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A PRELIMINARY STUDY ON TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITIES OF *CALYCOPTERIS FLORIBUNDA* ROOT EXTRACTS

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ABSTRACT: We evaluated the total phenolic acid content and antioxidant activity of methanolic and aqueous extracts of *Calycopteris floribunda* roots. Antioxidant activity of the extracts was determined using Reducing Power Assay and Total Antioxidant Capacity (TAC) assays. Preliminary phytochemical analysis of the extracts revealed the presence of various phytochemicals. The total phenolic content of the extracts was higher in methanol than compared to aqueous extracts and subsequently higher antioxidant activity was also observed in case of methanol extracts. The results revealed that the antioxidant activity of the extracts was positively associated with the total phenolic acid content.

INTRODUCTION: Oxidation is an important process for the management of energy in all living organisms and hence has been maintained and controlled by various cellular mechanisms. However, overproduction of free radicals and a lower antioxidant protection accelerate the ageing process and cause numerous diseases. The failure or inefficiency of internal enzymatic mechanisms during oxidative damage can be overcome by antioxidants which are considered as possible protective agents ¹. Due to this, there is a growing demand for natural antioxidants exhibiting antioxidant properties, which can be used as food compounds or preventive drugs for humans and animals.

Plants are a rich source of natural antioxidants. The secondary metabolites present naturally in fruits, vegetables and various herbs exhibit protection against oxidative stress. The protection inferred by these compounds is due to the presence of phenolic compounds and flavonoids that protect against oxidising agents and free radicals ².

Calycopteris floribunda Lam. (Combretaceae), is a scandent and climbing shrub distributed in the deciduous forests of the Western Ghats and having vines that abundantly store water. The plant has been used traditionally for healing wounds ³. The leaves of these plants have been reported to possess antidiabetic ⁴, hepatoprotective ^{5, 6} and antimicrobial activities ⁷. The extracts from young twigs have been used for the treatment of diarrhoea, dysentery and malaria ⁸. Phytochemical studies have reported the isolation of various flavonoids, calycopterin and quercetin ⁹. However there are no reports on the phytochemical constitution and antioxidant properties of the roots of *C. floribunda*.

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Hence, the present study was undertaken to determine the phytochemical composition and antioxidant activity of *C. floribunda* root extracts.

MATERIALS AND METHODS:

Chemicals and Glassware's: All the chemicals and solvents used were of analytical grade obtained from E-Merck (Germany), Sigma-Aldrich (USA) and "Ranbaxy" and "unless otherwise specified".

Plant Material and Sample Preparation: Roots of *Calycopteris floribunda* were collected from the Sathodi forest in Western Ghats, Karnataka during January 2017. The roots were rinsed with tap water followed by distilled water to remove any traces of soil on the surface. The roots were cut into small pieces, air dried for 2 - 3 days and freeze dried till constant mass was obtained. The dried roots were powdered using a blender and stored in desiccators until extraction. The extraction was carried out in a soxhlet apparatus for 8 hours in distilled water and methanol. The solvent was then evaporated using a rotary evaporator and crude extracts were stored in desiccators.

Phytochemical Screening: Preliminary phytochemical analysis was carried out using the standardised methods^{10,11}.

Total Phenolic Content: The total phenolic content in the root extracts were determined using the Folin-Ciocalteu calorimetric method¹². The phenolic acid content in the sample was calculated using the standard gallic acid (GA) curve and the results were expressed as μg gallic acid equivalent (GAE)/mg.

RESULTS AND DISCUSSION: The phytochemical analysis of *C. floribunda* roots revealed the presence of carbohydrates, glycosides, proteins, alkaloids, steroids, terpenoids and flavonoids in the root extracts **Table 1**. However, saponins were detected only in aqueous extracts. The preliminary phytochemical screening revealed the presence of bioactive agents in *C. floribunda* roots. The detected phytochemicals have proven medicinal properties like alkaloids have showed biological activities such as anti-malarial, cytotoxicity and anti-inflammatory^{15, 16, 17} steroids have shown hyper-cholesterolemic effects¹⁸, tannins have shown antiviral and antitumor activities¹⁹ and

glycosides have been used to treat cardiac arrhythmia²⁰. Sujit et al., (2005) have carried out biological and chemical studies on *C. floribunda* leaves and have found various phytochemicals²¹.

