Vegetos
https://doi.org/10.1007/s42535-024-00872-w

RESEARCH ARTICLES

Physico-chemical evaluation and phytochemical analysis of different leaf extracts of *Calycopteris floribunda* Lam. a multipotent medicinal climber by GC–MS

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Received: 24 April 2023 / Revised: 13 June 2023 / Accepted: 15 March 2024

Abstract
Calycopteris floribunda Lam. is a woody climber belongs to Combretaceae, commonly found in tropical and sub tropical forests of Southeast Asia. Leaves are used in the treatment of intestinal worms, colic, leprosy, malarial fever, snake bites and burned wounds. Flovonoids such as Calycopterin and Pachypodol isolated from leaves are reported to have anticancerous activity. Physico-chemical study of plant gives the standard parameters for the purity and quality of the drugs. Therefore, the present study is carried out to evaluate the Physico-chemical parameters and phytochemicals present in leaf extracts by using GC–MS. Different physico-chemical characters (ash values, loss of moisture, foaming index, pH and extractive yields) of the leaf were studied. The total phenolics and flavoinoid content of ethanolic leaf extract were found to be 472.66 ± 2.51 mg GAE/ g DW and 303.00 ± 3.60 mg QE/g DW, respectively. FTIR spectrum showed the presence of functional groups such as O–H, C = C, N–O S = O, and C-O. Further, GC-MS analysis showed the presence of over all 31 compounds, of which, 10 in chloroform extract, 9 in ethanol extract, 7 in petroleum ether extract and 5 compounds are in acetone extract. Few compounds were common in two and three extracts. 1,2-benzenedicarboxylic acid is one of the major compounds present in three extracts except ethanol, squalene (35.09%) is the major compound in the ethanol extract.

Keywords *Calycopteris floribunda* Lam. · FTIR · GC–MS · Physico-chemical · Phytochemical analysais · Secondary metabolites

Introduction

Medicinal plants have been used in treating various ailments and are natural sources for medicinally important secondary metabolites. Plant based medicines are reliable, cost effective with less side effects compared to chemical drugs (Zimila et al. 2020). About 80% of population of the world uses plant drugs as the primary treatment for various disorders. Many extracts from medicinal plants are used in traditional practices (Enerijiof et al. 2021). The World Health Organization (WHO) promotes the use of plant based medicine in developing countries, as secondary metabolites present in plants are having different biological activities like antioxidant, antimicrobial, anxiolytic and anti-inflammatory (Nagannawar and Jayaraj 2020). Analysis of secondary metabolites is very important to know the responses of the plants to environmental conditions. GC–MS analysis is used as one of the primary tools for secondary metabolite profiling of plant extracts, especially in the analysis of a single sample and in determining important metabolites like sugars, amino acids and organic acids (Abadie et al. 2022).

Calycopteris floribunda Lam. of Combretaceae is a large woody climber endemic to tropical forests of south east Asia and it is found in the central and southern parts of India. It is used in Asian medicinal practices like Ayurveda, folklore and Unani. The leaves have various medicinal properties and are used in the treatment of intestinal worms, colic, leprosy, malaria, dysentery, ulcers, vomiting (Bharat 2019), snake bites (Gomes et al. 2010), burned wounds (Shivanna and Rajakumar 2011). The stem and leaf extracts are used to prepare roborans tonic (Liu et al. 2009), and leaves are mixed with leaves of *Jasminum malabaricum* to calm the

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Published online: 15 April 2024

Asthmatic attack (Bhandary et al.1995). The aqueous extract of the leaves is reported to have antifungal activity (Vinayaka et al. 2009). Leaves also have anthelminthic activity (Ratnagiriswaran et al. 1934). Calycopterone, isocalycopterone and 4-demethylcalycopterone isolated from leaves showed activity against tumor cell lines (Mayer 1999). Calycopterin a flavonoid isolated from leaves showed anthelmintic, antiviral and anticancerous activity (Liu et al. 2009). Pachypodol is also flavonoid isolated from leaves showed anticancerous activity (Ali et al. 2008).

Though the leaves of *C. floribunda* have many medicinal properties, only phytochemical studies of the essential oils of leaf extracts and selected compounds from the leaf extracts have been reported (Mayer 1999; Ali et al. 2008; Liu et al. 2009). However, there are no reports on the overall phytochemical profiling of crude extracts of the leaves of *C. floribunda.* Hence, the present work is undertaken to study the physico-chemical parameters and to identify phytochemicals by GC–MS.

Material and methods

Plant material

The leaves of *Calycopteris floribunda* Lam. (Voucher specimen no: KUD/BOT/SY/MJ/02) were collected during the month of June 2021 from the plants grown in Karnatak University campus, Dharwad (15° 43′ 74.98″ N, 74° 98′ 36.86″ E), Karnataka, India (Fig. 1).

Extraction

The healthy leaves were collected and shade dried for 25 to 30 days to remove the moisture. Further, it is ground into powder by using electronic grinder and stored in an air tight zipper bag for further use (Fig. 2). Finely powdered sample (10 g) was used to extract the different organic solvents (the increasing polarity) such as petroleum ether, chloroform, acetone and ethanol for 18 h by using Soxhlet apparatus (Nagannawar and Jayaraj 2020).

Physico-chemical study

Physico-chemical parameters like ash value, moisture content, fat content, foaming index, pH and extractive values of leaf was studied by following standard procedures (Nissar et al. 2021).

