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An *In vtro* study on anti-diabetic and antiradical activities of ethanol extract from the leaves of *Calycopteris Floribunda*

N R Panneerselvam
Professor, Department of Kuzhanthai Maruthuvam, Sri Sairam Siddha Medical College and Research Centre, Poonthadalam, Chennai, Tamil Nadu, India

Abstract
The present study was attempted to investigate the antidiabetic and antioxidant activity of ethanol extract of *Calycopteris floribunda*. Phytochemical screening, total phenolic and to flavonoid subs tance were decided by using standard methods. The invitro antidiabetic recorded by inhibition of amylase and glucosidase. The antioxidant inaction was measured by ABTS free radical assay, lipid peroxidation and nitric oxide. The result about appeared that the phytochemical screening presence of triterpenoids, flavonoids, glycosides, saponins, and tannins that were contribute to organic activity. In ABTS scavenging assay, lipid peroxidatioin and nitric oxide scavenging EC_{50} values of ethanol extract of *C. floribunda* were found 58.32 µg/ml, 64.32 µg/ml and 69.32 µg/ml, separately. These results demonstrated that ethanol extract of *C. floribunda* have solid anti-diabetic and antioxidant possibilities and back conventional restorative utilize for the treatment of diabetes mellitus and good source for natural antioxidants.

Keywords: *Calycopteris floribunda*, antidiabetic, antioxidant, phytochemical screening

Introduction
Plants have always been a great source of medications, and many of the drugs that are currently available were deduced directly or by inference from them. Nearly 800 plants have anti-diabetic potential, according to ethnobotanical data. When tested by and using available test methods, a handful of these plants proved to have anti-diabetic properties (Jafri et al., 2000). A large number of plant-derived dynamic standards for diverse chemical substances have shown movement compatible with their potential use in the treatment of diabetic. Alkaloids, glycosides, glycopeptides, terpenoids, amino acids, and inorganic particles are among these substances. As a result, plants are a possible source of anti-diabetic medications, although this fact has yet to be recognised. There are a variety of reasons for this, including a lack of belief among traditional medicine specialists in alternative medicine, elective pharmaceutical shapes that are not well-defined, the possibility of quacks honing such pharmaceutical giving charming and enchanted cures, and the fact that normal drugs may change dramatically in substance, quality, and security.
Diabetes mellitus is a metabolic syndrome with multiple etiologies that is characterised by persistent hyperglycemia alongside unsettling influences in the carbohydrate, protein, and fat digestion system. Diabetes mellitus is caused by a decrease in circulating affront concentrations (affront deficiency), a decrease in the reaction of fringe tissues to affront (affront resistance), or both. Diabetes mellitus is the most common clinical condition, affecting almost 10% of the world's population. At now, there are an estimated 246 million diabetics worldwide, with nearly 80% of them living in developing countries (Lord et al., 1998). Diabetes is a global disease that has a significant negative impact on health and death, particularly from cardiovascular diseases. Controlling not only blood glucose levels but also lipid levels is essential to avoid serious consequences from diabetes, such as heart and blood vessel infections (Markuszewski et al., 2006).
Because of their efficiency and low toxicity, there is an increasing trend toward the use of common antioxidants derived from plant sources. As a result, the antioxidant and radical rummaging activities of restorative plants have been extensively studied. Plant phenolics contain antioxidant, hypocholesterolemic, hypolipidemic, antihypertensive, antidiabetic, and anticancer effects. Phenolics are powerful antioxidants that break up chains and contribute to antioxidant activity. The close proximity of hydroxyl gather could explain phenolics' extraordinary antioxidant capability. Another group of polyphenol flavonoids is extremely important for human health, and they work by rummaging or chelating metal ions (Manach et al., 2004). Vegetables and medicinal plants that contain phenolic compounds are aware of the antioxidant movement.
Despite the fact that the antioxidant examination of medicinal plants has been widely explored in recent years all over the world, very few studies have been conducted to survey medicinal plants and vegetables developed in India and used locally or traded to a few countries (Assimopoulou et al., 2005). Lam. *Calycopteris floribunda* is an evergreen bush, locally known as enjir. Timberland renters usually rely on this vine during the summer when streams dry up, and it is commonly referred to as a lifesaver. Water is stored in areas of the vine, which people use to relieve their thirst. Intestinal worms, colic, illness, malarial fever, diarrhoea, ulcers, and heaving are only a few of the ailments for which the plant parts are used medicinally. Natural items can help with jaundice, ulcers, pruritus, and skin conditions. The plant's sensitive copper-colored leaves were mashed into glue or dry powders administered for the elimination of microscopic organisms, free radicals, and round worms,

yielding a number of phenolic and non-phenolic flavonoids with cytotoxic, anthelmintic, and antiviral activities. The most common focus of the work display was to consider the anti-diabetic and anti-radical effects of an ethanol extract of Calycopteris floribunda.

