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PRELIMINARY PHYTOCHEMICAL SCREENING OF VARIOUS EXTRACTS OF WHOLE PLANT OF *CALYCOPTERIS FLORIBUNDA* LAM.

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ABSTRACT

Medicinal plants are a rich source of bioactive phytochemicals or bionutrients. Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since entiquity.The present study was designed to investigate the presence of various phytochemicals in the three different extracts of the whole plant of *Calycopteris floribunda* which evokes various therapeutic effect. The whole plant of *Calycopteris floribunda* were extracted with the appropriate three solvents (Harborne, 1984) and these were evaluated for the qualitative phytochemical analysis and these can be further studied for the novel compounds and their biological activities. The petroleum ether and methanol extracts have much lesser compounds when compared with ethyl acetate extracts. When compared to the three extracts, ethyl acetate extracts have high affinity towards the biological activities. The qualitative phytochemical analysis mainly focused on different chemical compounds which can be useful for the drug discovery and effective medicine improvement from the natural resources. The ethyl acetate extract has more phyto constituents than the remaining solvents ,contains phenols, flavonoids, steroids, tannins, saponins and reducing sugars.Our findings provided evidence that ethyl aceate extracts of the tested plant *Calycopteris floribunda* contain

medicinally important bioactive compounds and it justifies their use in the traditional medicine for treatment of different diseases.

Key words: *Calycopteris floribunda*, Phytoconstuients, Whole plant, Phytochemical Qualitative Tests.

Introduction

The importance of plants is known to us well. The plant kingdom treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substance include tannin alkaloids, carbohydrate, terpenoids, steroids and flavonoids (Mathai 2000 and Mann 1978) These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxificationen enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. Herbal medicines have been used for so many years that have lesser side effects and less priced than the synthetic medicine. The Active compounds of many drugs found in plants are secondary metabolites (Dobelis 1993 and Edoga 2005) For a long period of time medicinal plants or their secondary metabolites have been directly or indirectly playing an important role in the human society to combat diseases. In the early century of mankind, plant derived secondary metabolites have been used by humans to treat acute infections, health disorders and chronic illness. Only during the last 100 years natural products have been largely replaced by synthetic drugs (Gibson 1998)

Phytochemicals are not essential nutrients and are required by the human body for sustaining life, but have important properties to prevent or to fight some common diseases.They protect plants from disease and damage and contribute to the plants color, aroma and flavor. In general, the plant chemicals from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals. Natural products are the source of synthetic and traditional herbal medicine.The medicinal importance of plant due to the presence of some special substances like alkaloids, glycosides, tannins, flavonoids, saponins etc., Soxhlet apparatus was used for the organic extraction. Solvents used were petroleum ether , ethyl acetate and methanol.

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(Chopra 1956,WHO 2001 Orient Longman 1995) The leaves have reported to posses anti-diabetic activity(Sreenu *et al* .,2012 The hepato protective activity of various stem and

leaf extracts have been reported(Chinna *et al* .,2010, Thalla *et al.*, 2011) and even fruits claimed to treat jaundice. Calycopterone, Isocalycopterone and 4-dimethyl-calycopterone showed a wide range activity against solid cell lines(Wall *et al.*, 1994)

As far as our literature survey could ascertain, no reports concerning the phytochemical investigation of the whole plant of *C.floribunda* given here. Therefore we undertook the present work to screen qualitatively the various extracts of the whole plant of *C.floribunda* through various methods. The Main objective of the present study was to investigate the active phytoconstituents present in various extracts *Calycopteris floribunda* (Lam.)

Materials and methods

Collection and identification of *Calycopteris floribunda* (Lam.)

The whole plant of *Calycopteris floribunda* (Lam.), was collected from, Pulliyankudi, Nellai District of Tamil nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India, Palayamkottai. The whole plant of *Calycopteris floribunda* (Lam.) was dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered plant materials were stored in an airtight container.

Preparation of Various extracts from *Calycopteris floribunda* (Lam.)

The powdered plant materials were successfully extracted with petroleum ether (40-60 ºC) by hot continuous percolation method in Soxhlet apparatus (Harborne, 1984). for 24 hrs. Then the marc was dried and then subjected to ethyl acetate extraction (76-78ºC) for 24 hrs, then marc was dried and then it was subjected to methanol extraction (80ºC) for 24 hrs. The solvent from the extracts was recovered under reduced pressure using rotary evaporator and subjected to freeze drying in a lyophilizer until dry powder was obtained.