Preliminary phytochemical analysis

Leaf extracts in different organic solvents (petroleum ether, chloroform, acetone and ethanol) are used for preliminary analysis of secondary metabolites by using the standard procedures. Tests are conducted for the analysis of carbohydrates (Molisch's test), reducing sugar (Fehling's test and Benedict's test), protein (Million's test and Biuret's test), amino acids (ninhydrin test), glycosides (anthrone test), phenols (ferric chloride test), tannins (gelatin test), flavonoids (Shinoda's test) and alkaloids (Dragendorff's test, Wagner's test and Mayer's test) (Banni and Jayaraj 2022).

Determination of total phenolic content

Phenolic content of leaf extract was determined by using Singleton et al. (1999) method. About 0.1 mL of leaf extract was diluted using distilled water (3 mL) and mixed with Folin-Ciocalteu reagent (0.5 mL) and incubated for 3 min. Further, 20% sodium carbonate (2 mL) was added and the absorbance was recorded at 760 nm after 30 min of incubation using UV–visible spectroscopy (Hitachi U-3000). The results obtained were expressed in mg of gallic acid

Fig. 1 Branch with leaves of *Calycopteris floribunda* Lam.

Fig. 2 Leaf powder of *Calycopteris floribunda* Lam.

Table 1 Physico-chemical parameters of leaf of *Calycopteris floribunda* Lam.

Parameters	Observations
Total ash (%)	8.433 ± 0.305
Acid insoluble ash (%)	0.233 ± 0.057
Water insoluble ash (%)	4.10 ± 0.264
Loss on drying (%)	12.340 ± 0.250
Foaming index	100
pH (1%)	5.65 ± 0.030
pH (10%)	5.153 ± 0.015

Result expressed as mean ± SD of n = 3

equivalent to per gram of extract (mg GAE/g) using calibration curve for gallic acid standard solutions.

Determination of total flavonoid content

Total flavonoid content of the leaf extract was determined using the aluminium chloride colorimetric method. In this method, 0.1 mL of leaf extract, 2% (w/v) $AlCl_3$ (1 mL), 5% (w/v) and sodium acetate (3 mL) were mixed thoroughly. After incubation for 120 min, the absorbance was read at 440 nm. Results were expressed as mg of quercetin equivalent to per gram of extract (mg QE/g). Quercetin was used to make a standard calibration curve (Safari and Ahmady-Asbchin 2019).

FTIR spectroscopic analysis

1.0 mg of leaf powder was compressed into KBr pellets and scanned using FTIR within the range of 400 to 4000 cm^{-1} with resolution of 4.0 cm^{-1}. The functional groups were

Table 2 Extractive yield (%) of leaf of *Calycopteris floribunda* Lam.

Solvents	Cold extraction (%)	Hot extraction (%)	Successive extraction (%)
Petroleum ether	1.196 ± 0.035	2.0 ± 0.100	1.853 ± 0.251
Chloroform	3.633 ± 0.152	6.900 ± 0.200	4.800 ± 0.100
Acetone	9.033 ± 0.152	17.533 ± 0.025	5.800 ± 0.030
Alcohol	10.843 ± 0.025	18.856 ± 0.020	4.066 ± 0.208
Water	6.366 ± 0.251	19.966 ± 0.105	0.660 ± 0.144

Result expressed as mean ± SD of n = 3

Table 3 Preliminary phytochemical analysis of leaf extracts of *Calycopteris floribunda* Lam.

Sl. no	Test		Petroleum Ether	Chloroform	Acetone	Alcohol	Water
1	Carbohydrates	Molish's test	+	+	+	+	+
2	Reducing Sugars	Fehling's test	−	+	+	−	+
		Benedict's test	−	+	+	+	+
3	Hexose Sugar	Tollen's Phloroglucinol test	−	−	−	−	−
		Iodine test	−	−	−	−	−
4	Proteins	Biuret's test	+	−	−	−	−
		Million's test	+	−	−	−	−
5	Amino Acid	Ninhydrin's test	+	−	−	−	−
6	Protein containing Sulphur	Lead Acetate test	−	−	−	−	−
7	Steroids	Salkwaski's test	+	+	+	+	+
8	Glycosides	Anthrone test	−	-	−	−	−
9	Saponin's test		+	+	−	−	+
10	Coumarin's glycoside	NaOH test	+	+	+	+	-
11	Alkaloids	Dragondroff's test	+	+	+	+	+
		Wagner's test	+	+	+	+	+
		Mayer's test	+	+	+	+	+
12	Phenols	Ferric Chloride test	+	+	+	+	+
13	Tannins	Gelatin's test	+	−	−	−	+
14	Organic Acids		−	−	−	+	+
15	Triterpenoids		+	+	+	+	+

Table 4 Total Phenolics content,and flavonoids content of ethanolic leaf extract of *Calycopteris floribunda.* Lam.

Sample	TPC (mg GAE/g DW)	TFC (mg QE/g DW)
Ethanol leaf extract	472.66 ± 2.51	303.00 ± 3.60

Result expressed as mean ± SD of n = 3

identified using sigma Aldrich IR spectrum table (Silva da Costa et al. 2022).

GC-MS analysis

GC–MS analysis of different leaf extracts of *C. floribunda* was carried out using Shimadzus (QP2010S) GC–MS equipped with ELITE-5MS column which is 30 m long, having 0.25 mm inner diameter and a 0.25 µm thickness. Helium was used as a carrier gas with the flow rate of 1 mL/min and an injection temperature of 280 °C. The initial temperature of the analysis was kept at 80 °C for 5 min and then the temperature was gradually increased at a rate of 10 °C/min till it reached 280 °C and the same temperature was maintained for 5 min. Samples were kept for analysis for 50 min in GC–MS column. The samples were injected in split mode with a pressure of 65.0 kPa of the carrier gas with the split ratio of 1:20. All data obtained are within the scan range of 50–500 m/z. The NIST 11 and WILEY 8 libraries were used to identify the compounds by comparing their retention values (Nagannawar and Jayaraj 2020; Banni and Jayaraj 2022).