Materials and Methods

Plant Collection

Calycopteris floribunda leaves were caught from home grown garden of Government Siddha Medical College, Arumbakkam, Chennai, Tamil Nadu, India. A plant taxonomist verified the plant and samples were kept within the Medicinal Botany herbarium with voucher specimen numbers MB/GSMC-345/2021.

Extraction

Calycopteris floribunda leaves, 100 g of the dried plant was smashed utilizing blender to a paste-like state for 1 min. The homogenized sample was firstly solidify dried in arrange to decrease dampness substance of the test for a more proficient extraction process. The powder was at that point drenched in n-Hexane to defat for 24 h. It was at that point doused in ethanol for 72 h to get ethanol unrefined extract, which was concentrated employing a rotatory evaporator at 40 °C (Dasgupta et al., 2014).

Phytochemical Screening

The aqueous extract of from the leaves *Calycopteris floribunda* were subjected to phytochemical screening to decide the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins and polyphenols utilizing standard methods (Trease and Evans 1983).

Glucose Uptake in Yeast Cells

The commercial baker's yeast in distilled water was subjected to rehashed centrifugation (3,000×g, 5 min) until clear supernatant liquids were obtained and a 10% (v/v) of the suspension was arranged in distilled water. Different concentrations of ethanol extracts (25-100µg/mL) were included to 1mL of glucose arrangement (25mM) and incubated together for 10 min at 37 °C Response was begun by including 100µL of yeast suspension taken after by vortexing and encourage incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and sum of glucose was estimated within the supernatant (Cirillo, 1962). glycomet was utilized as standard drug. The rate increment in glucose uptake by yeast cells was calculated utilizing the taking after forumla:

$$\textbf{Increase in glucose uptake (\%)} = \frac{\textbf{Abs sample} - \textbf{Abs control}}{\textbf{Abs sample}} \times 100$$

α-Amylase Inhibition Activity

Alpha amylase is an enzyme that hydrolyses alpha-bonds of huge alpha linked polysaccharide such as glycogen and starch to abdicate glucose and maltose.

Alpha amylase inhibitory activity was based on the starch iodine method that was initially created by (Lena et al., 2008) and afterward utilized by others for assurance of amylaseb movement in plant extracts with a few alterations.

$$\text{Inhibition of alpha- Amylase (\%)} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \times 100$$

β-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory activity was evaluated by the standard method (Dong et al., 2012), with slight alterations. Briefly, a volume of 25, 50, 75 and 100 µg/ml of sample solution and 50 µl of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.2 U/ml) was incubated in test tube at 37 ºC for 20 min. After pre-incubation, 50 µl of 5 mM p-nitrophenyl-α-D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at 37 ºC for another 20 min. At that point the response was halted by including 160 µl of 0.2 M $NaCO_3$ into each well, and absorbance readings (A) were recorded at 405 nm by micro-plate reader and compared to a control which had 60 µl of buffer solution input of the extract. For clear incubation (to permit foabsorbance delivered bythe extricate), enzyme solution was supplanted by buffer arrangement and absorbance recorded.

Abts (2,2'-Azino-Bis-3-Ethyl Benzthiazoline-6-Sulphonic Acid) Radical Scavenging Assay

ABTS radical scavenging movement of ethanol extract of Calycopteris floribunda was taken after by Re et al. (1999). ABTS radical was recently arranged by addition 5 ml of 4.9 mM potassium persulfate solution to 5 ml of 14 mM ABTS solution and kept for 16 h in dull. This solution was diluted with distilled water to produce an absorbance of 0.70 at 734 nm and the same was utilized for the antioxidant movement.

Nitric Oxide Radical Scavenging Assay

The nitric oxide radical scavenging assay taken after by Panda et al. (2009). The extracts were prepared from a 10 mg/mL ethanol crude extract. These were at that point serially diluted with distilled water to
create concentrations from 25-100 µg/mL of ethanol extract of *Calycopteris floribunda* and standard.