Calculation of percentage

The percentage yield was calculated for the extracts and major compounds with reference to the crude material taken using the formula given below.

$$\text{Percentage yield with reference to crude plant material} = \frac{\text{Weight in grams of extracts obtained}}{\text{Weight in grams of plant material taken}} \times 100$$

The extract was subjected to preliminary phytochemical screening for the detection of various plant constituents present. The term qualitative analysis refers to the establishing and providing the identity of a substance. The pharmacological action of crude drugs was determined by the nature of their constituents. The phytoconstituents are responsible for the desired therapeutic properties. To obtain these pharmacological effects, the plant materials itself or extract in a suitable solvent or isolated active constituent may be used. The various extracts of *Calycopteris floribunda* were subjected to the following chemical tests separately for the identification of various active constituents (Evans, 1997).

Tests for Alkaloids (Evans, 1997)

a) Dragondroff's test

A fraction of the extract was treated with Dragondroff's reagent and observed for the formation of yellow colored precipitate.

b) Wagner's test

A fraction of the extract was treated with Wagner's reagent and observed for the formation of yellow colored precipitate.

c) Mayer's test

A fraction of the extract was treated with Mayer's reagent and observed for the formation of yellow colored precipitate.

d) Hager's test

A fraction of the extract was treated with Hager's reagent and observed for the formation of yellow colored precipitate.

Test for Carbohydrates (Evans, 1997)

a) Molisch test

To 2ml of the extract, 1 ml of α-naphthol solution was added, and concentrated sulphuric acid through the sides of test tube. Purple or reddish violet color at the junction of the two liquids revealed the presence of carbohydrates.

b) Fehling's test

To 1ml of the extract, equal quantities of Fehling's solution A and B were added, upon heating formation of a brick red precipitate indicated the presence of carbohydrates.

c) Benedict's test

To 5 ml of Benedict's reagent, 1ml of extract solution was added and boiled for 2 minutes and cooled. Formation of a red precipitate showed the presence of carbohydrates

Tests of Glycosides (Evans, 1997)

a) Legal test

The extract was dissolved in pyridine and sodium nitroprusside solution was added to make it alkaline. The formation of pink red to red color showed the presence of glycosides.

b) Baljet test

To one ml of the test extract was added with one ml sodium picrate solution and the yellow to orange color revealed the presence of glycosides.

c) Borntrager's test

A few ml of dilute HC1 was added to 1ml of the extract solution. It was then boiled, filtered and the filtrate was extracted with chloroform. The chloroform layer was they treated with 1 ml of ammonia. The formation of red color showed the presence of anthraquinone glycosides

d) Keller Killiani test

The extract was dissolved in acetic acid containing traces of ferric chloride and it was then transferred to a test tube containing sulphuric acid. At the junction, formation of a reddish brown color, gradually became blue, confirmed the presence of glycosides.

Tests for Phytosterol (Finar, 1986)

a) Libermann Burchard test

3 ml of test solution was mixed with 3 ml of acetic acid anhydride was heated and then cooled. Few drops of concentrated sulphuric acid were added. A blue color appeared.

b) Salkowski test

The extract was dissolved in chloroform and equal volume of concentrate sulphuric acid was added. Formation of bluish red to cherry red color in chloroform layer and green fluorescence in the acid layer represented the steroid components in the tested extract.

Test for Flavonoids (Dey and Harborne, 1987 Evans, 1989

a) Shinoda test

The dried powder or extract was treated with 5 ml of 95% ethanol. Few drops of concentrated hydrochloric acid and 0.5 g of magnesium turnings were added. Development of pink color indicates the presence of flavonoids.

Test for Tannins and phenolic compounds (Mace, 1963)

a) Ferric chloride test

One ml of the extract was added with ferric chloride and observed the formation of a dark blue or greenish black color.

b) Gelatin test (Evans, 1997)

A fraction of the extract was treated with 1% gelatin containing 10% NaCl and observed for the precipitation.

Test for proteins and Amino acids (Evans, 1997)

a) Biuret test

One ml of the extract was treated with 1ml of 40% sodium hydroxide solution followed by 2 drops of 1% copper sulphate solution. Formation of a violet color showed the presence of proteins.

b) Xanthoprotein test

One ml of the extract was treated with 1ml of concentrated nitric acid. A white precipitate formed was boiled and cooled. Then 20% of sodium hydroxide or ammonia was subsequently added. Formation of orange color indicated the presence of aromatic amino acids.

c) Lead Acetate test

A fraction of extract was treated with 1 ml of lead acetate. Formation of white precipitate indicated the presence of proteins.

Test for Saponins (Evans, 1997)

About 1 ml of methanol extract was diluted separately with distilled water to 20 ml, and shaken in a graduated cylinder for 15 minutes. One cm layer of foam indicated the presence of saponins.

Test for Fixed Oils

a) Spot test

A small quantity of extract was pressed between two filter papers. Oil stains on the filter paper indicated the presence of fixed oils.

b) Saponification test

One ml of extract was added with few drops of 0.5 N alcoholic potassium hydroxide along with a drop of phenolpthalein. The mixture was heated on a water bath for 1-2 hours. The formation of soap or partial neutralization indicated the presence of fixed oils.