Statistical analysis

Results were obtained in triplicate and expressed as mean ± standard deviation (SD). GC–MS results obtained in this study were analyzed by principal component analysis (PCA) to determine correlations between the biological activities of secondary metabolites of leaf extracts using IBM SPSS ver. 20 software.

Results and discussion

Physico-chemical study of plants gives the standard parameters for the purity and quality of the drugs. The ash content of the drugs helps to determine the inorganic chemicals like the oxides of Ca, Mg, K, Na, Si, P and Fe. These can be used as the parameters for the purity and authentication of drugs (Papiya, et al. 2014). In the present study the total ash value, acid insoluble ash value and water insoluble ash value of the leaves of *C. floribunda* were calculated, the results obtained show that the water soluble ash value is higher than to the acid insoluble ash value which indicated that leaf powder of the plant has more organic contents (Table 1).

Loss of moisture on drying is one of the basic parameters used to determine the quality of the drug. Thus the minimum value of the moisture content of the crude drug should be less than 14% to avoid any microbial contamination. The loss of moisture on drying of the leaf powder is below 14%. The formation of foam by crude drugs when dissolved in water is due to the presence of saponins, this foam formation is measured as the foaming index and

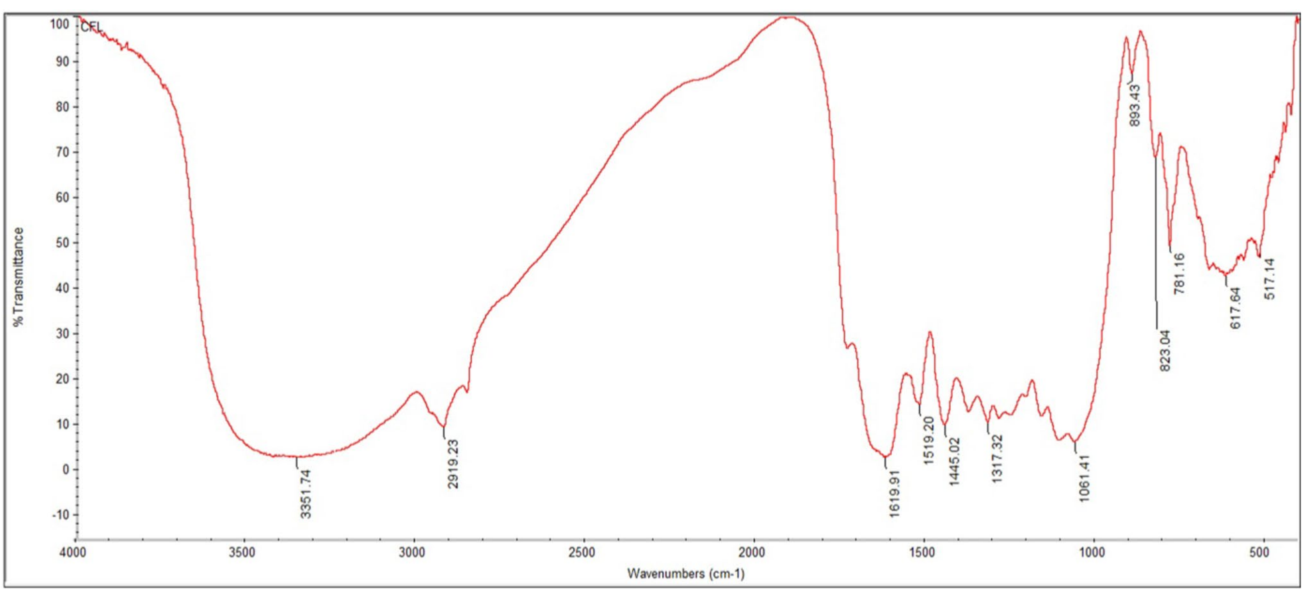


Fig. 3 FTIR spectrum of *Calycopteris floribunda* Lam. leaf powder

Table 5 GC–MS analysis of petroleum ether leaf extract of *Calycopteris floribunda* Lam.

Peak	R. Time	Area %	Molecular Formula	Molecular Weight (g/mol)	Compound Name
1	27.946	1.40	$C_{20}H_{40}O$	296.5	Phytol
2	32.883	6.83	$C_{30}H_{50}O$	426.7	Lupeol
3	35.203	34.09	$C_8H_6O_4$	166.13	1,2-benzenedicarboxylic acid
4	39.188	24.23	$C_{30}H_{50}$	410.7	Squalene
5	40.063	9.49	$C_{25}H_{52}$	352.7	Pentacosane
6	43.263	17.56	$C_{44}H_{90}$	619.2	Tetratetracontane
7	48.950	6.41	$C_{29}H_{50}O$	414.7	Gamma.-Sitosterol
		100			

Table 6 GC–MS analysis of chloroform leaf extract of *Calycopteris floribunda* Lam.