Inhibition of Lipid Peroxidation Activity

Lipid peroxidation initiated by Fe2+ascarbate framework in egg yolk was surveyed as thiobarbituric acid responding substances (TBARS) by the method of Ohkawa et al. (1979). The experimental mixture contained 0.1 ml of egg yolk (25% w/v) in Tris-HCl buffer and different concentrations of ethanol extract in a last volume of 0.5 ml. The test blend was brooded at 37°C for 1 h. After the incubation period, 0.4 ml was collected and treated with 0.2 ml sodium dodecyl sulphate (SDS) (1.1%); 1.5 ml thiobarbituric acid (TBA) (0.8%); and 1.5 ml acidic acid (20%, pH 3.5). The ultimate volume was made up to 4.0 ml with distilled water and after that kept in a water shower at 95 to 100 °C for 1 hour.

Statistical analysis

All comes about were communicated as mean ± standard deviation (n=3). Centrality of differences from the control was determined by Duncan's test and a p esteem < 0.05 was considered noteworth

Result and Discussion

Phytochemical screening

The phytochemical screening of aqueous extract of *Calycopteris floribunda* were considered by and by appeared the presence of alkaloids, flavonoids, polyphenol, terpenoids, and absence of glycosides and tannin (Table-1).

Table 1: Phytochemical screening of aqueous extract of *Calycopteris floribunda*

Sl. No.	Phytochemical Constituents	Observation	Aqueous extract of *C. floribunda*
1	**Alkaloids** -Dragendorff's Test -Mayers test	Orange / red precipitate Yellow or white precipitate	+ +
2.	**Flavonoids** -Alkalai Reagent -Lead acetate test	Intense yellow colour Precipitate formed	+ +
3.	**Glycosides** Keller-Killiani test	Reddish brown colour ring formed	-
4.	**Tannin** -$FeCl_3$ test	Blue black coloration	-
5.	**Saponins** -Frothing test	Foam	-
6.	**Terpenoids** *-Salkowski test*	Dark reddish brown color in interface	-
7.	**Polyphenols** -Ferrozine test	Raddish blue	+
8.	**Anthocyanin test** **Ammonia**	Ammonia layer yellow in color	+

TLC Profile

The ethanol extract of C. floribunda loaded on Pre-coated TLC plates (60 F2 54 Merck) and created with a solvent framework of petroleum ether, chloroform and methanol within the proportion of 1:0.5:0.1 were productive to extract the antioxidant and anti-obesity compound it is utilized for encourage studies. The created plate was seen beneath UV 240nm and 360nm (Fig-1).

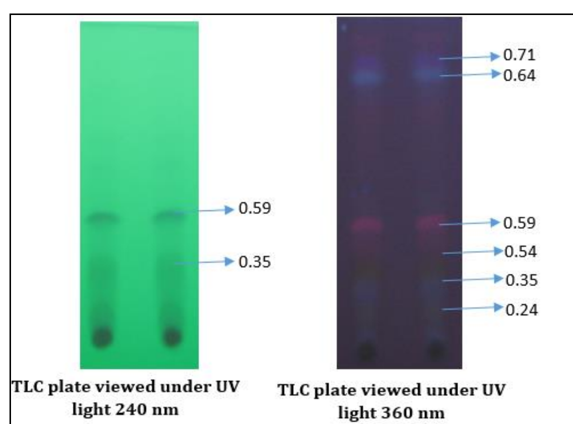
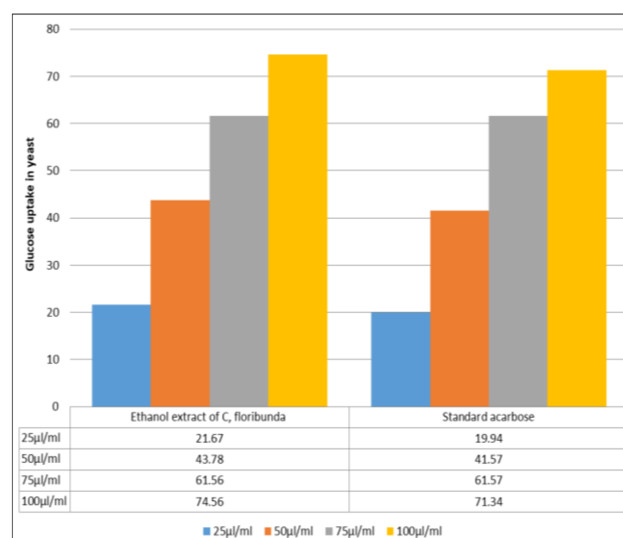


Fig 1: Partial characterization of ethanol extract of *Calycopteris floribunda* by TLC

Glucose Uptake in Yeast Cells

Distinctive concentrations of that ethanol extract of *C. floribunda* was subjected to in vitro glucose uptake test utilizing yeast as show. The rate of glucose take-up in yeast cells by that ethanol extract of *C. floribunda* was compared with standard drug glycomet. Ethanol extract of *C. floribunda* shown higher action than the standard (Graph-1). There was concentration subordinate increment in rate of glucose take-up with expanding in concentration of ethanol extract of *C. floribunda*.