TABLE 1: PRELIMINARY PHYTOCHEMICALS PRESENT IN THE CALYCOPTERIS FLORIBUNDA ROOTS EXTRACTS

Phytochemicals	Extracts	
	Aqueous	Methanol
Carbohydrates	+	+
Glycosides	+	+
Proteins	+	+
Alkaloids	-	-
Steroids	+	+
Terpenoids	+	+
Flavonoids	+	+
Saponins	+	-
Phenolics	+	+

The total phenolic content of methanolic and aqueous root extract calculated from the calibration curve ($R^2 = 0.9996$) was 343.77 ± 2.02 and 87.87 ± 6.06 gallic acid equivalent/mg. The redox properties and chemical structures of phenolic compounds allow them to act as antioxidants²². The hydroxyl groups in them facilitate their free radical scavenging activity and thus can be used for rapid screening of antioxidant activity. Reductants present in the plant extracts leads to the reduction of ferricyanide complex to ferrous form. The values of crude extracts increase significantly changes with increasing concentration (50 - 350 mg/ml). The absorbance values for methanolic root extracts ranged from 0.09 - 0.78 and for aqueous extracts the values were between 0.06 - 0.67 **Fig. 1**.

Similarly, the TAC obtained by the phosphor-molybdenum assay indicates an increase in value with increasing concentrations (50 - 350 mg/ml). The aqueous extracts exhibit lower antioxidant activity when compared to methanol and ascorbic acid standard **Fig. 2**. These results indicate that the antioxidant activity is attributed to the presence of phenolic content, which was higher in case of methanolic extracts leading to higher antioxidant activity. Our results are consistent with previous reports^{23, 24}.

Our study revealed that the methanolic extracts of roots showed higher antioxidant capacity compared to aqueous extracts in accordance to the phenolic acid content.

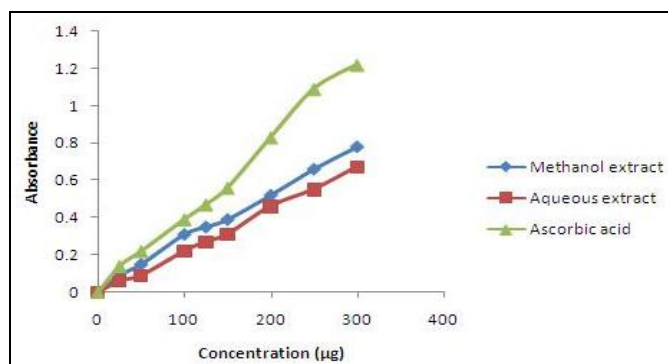


FIG. 1: REDUCING POWER ASSAY OF ASCORBIC ACID AND METHANOL AND AQUEOUS EXTRACTS OF CALYOPTERIS FLORIBUNDA ROOTS. Values are average of triplicate experiments and expressed as mean \pm standard deviation

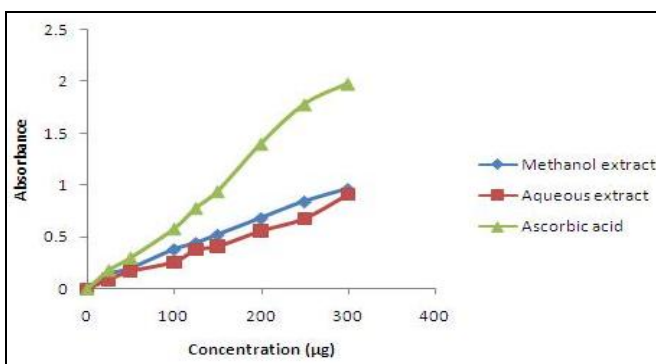


FIG. 2: TOTAL ANTIOXIDANT CAPACITY (TAC) OF ASCORBIC ACID AND METHANOL AND AQUEOUS EXTRACTS OF CALYOPTERIS FLORIBUNDA ROOTS. Values are average of triplicate experiments and expressed as mean \pm standard deviation

Determination of Antioxidant Activity Using Reducing Power Assay and Total Antioxidant Capacity: The reducing power assay and total antioxidant capacity of the root extracts was determined as reported elsewhere^{13, 14}. The assays were carried out in triplicates and the results are expressed as mean \pm standard deviation. Ascorbic acid was used as standard.

CONCLUSION: The results suggest that the *C. floribunda* is a potential source of various phytochemicals and antioxidant molecules. The roots can thus be used in isolation of various phytochemicals and can be used as a source of natural antioxidants. However, further analysis is required for isolation of bioactive compounds and its use in various pharmacological activities.

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CONFLICT OF INTEREST: The authors hereby declare no conflict of interest.

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