Peak	R. Time	Area %	Molecular Formula	Molecular Weight (g/mol)	Compound Name
1	17.518	2.91	$C_{14}H_{22}O\ _6$	206.32	2,4-ditert-butylphenol
2	23.621	1.53	$C_{19}H_{38}$	266.5	1-nonadecene
3	24.596	2.75	$C_{20}H_{38}$	278.5	Neophytadiene
4	27.330	1.89	$C_{16}H_{22}O_4$	278.34	Dibutyl phthalate
5	27.841	2.27	$C_{17}H_{32}O$	252.4	E-15-heptadecenal
6	34.044	1.90	$C_{21}H_{42}$	294.6	10-heneicosene (c,t)
7	39.152	1.62	$C_{19}H_{35}F_3O_2$	352.5	Heptadecyl trifluoroacetate
8	42.319	70.65	$C_8H_6O_4$	166.13	1,2-benzenedicarboxylic acid
9	44.640	6.91	$C_{29}H_{50}O$	414.7	Beta.-sitosterol
10	45.580	7.58	$C_{29}H_{50}O$	414.7	Methyl commate c
		100			

Table 7 GC–MS analysis of acetone leaf extract of *Calycopteris floribunda* Lam.

Peak	R. Time	Area %	Molecular Formula	Molecular Weight (g/mol)	Compound Name
1	17.433	1.37	$C_{20}H_{38}$	278.5	Neophytadiene
2	17.882	0.55	$C_{20}H_{38}$	278.5	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
3	20.169	4.73	$C_{20}H_{40}O$	296.5	Phytol
4	24.321	85.77	$C_8H_6O_4$	166.13	1,2-benzenedicarboxylic acid
5	28.745	7.59	$C_{30}H_{50}$	410.7	Squalene
		100			

used as a parameter for standardizing the quality of the drug (Nissar et al. 2021). In the present study, the foaming index of leaf powder is 100. The pH of the drug gives the ionic nature of the drug, in the present study on the leaf powder pH value is slightly acidic in nature (Table 1).

Extractive yield of the plant extracts is considered one of the important physico-chemical parameters for standardizing for quality of the plant drugs, the extractive yield gives information regarding the phyto-constituents of the extracts and in selection of the better suitable solvents for the extraction of the crude drugs (Aslam and Afridi 2018). In the present work three types of extraction were carried out. The results obtained showed the presence of more polar compounds in the leaves of *C. floribunda* have (Table 2).

Preliminary phytochemical analysis

The preliminary phytochemical analysis of leaf extracts showed the presence of carbohydrates, steroids, alkaloids, phenols and triterpenoids in all the solvents used in the present study (Table 3). Reducing sugars were present in chloroform, acetone and ethanol extracts, protein and amino acids are present only in petroleum ether. Saponins

Table 8 GC–MS analysis of ethanol leaf extract of *Calycopteris floribunda* Lam.

Peak	R. Time	Area %	Molecular Formula	Molecular Weight (g/mol)	Compound Name
1	16.658	13.53	$C_{20}H_{38}$	278.5	Neophytadiene
2	17.332	4.88	$C_{20}H_{40}O$	296.5	3,7,11,15-tetramethyl-2-hexadecen-1-ol
3	19.041	6.64	$C_{16}H_{22}O_4$	278.34	Dibutyl phthalate
4	422.339	5.35	$C_{20}H_{40}O$	296.5	Phytol
5	33.026	2.70	$C_{44}H_{90}$	619.2	Tetratetracontane
6	35.008	35.09	$C_{30}H_{50}$	410.7	Squalene
7	35.930	3.86	$C_{23}H_{48}$	324.6	Tricosane
8	44.818	14.19	$C_{29}H_{50}O$	414.7	Gamma.-sitosterol
9	47.321	13.75	$C_{30}H_{50}O$	426.7	Lupeol
		100			

are present in petroleum ether, chloroform and aqueous extracts. Tanin is present in petroleum ether and aqueous extracts. Organic acids are present in ethanol and aqueous extract (Table 3). Results obtained were compared with the preliminary phytochemical study carried out by Glory et al. (2016) on the leaves of *C. floribunda.* The petroleum extract showed presence of alkaloid, flavonoid, steroid, saponin and tannin in the present study is similar to study of Glory et al. (2016). However, the chloroform extract of the present study showed presence of more phytochemicals.

Total phenolics and flavonoids

Total phenolics and total flavonoids are considered as the indicators of antioxidant properties of plant extracts. Earlier studies reported that *C. floribunda* has biologically important phytochemicals belonging to flavonoids (Liu et al. 2009). The ethanolic leaf extract showed 472.66 ± 2.51 mg GAE/ g dw of phenolics and 303.00 ± 3.60 mg QE/g dw of flovonoids (Table 4). The results obtained showed better results compared to previous study (Vantamuri et al. 2018) in methanolic and aqueous root extracts (343.77 ± 2.02 and 87.87 ± 6.06 gallic acid equivalent/ mg), the study of whole plant extracts of *C. floribunda* by Santharam et al. (2015) showed that the presence of total flavonoids is 0.038 ± 0.076 mg/g in petroleum ether extract, 3.545 ± 0.023 mg/g in ethyl acetate extract and 1.489 ± 0.043 mg/g in methanolic extract.