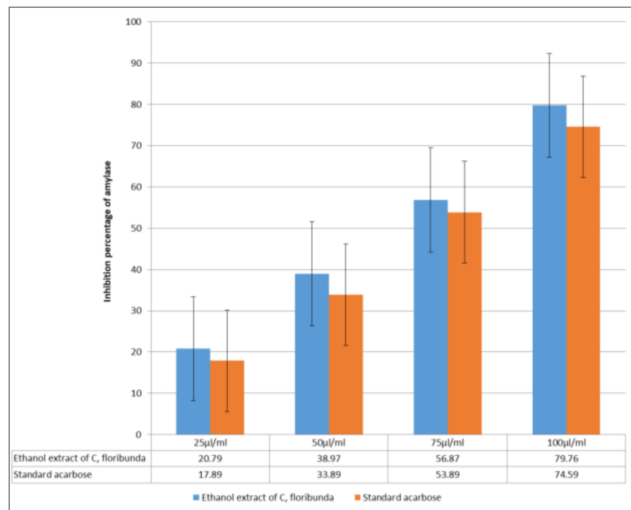
Ethanol extract of *C. floribunda* shown most noteworthy rate of glucose take-up i.e. 74.56%, which was nearly close to the standard i.e. 71.34% at 100 µg/ml concentration. Medicinal plants having anti-diabetic properties can give a valuable source for the uncovering of more secure financial anti-diabetic drug Benalla et al. (2010)



Graph 1: Glucose Uptake In Yeast Cells By Ethanol Extract Of *Calycopteris Floribunda*

Alpha-Amylase Inhibition

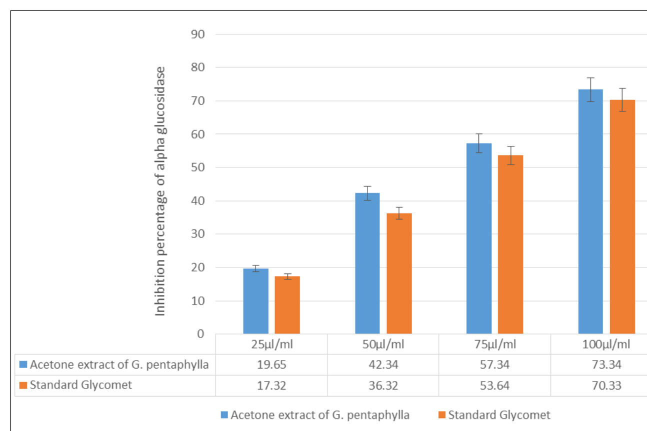
The outcomes of result demonstrated that ethanol extract of *C. floribunda* shown no noteworthy impact on alpha-amylase at all the tested concentrations (Graph-1). At the most elevated concentration (100 µg/ml) examined, the ethanol extract of *C. floribunda* shown calculable impact on alpha-glucosidase by 79.76%. In any case, acarbose as positive controls, were distant more viable within the particular measures than the extricate, showing rate inhibitory activities of EC_{50} esteem 59.89 µg/ml against alpha-amylase.



Graph 2: Alpha-amylase inhibition by ethanol extract of *Calycopteris floribunda*

α-Glucosidase Inhibitory Activity

The outcome of in-vitro α-glucosidase inhibitory study are appeared in Graph-3. The ethanol extract of *C. floribunda* appeared a concentration-dependent inhibition of enzyme. The most noteworthy concentration of 100 µl/ml tried appeared a maximum inhibition of about 73.34% (EC_{50} 64.32µg/ml) in that ethanol extract of *C. floribunda* appears to be less strong in α-glucosidase inhibitory potential compared to Glycomet. It may be that α-glucosidase is more delicate towards glycomet with the concentration required for 50% inhibition (EC_{50}) found to be 67.23µg/ml. Separated from that polyphenolic compounds were found in plant extract, may associated or hinder particular positions in enzymes subsequently diminishing the power of α-amylase and α-glucosidase (Shai et al., 2011).