RESULTS AND DISCUSSION

Preparation of Extracts and the Calculation of Percentage Yield of *Calycopteris floribunda*

The various extracts of whole plant of *Calycopteris floribunda* screened for the presence of various bioactive phytochemical compounds. Specific qualitative tests were performed to identify bioactive compounds of pharmacological importance through standard methods. The powdered plant materials were successively extracted with petroleum ether (40-60°C) by hot continuous percolation method in soxhlet apparatus for 24hrs. The extracts were stored in screw cap vials until further use. The percentage yields of the whole plant of *Calycopteris floribunda* are shown in Table1. Continuous hot percolation method of extraction of the whole plant of *Calycopteris floribunda* yields5.16% w/w, 14.52%w/w and 9.70%w/w in petroleum ether, ethyl acetate and methanol extracts respectively. The highest yield was obtained in ethyl acetate extract followed by methanol extract.

The various extracts of *Calycopteris floribunda* screened for the presence of various bioactive photochemical compounds. Specific qualitative tests were performed to identify bioactive compounds of pharmacologically importance through standard methods. These tests were helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compound. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, phytosterols etc.

Phytosterols, fixed oil and fats were present in the petroleum ether extracts of *Calycopteris floribunda* . The ethyl acetate extractrevealed the presence of alkaloids, flavonoids, carbohydrates ,glycosides, phenols, saponins, phytosterols, protein, amino acid and fixed oils.. Alkaloids, carbohydrates and glycosides, saponins, phenolic compounds and tannins, protein and amino acid and flavonoids were found in the methanol extract of whole plant of *Calycopteris floribunda.* From this analysis, ethyl acetate extract of *C.floribunda* was found to have more constituents compared to other two extracts. So, the ethyl acetate extract was ascertained as suitable solvent for the extraction of most of the phytochemicals in this experimental plant (Table 1.2).

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. The active principles usually remain concentrated in the storage organs of the plants (Sony *et al.,* 2011) Phytochemicals give plants their color, flavour, smell and are part of a plant`s natural defense system and protect them against herbivorous insects and vertebrates, fungi, pathogens, and parasites(Ibrahim *et al.,* 2010 Sherman and Billing 1999

Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups (Sandhyarani *et al.,* 2011)Therefore, the phenolic content of plants may contribute directly to their antioxidant action. Literature survey has revealed a direct relationship between antioxidant activity and total phenolic content. This study has supported that phenols present contribute to antioxidant activity. Phenolics are secondary metabolites that play a role in the maintenance of the human body. This study reveals presence of phytoconstituents, such as phenols, flavonoids and tannin in plants, indicates the possibility of antioxidant activity and this activity will helps in preventing a number of diseases through free radical scavenging activity. Phenolics compound the principal antioxidant constituents of natural plant products are composed of phenolic acid and flavonoids (Gulcin *et al.,* 2004) In this respect, polyphenolic compounds commonly found in plants have been reported to have multiple biological effects like anticancer, antiproliferative, antimicrobial, wound healing and antibacterial activities including antioxidant activity (Sreeram *et al.,* 2005 Supayarg *et al.,* 2004) The plant undertaken for this study also has all the above mentioned activities.

The polyphenolic compound like flavonoids commonly found in plants have been reported to have multiple biological effects, including

antioxidant activity (Brown *et al.*1998, Gil *et al.*, 1999, Kahkonen *et al.*, 1999, Vinson *et al.*, 1995). It is reported that polyphenols the secondary plant metabolites play a role in the protection of plants against ultraviolet radiation, pathogens and herbivores (Harborne and Williams, 2000). The flavonoids commonly found in this plants *Calycopteris floribunda* to have several biological activities including antioxidant properties.The flavonoids have been reported to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities (Aiyelaagbe and Osamudiamen, 2009)

Rajasekaran and Periasamy 2012) reported the presence of terpenes, steroids, tannins and flavonoids in alcoholic leaf extract of *Calycopteris floribunda*. In this present study, alkaloids, saponins and carbohydrates were also identified along with the above mentioned plant constituents. Five bioflavonoids were identified by Mayer (2004) from the green leaves have been isolated from *Calycopteris floribunda* (Mayer 1999; Wall *et al.*, 1994, Dey *et al.*, (2003) isolated steroid and terpene from the leaves of *Calycopteris floribunda*. Thalla and Pentela (2011) revealed the presence of alkaloids, carbohydrates, phytosterols, glycosides, saponins, tannins and phenolic compounds in hydroalcoholic leaf extracts of *C.floribunda.*Secondary metabolites may be used for the preparation of drug in a systematic way which may lead to the cure of many ailments in the future (Shanthi and Amudha, 2010).