FTIR spectroscopic analysis

FTIR analysis is used conventionally to identify the components of phytochemicals in medicinal plants. The characteristic absorption peaks of FTIR spectrum show specific functional groups present in the chemical components. The IR fingerprint is used to analyze the integrity and universality of chemical components (Bai et al. 2021). The IR spectrum of *C. floribunda* leaf powder was analyzed for the identification of functional groups. FTIR spectrum was obtained in between the range of 4000–400 cm^{-1}. The peak observed at 3351.74 cm^{-1} was due to strong O–H bond stretching (alcohol group). Band at 2919.23 cm^{-1} (strong broad) was due to O–H bond stretching (carboxylicacid), and the band at 1619.91 cm^{-1} (strong broad) is due to C = C stretching (α, β unsaturated ketone). Bands at 1519.20 and 1445.02 (strong) were denoted as nitro compound (N–O) stretch. The bands observed at 1317.32 and 1061.41 cm^{-1} (strong) were due to S = O (sulfone) and C-O (primary alcohol) stretching, the medium sharp bands at 823.04 cm^{-1} and 781.16 cm^{-1} indicated the stretching of C = C bending of alkene trisubstuted group. These functional groups infer the possible presence of several bioactive metabolites in the leaves of *C. floribunda* (Fig. 3). The FTIR study which showed strong O–H, C = C, N–O, S = O (sulfone), C-O and C = C bonds stretching similar to the study (Selvaraju et al. 2021) on *Ocimum gratissimum*.

GC-MS analysis

GC–MS study is used to detect the potential phytochemicals in *C. floribunda* leaf extracts by comparing their mass spectra and retention time with the NIST 11 and WILEY 8 libraries. The results obtained from leaf extracts of *C. floribunda* showed the presence of various phytochemicals of the different classes viz. long chain alkanes, phytosterols, phenols, diterpenes and triterpenoids contributing to the medicinal properties of the plant. The identified compounds with their peak number, retention time (RT), peak area (%), molecular formula and molecular weight in each extract presented (Tables 5, 6, 7, 8) (Fig. 4). Comparative GC–MS analysis of different solvent extracts of leaf showed the presence of 18 compounds in the present study (Table 9). However, GC – MS study by Liu et al. (2009) on volatile oils of leaf of

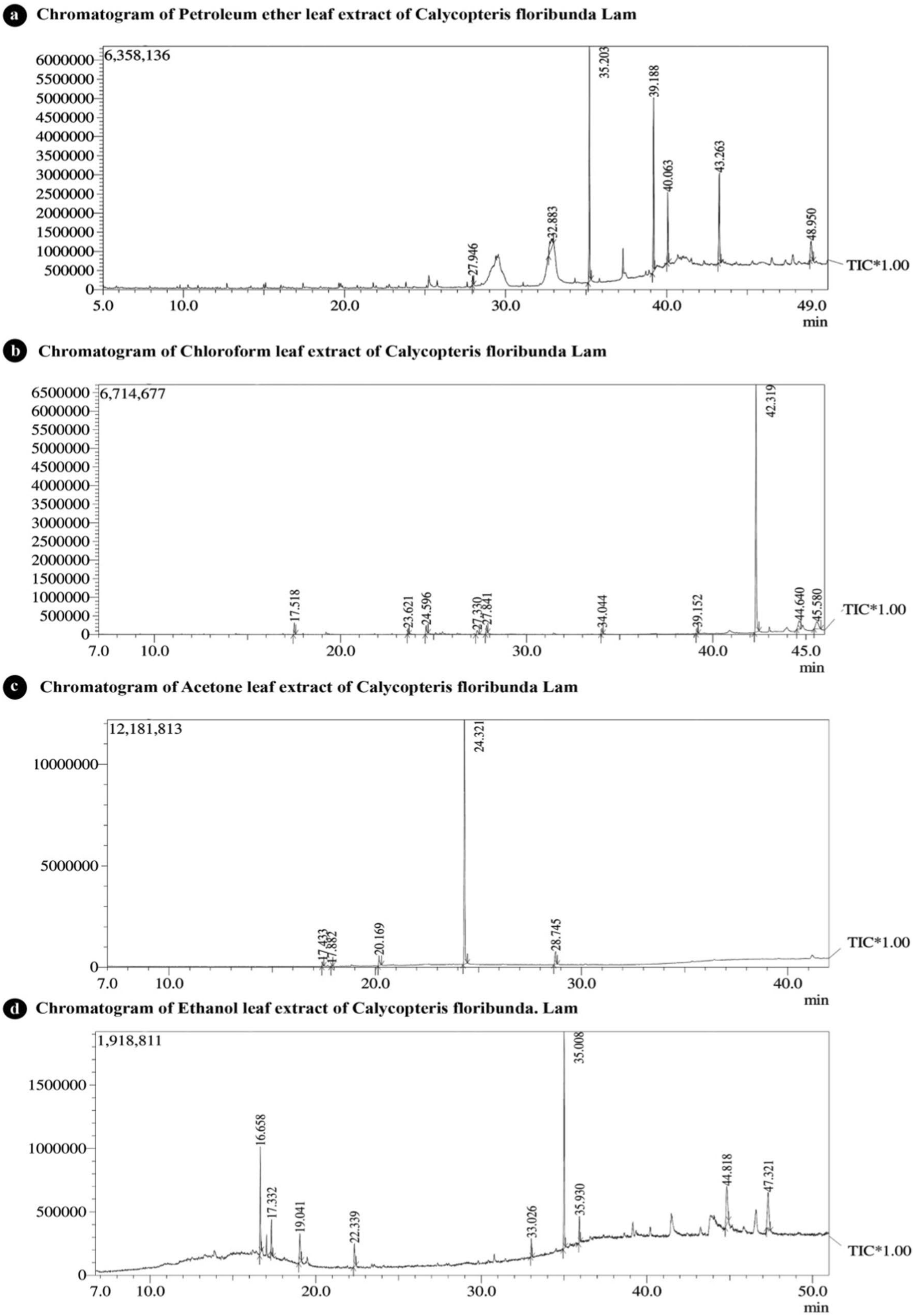


Fig. 4 Chromatograms of leaf extracts in different solvents

Table 9 Comparative GC–MS analysis of the bioactive Components of Leaf extracts of *Calycopteris floribunda* Lam.