Graph 3: A-Glucosidase Inhibitory Activity of Ethanol Extract of *Calycopteris Floribunda*

Radical Scavenging Activity ABTS

The antioxidant activity of ethanol extract of *C. floribunda* was essentially evaluated by ABTS, which is based on the capacity of ABTS to respond with proton benefactors such as phenols. Be that as it may, ethanol extract of *C. floribunda* free radical scavenging capacity remains obscure. The display study appeared that ethanol extract of *C. floribunda* shows noteworthy free radical scavenging potential (EC_{50} 58.32±1.78 µg/mL) (Table-2). The rates of free radical scavenging are given in Table-4. This property has been broadly utilized to assess the free radical scavenging impact of common cancer prevention agents (Choi et al., 2002).

Table 2: ABTS Radical scavenging activity by ethanol extract of *Calycopteris floribunda*

Different concentration of extract	Ethanol extract of *C. floribunda*	Standard Vitamin-C
25 µl/ml	22.31±0.37	18.34±1.34
50 µl/ml	39.64±1.78	36.34±0.37
75 µl/ml	58.31±0.36	53.64±2.37
100 µl/ml	77.32±2.34	73.61±1.78
EC_{50} Value	58.32±1.78	61.34±1.59

Results are expressed as percentage radical scavenging activity ABTS formation with respect to control. Each value represents the mean+SD of three experiments.

Inhibition of Lipid Peroxidation

Within the display consider, egg yolk was used as substrate for gratis radical interceded lipid peroxidation, which may be a non-enzymatic method. Ethanol extract of *C. floribunda* a too repressed the lipid peroxidation initiated by ferrous sulfate in egg yolk homogenates. Greatest inhibition was recorded in ethanol extract of C. floribunda 72.32% with EC_{50} esteem 64.32 µl/ml and least inhibition rate ascorbic acid 67.32% with EC_{50} 66.32 (Table-3). Because it is recognized that lipid peroxidation is the net result of any free radical attack on layer and other lipid components show within the framework, the lipid peroxidation may be enzymatic (Fe/NADPH) or non-enzymatic (Fe/ascorbic acid). Ordinarily, the instrument of flavonoid compounds for neutralizing lipid free radicals and avoiding decay of hydroperoxides into free radicals (Mill operator, 1996).

Table 3: Inhibition of lipid peroxidation activity of ethanol extract of *Calycopteris floribunda*

Different concentration of extract	Inhibition percentage of Lipid peroxidation	
	Ethanol extract of *Calycopteris floribunda*	Standard Vitamin-C
25 µl/ml	18.32±1.89	16.34±0.36
50 µl/ml	33.64±2.78	30.34±0.78
75 µl/ml	54.32±2.37	48.32±1.78
100 µl/ml	72.32±1.89	67.32±0.23
EC_{50} value	64.32	66.32

[a] Results are expressed as percentage inhibit of lipid peroxidation with respect to control. Each value represents the mean+SD of three experiments.

Nitric Oxide Radical Scavenging

Ethanol extract of *C. floribunda* appeared a solid nitric oxide scavenging activity which was comparable to the standards ascorbic acid.

The EC_{50} esteem 69.32 of ethanol extract of C. floribunda was less than ascorbic acid 74.32%. Rate of Nitric oxide radical scavenging movement ethanol extract of C. floribunda and benchmarks were displayed in Table-4. Within the current consider, nitrite was create by brooding of sodium nitroprusside in standard phosphate saline buffer at 25°C was diminished by ethanol extract of C. floribunda. Noteworthy rummaging action may be due to the antioxidant property of flavonoid, compounds display in ethanol extract of *C. floribunda*, which compete with oxygen to respond with nitric oxide, driving to less generation of nitric oxide.

Table 4: Nitric oxide radical scavenging assay of ethanol extract of *Calycopteris floribunda*

Different concentration of extract	Percentage of Nitric oxide radical scavenging activity	
	Ethanol extract of *Calycopteris floribunda*	Standard Vitamin-C
25 µl/ml	23.64±2.34	19.34±1.48
50 µl/ml	41.34±1.37	38.64±1.37
75 µl/ml	63.32±1.78	55.34±2.37
100 µl/ml	79.32±0.88	76.32±0.34
EC_{50} value	69.32	74.32

[a] Results are expressed as percentage of Nitric oxide radical activity with respect to control. Each value represents the mean + SD of three experiments.

Conclusions

The display consider shown that the ethanol extract of C. floribunda had most noteworthy phenolic and flavonoid compound and exhibuted solid antidiabetic and antioxidant activities, which were comparable to the commercial antidiabetic medicate glycomet and antioxidant ascorbic acid. This appears that the ethanol extract of C. floribunda can be utilized as common antidiabetic and antioxidant specialist.

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