana K, Shridhar NB. Toxicity Studies of Calycopteris floribunda in Calf, rabbit and rat. J Ethnopharmacol *2006;* 107(2): 229-33.
24. Harborne JB. Phytochemical methods 2nd ed. New York: In Chapman & Hall; 1984.
25. Mensor LL, Meneze FS, Leitao GG, Reis AS, Dos SJC, Coube CS, Leitao SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res 2001*;* 15*:* 127-130.
26. Winterbourne CC, Hawkins RE, Brain M, Carrel RW. The estimation of red cell superoxide dismutase activity. J Lab Chem Med 1975; *85:* 337-41.
27. Benzie IEF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996; *239:*70-6.
28. Wanasundara PK, Shahidi F. Optimigation of hexametaphosphate-assisted extraction of flaxseed proteins using response surface methodology J Food Sci 1996; 61: 604-7.
29. Roy H, Burdon. Free Radical Damage and Its Control. Amsterdam: Elsevier Science; 1994.
30. Hadjigogos K. The role of free radicals in the pathogenesis of rheumatoid arthritis. Panminerva Med 2003; 45(1):7-13.
31. Hristensen LP. Tuliposides from Tulipa sylvestris and T. turkestanica. Phytochem. 1999; 51(8): 969-9.
32. Jayaprakasha GK, Selvi T, Sakariah KK. Antibacterial and antioxidant activities of grape (Vitis vinifera) seed extract. Food Res Int 2003; 36:117-122.
33. Soares JR, Dins TCP, Cunha AP, Ameida LM. Antioxidant activity of some extracts of Thymus Zygis. Free Rad Res 1997; 26: 469-78.
34. Rattanachitthawat S, Suwannalert P, Riengrojpitak S, Chaiyasut C, Pantuwatana S. Phenolic content & Antioxidant activities in red unpolished Thai rice prevents oxidant stress in rats. J Med Plants Res *2010;* 4(9): 796-801.
35. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. 4th ed. Oxford: Clarendron press; 2007.
36. Shirwaikar A, Punitha ISR. Antioxidant studies on the methanol stem extract of Coscinium fenestratum. Nat Prod Sci 2007; 1: 40-45.
37. Zheng W, Wang SY Antioxidant activity and phenolic compounds in selected herbs. J Agri Food Chem 2001; 49: 5165-70.
38. Duh PD, Tu YY, Yen GC. 1999. Antioxidant activity of water extract of Harng Jyur (Chrysanthemium morifolium Ramat) LMT- Food Sci. Tech. 32: 269-277.
39. St Angelo AJ. Lipids oxidation in food. ACS Symposium Series. 1st ed. Washington DC: American Chemical Society; 1992.
40. Smith C, Halliwell B, Aruoma OI. Protection by albumin against the pro-oxidastion actions of phenolic dietary components. Food Chem Toxicol. 1992; 30:483-9.
41. Gordon MH. The mechanism of antioxidant action in vitro. In food Antioxidants; Elsevier Applied Science: London; 1990.
42. Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR. Iron chelating activity, phenol and flavanoid content of some medicinal plants from Iran. Afr J Biotechnol. 2008; 7(18): 3188-92.