Sl No	Compound Name	Petroleum ether	Chloroform	Acetone	Alcohol
1	Phytol	+	−	+	+
2	Lupeol	+	−	−	+
3	1,2-benzenedicarboxylic acid	+	+	+	-
4	Squalene	+	−	+	+
5	Pentacosane	+	−	−	−
6	Tetratetracontane	+	−		+
7	Gamma.-Sitosterol	+	−	−	+
8	2,4-ditert-butylphenol	−	+	−	−
9	1-nonadecene	−	+	−	−
10	Neophytadiene	−	+	+	+
11	Dibutyl phthalate	−	+	-	+
12	E-15-heptadecenal	−	+	−	−
13	10-heneicosene (c,t)	−	+	−	−
14	Heptadecyl trifluoroacetate	−	+	−	−
15	Beta.-sitosterol	−	+	−	−
16	Methyl commate c	−	+	−	−
17	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	−	-	+	+
18	Tricosane	−	−	−	+

C. floribunda showed 52 compounds in which only phytol and dibutyl phthalate are recorded for the leaf extracts of *C. floribunda* of the present study is similar to the earlier work.

1, 2-benzenedicarboxylic acid is one of the major peaks in petroleum ether, chloroform and acetone extracts. 1, 2-benzenedicarboxylic acid and its derivatives have anticancerous, antimicrobial and antiarthritic activities (Reddy et al. 2020). Squalene and phytol are present in petroleum ether, acetone and alcohol extracts. Tulika et al. (2017) in their study on GC–MS analysis of bioactive constituents in the extract of *Pistia stratiotes* and extracts of *Eichhornia crassipes* are also reported to have antibacterial, antioxidant, antitumor, antiinflammatory, antinociceptive, potential antiplatelet components, hypoglycemic, and hypolipidemic activities of squalene. Antinociceptive, antioxidant, anti-inflammatory, antiallergic, hypolipidemic, anticancer, antimicrobial, cytotoxic, anti-teratogenic, antidiabetic, antispasmodic, anticonvulsant, disinfectant and antidiuretic activities of phytol were studied (Chirumamilla et al. 2022). Neophytadiene is present in chloroform, acetone and alcohol extracts. Ahmad et al. (2022) reported the presence of neophytadiene in aerial parts of *Leucophyllum frutescens* and mentioned its analgesic, antipyretic, anti-inflammatory, antimicrobial and antioxidant activities.

Tetratetracontane is identified in petroleum ether and ethanol leaf extracts in the present study. Varsha Jayakar et al. (2020) reported its anti-inflammatory, hypocholesterolaemia, cancer preventive, hepatoprotective and antioxidant activities in their study on leaf extracts of *Garcinia cambogia and Garcinia indica*. Gamma.-sitosterol is a steroid identified in both petroleum ether and ethanol extracts. Vats and Gupta (2017) reported gamma-sitosterol in the hydro-ethanolic extract of *Moringa oleifera* and documented its anti-diabetic, antiangeogenic, anticancerous, anti-inflammatory and antimicrobial activities. Dibutyl phthalate is a phthalate ester present in chloroform and ethanol extracts which is reported to have antifungal, antimicrobial and antimalarial activities (Akpuaka et al. 2013).

GC–MS analysis of leaf extracts *C. floribunda* of present study showed phytochemicals which were present in one of the extracts, pentacosane in petroleum ether, 2, 4-ditert-butylphenol, 1-nonadecene, E-15-heptadecenal, 10-heneicosene, heptadecyl trifluoroacetate, beta-sitosterol and methyl commate c in chloroform extract and tricosane in ethanol extract were indentified. These phytochemicals are reported to have antibacterial, anti inflammatory, antifungal, antioxidant and anticancerous activites (Table 10).

Principle component analysis (PCA)

Phytochemicals are identified by GC–MS have biological activities and were subjected to a principal component analysis (PCA) (Fig. 5). The resulted data showed the relationship between the secondary metabolites having different bioactivities like antioxidant, anticancerous, anti inflammatory and antimicrobial with the solvents used for extraction (Fig. 5). The PCA showed 95.31% of the overall data variation and the PC 1 showed 68.29% of data variation and composed of antioxidant, antifungal, antibacterial and anticancerous

Table 10 Biological activities of the compounds of leaf extracts of *Calycopteris floribunda* Lam.