The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. The steroids are known to be important for their cardiotonic activities and also insecticidal and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics (Callow, 1936).

Tannins and flavonoids are compounds that act as primary antioxidants or free radical scavengers. Since these compounds were present in the extract it might be responsible for the potent antioxidant capacity of *Calycopteris floribunda.* The Tannins were reported to exhibit antiviral, antibacterial and anti-tumour activities. It was also reported that certain tannins were able to inhibit HIV replication selectively and was also used as diuretic (Callow,1936).The plant selected for this may have the mentioned activity due to its presence of tannins and flavonoids.

The secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value.For example, Saponins have hypotensive and cardiodepressant properties (Olaleye, 2007) In medicine, it is used in hypercholestrolaemia, hyperglycaemia, antioxidant, anticancer, antiinflammatory, weight loss, etc. It is also known to have antifungal properties(Haslam,1989). Glycosides are naturally cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythnica (Brian *et al.*, 1985)The presence of saponins and glycosides in the extracts might play a role in the cardioprotective potential of *Caycopteris floribunda.*

It has been evaluated that phytosterols can reduce cancer risk, by various trials.Various studies suggests

that phytosterols can play an important role in the prevention of several types of cancer such as lung, stomach, prostate, ovarian and breast cancer (Santans *et al.,* 2013). It is reported that many mechanisms by which phytosterols show protective effect against cancer such as inhibition of the production of carcinogens, cancer cell growth, invasion and metastasis and promote apoptosis of cancerous cells (Woyengo *et al.,* 2009) This plant *Calycopteris floribunda* might have cancer protective effects due to the presence of phytosterol in its extracts. Research work done on cholesterol lowering ability of phytosterols at the intestinal level (Santans *et al.,* 2013). So this plant might possess antihyperlipidemia activity and can be recommended for its usage as lipid reducing medicine. The research article explained in detail the potential concern and benefits of phytosterols in prevention of various diseases (Dhande *et al.,*2015) In addition to being rich in fiber and plant protein, the diets of our ancestors were also rich in phytosterols—plant-derived sterols that are similar in structure and function to cholesterol. There is increasing evidence that the reintroduction of plant foods providing phytosterols into the modern diet can improve serum lipid (cholesterol) profiles and reduce the risk of cardiovascular disease (Kendall and Jenkins, 2004). Cholesterol in human blood and tissues is derived from the diet as well as endogenous cholesterol synthesis. In contrast, all phytosterols in human blood and tissues are derived from the diet because humans cannot synthesize phytosterols(Sudhop *et al.,* 2005)

Our results were in accordance with the findings who reported ethyl acetate to be the best solvent for the extraction of antioxidant compounds (Shah *et al.,* 2015). It would thus mean that in this study, the ethyl acetate had the highest number of bioactive compounds. Since the yield of bioactive metabolites in a plant extract also varies considerably with the method/solvent of extraction (Clark *et al.,* 1997 and Marston *et al.,* 1993). It is plausible that ethyl acetate were generally more potent than other two extracts probably because the active principles in the plant dissolved more readily in better extracted by the solvent.

Table 1.1. Percentage yield of the whole plants of *Calycopteris floribunda* by continuous hot percolation method

Solvent system	Percentage yield (%w/w)
Petroleum ether	5.16
Ethyl acetate	14.52
Methanol	9.70

Table 1.2.Phytochemical analysis of various extracts of *Calycopteris floribunda*

S.No.	Test	Petroleum ether	Ethyl acetate	Methanol
1.	Alkaloids	-	+	+
2.	Carbohydrates and glycosides	-	+	+
3.	Phytosterols	+	+	-
4.	Fixed oil and fats	+	+	-
5.	Saponins	-	+	+
6.	Phenolic compounds and tannins	-	+	+
7.	Protein and Amino Acid	-	+	+
8.	Gum and Mucilage	-	-	-
9.	Test for flavonoids	-	+	+

+ Present
- Absent

Conclusion

In the present study, we have found that most of the biologically active phytochemicals were present in ethyl acetate extract of the whole plant of *Calycopteris floribunda* .Since the ethyl acetate extract of the whole plant of *Calycopteris floribunda* contains more constituents it can be considered beneficial for further investigation.With the advances in synthetic methodology and the development of more sophisticated isolation and analytical techniques, all the above mentioned phytochemicals present in the extract could be isolated. So it is concluded that the presence of phytoconstituents make this plant useful for treating different ailments and have a potential of providing useful drugs of human use. 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. In Conclusion , the results obtained herein have been discussed in details to document merits of ethyl acetate of *Calycopteris floribunda* as a therapeutic agent which can be used in preventing the progress of oxidative stresses may be due to the presence of large amount of phenolic compounds.

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