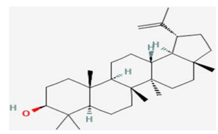
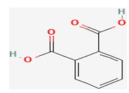
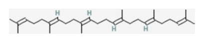
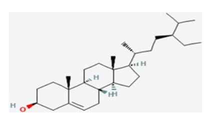
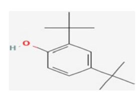
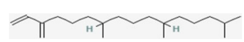
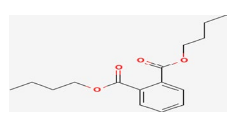
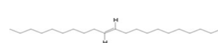
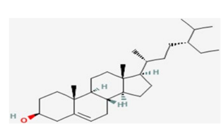
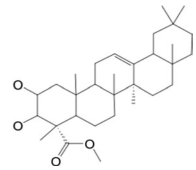
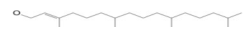
Sl. No	Name of the compound and Pubchem C.I.D	Chemical Nature	Chemical Structure	Biological Activity
1	Phytol (5,280,435)	Diterpene		antinociceptive, antioxidant, anti-inflammatory, antiallergic, hypolipidemic, anticancerous, antimicrobial, cytotoxic, anti-teratogenic, antidiabetic, antispasmodic, anticonvulsant, disinfectant and antidiuretic (Chirumamilla et al. 2022)
2	Lupeol (259,846)	Pentacyclic triterpenoid		Antiprotozoal, Anti-inflammatory, Antitumor, Nutraceutical, Antimicrobial (Gallo and Sarachine 2009)
3	1,2-benzenedicarboxylic acid (90,531)	Derivative phthalic anhydride		Anticancerous, antimicrobial and antiarthritic (Reddy et al. 2020)
4	Squalene (638,072)	Triterpene		Antibacterial, antioxidant, antitumor, anti-inflammatory, Antinociceptive, potential antiplatelet components, hypoglycemic and hypolipidemic (Tyagi and Agarwal 2017)
5	Pentacosane (12,406)	Long-chain alkane		Anti bacterial activity (Arora and Meena 2017)
6	Tetratetracontane (23,494)	Long-chain alkane		Anti-inflammatory, hypocholesterolaemia, cancer preventive, hepatoprotective, anti-oxidant and hypocholesterolaemia (Jayakar et al. 2020)
7	Gamma.-Sitosterol (133,082,557)	Steroid		Anti-diabetic, antiangeogenic, anticancerous, anti-inflammatory and antimicrobial (Vats and Gupta 2017)
8	2,4-ditert-butylphenol (7311)	Phenols		Anti- inflammatory (Nair et al. 2020), antifungal, antibacterial, antioxidant anticancerous and herbicidal (Udupa et al. 2021)
9	1-nonadecene (29,075)	Alkene		Antimicrobial, Antioxidant, Anticancerous (Banakar and Jayaraj 2018)
10	Neophytadiene (10,446)	Diterpene		Analgesic, antipyretic, anti-inflammatory, antimicrobial and antioxidant (Ahmad et al. 2022)
11	Dibutyl phthalate (3026)	Phthalate ester		Antimicrobial, antimalarial and antifungal (Akpuaka et al. 2013)
12	E-15-Heptadecenal (5,363,097)	Long chain alkane		Anti oxidant, anti bacterial, anticancerous, anti fungal, anti- inflammatory and anti microbial (Zaheer et al. 2021)
13	10-heneicosene (5,364,553)	Long chain alkane		Antibacterial, antioxidant (Delyan 2016)
14	Heptadecyl trifluoroacetate (14,574,252)	Long chain alkane		Antimicrobial (Ferdosi et al.2022)
15	Beta-sitosterol (222,284)	Phytosterols		Anticancerous (Rashed 2020)

Table 10 (continued)

Sl. No	Name of the compound and Pubchem C.I.D	Chemical Nature	Chemical Structure	Biological Activity
16	Methyl commate c	Glycol		Cytotoxic, antibacterial, antimicrobial, antiviral, insecticide, nematicide anticoagulant, hemolytic, antiparasitic, wound healing and antitumor (Srinivasan and Priya 2019)
17	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (5,366,244)	Long chain alkane		Antimicrobial, anticancerous and anti-inflammatory (Madhavan 2015)
18	Tricosane (12,534)	Alkane		Antifungal, antibacterial and antimicrobial (Kaplan and Celikoğlu 2020)

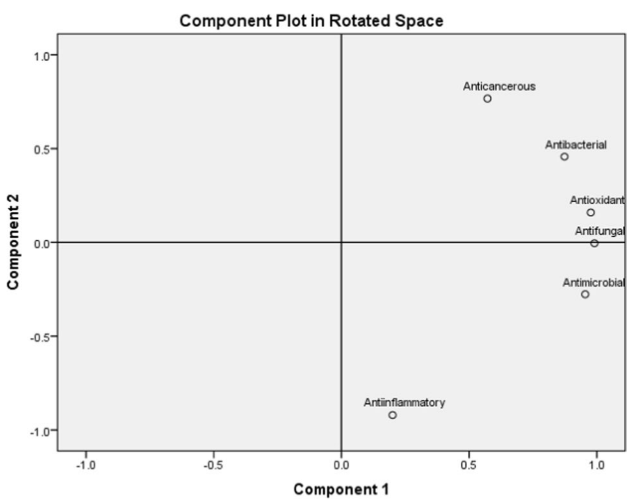


Fig. 5 PCA map showing the relationship among different solvent leaf extracts of *Calycopteris floribunda* Lam. and biological activities of secondary metabolites. AF, antifungal; AB, antibacterial; AM, antimicrobial; AML, antimalarial; AO, antioxidant; AI, anti-inflammatory; AC, anticancerous

activities. The PC 2 inferred anti-inflammatory and antimicrobial activities with 27.01% of the data variation.

The principal component analysis (PCA) of the bioactivities of secondary metabolites of each extract showed that the leaf of *Calycopteris floribunda* Lam. is rich in secondary metabolites having antioxidant, antifungal and antibacterial activities.

Conclusion

GC–MS analysis of leaf extracts of *Calycopteris floribunda* Lam. showed the presence of 31 major bioactive compounds from the four different solvent extracts that are having greater number of biological activities. Present study concludes that the leaves of *Calycopteris floribunda* Lam. are rich in phytochemicals having antioxidant, antibacterial and antifungal activities. These phytochemicals can be extracted individually and they can be used to develop new classes of drugs to treat various disorders with respect to their biological activities by pharmaceutical industry.

Acknowledgements Authors thank the Chairman, PG department of studies in Botany, Karnatak University, Dharwad and University scientific and instrumentation centre (USIC) Karnatak University, Dharwad. KFRI Trissur, Kerala for the GC-MS analysis.

Declarations

Conflict of interest The authors declare that there are no conflicts of interest relevant to this article.

References

Abadie C, Lalande J, Tcherkez G (2022) Exact mass GC-MS analysis: protocol, database, advantages and application to plant metabolic profiling. Plant Cell Environ 45(10):3171–3183. https://doi.org/10.1111/pce.14407

Ahmad I, Ahmed S, Akkol EK, Rao H, Shahzad MN, Shaukat U, Fatima M (2022) GC–MS profiling, phytochemical and biological investigation of aerial parts of *Leucophyllum frutescens* (Berl.) IM Johnst (Cenizo). S Afr J Bot 148:200–209. https://doi.org/10.1016/j.sajb.2022.04.038

Akpuaka A, Ekwenchi MM, Dashak DA, Dildar A (2013) Biological activities of characterized isolates of n-hexane extract of *Azadirachta indica* A. Juss (Neem) leaves. Nat Sci 11(5):141–147

Ali HA, Chowdhury AA, Rahman AK, Borkowski T, Nahar L, Sarker SD (2008) Pachypodol, a flavonol from the leaves of *Calycopteris floribunda*, inhibits the growth of CaCo 2 colon cancer cell line in vitro. Phytother Res 22(12):1684–1687. https://doi.org/10.1002/ptr.2539

Arora S, Meena S (2017) GC-MS Profiling of *Ceropegia bulbosa* Roxb. Var. bulbosa, an endangered plant from Thar Desert, Rajasthan. Pharma Innov J 6(11):568–573

Aslam I, Afridi MSK (2018) Pharmacognostic characterization of *Beaumontia grandiflora* (Roxb.) Wall. leaf for taxonomic identification for quality control of a drug. J Appl Res Med Aromatic Plants 8:53–59. https://doi.org/10.1016/j.jarmap.2017.11.002

Bai M, Jin X, Cen Z, Yu K, Yu H, Xiao R, Li Y (2021) GC–MS and FTIR spectroscopy for the identification and assessment of essential oil components of five cinnamon leaves. Br J Bot 44(3):525–535. https://doi.org/10.1007/s40415-021-00751-7

Banakar P, Jayaraj M (2018) GC–MS analysis of bioactive compounds from ethanolic leaf extract of *Waltheria indica* Linn. and their pharmacological activities. Int J Pharm Sci Res 9(5):2005–2010. https://doi.org/10.13040/IJPSR.0975-8232.9(5).2005-10

Banni M, Jayaraj M (2022) Identification of bioactive compounds of leaf extracts of *Sida cordata* (Burm. f.) Borss. Waalk. by GC/MS Analysis. Appl Biochem Biotechnol. https://doi.org/10.1007/s12010-022-04115-z

Bhandary MJ, Chandrashekar KR, Kaveriappa KM (1995) Medical ethnobotany of the siddis of Uttara Kannada district, Karnataka, India. J Ethnopharmacol 47(3):149–158. https://doi.org/10.1016/0378-8741(95)01274-H

Bharat BV (2019) Medicinal profile of *Calycopteris floribunda* Lam: a review. J Med Plants 7(3):130–133

Chirumamilla P, Dharavath SB, Taduri S (2022) GC–MS profiling and antibacterial activity of *Solanum khasianum* leaf and root extracts. Bull Natl Res Centre 46(1):1–10. https://doi.org/10.1186/s42269-022-00818-9

Delyan E (2016) Analisys of component composition of volatile compounds of field sow thistle (*Sonchus arvensis* L.) leaves using the method of gas chromatography with mass-detection. Pharma Innov 5(10):118

Enerijiofi KE, Akapo FH, Erhabor JO (2021) GC–MS analysis and antibacterial activities of *Moringa oleifera* leaf extracts on selected clinical bacterial isolates. Bull Natl Res Centre 45(1):1–10. https://doi.org/10.1186/s42269-021-00640-9

Ferdosi MF, Javaid A, Khan IH (2022) Phytochemical profile of n-hexane flower extract of *Cassia fistula* L. Bangladesh J Bot 51(2):393–399. https://doi.org/10.3329/bjb.v51i2.60438

Gallo MB, Sarachine MJ (2009) Biological activities of lupeol. Int J Biomed Pharm Sci 3(1):46–66

Glory A, Judin J, Vasudevan R, Sumathi P (2016) Preliminary phytochemical content and antibacterial activity of Ukshi (*Calycopteris floribunda* Lam.) leaves. J Med Plants Stud 4(2):57–59

Gomes A, Das R, Sarkhel S, Mishra R, Mukherjee S, Bhattacharya S, Gomes A (2010) Herbs and herbal constituents active against snake bite. Indian J Exp Biol 48:865–878

Jayakar V, Lokapur V, Shantaram M (2020) Identification of the volatile bioactive compounds by GC-MS analysis from the leaf extracts of *Garcinia cambogia* and *Garcinia indica*. Med Plants 12(4):580–590. https://doi.org/10.5958/0975-6892.2020.00070.2

Kaplan A, Celikoğlu U (2020) Evaluation of phytochemical constituents in the whole plant parts of hexane extract of some traditional medicinal plants by GC-MS analysis. Middle East J Sci 6(2):57–67

Liu JJ, Yang DL, Zhang Y, Yuan Y, Cao FX, Zhao JM, Peng XB (2009) Chemical component and antimicrobial activity of volatile oil of *Calycopteris floribunda*. J Cent South Univ Technol 16(6):931–935. https://doi.org/10.1007/s11771-009-0155-7

Madhavan M (2015) Phytochemical constituents of leaves of *Spatholobus parviflorus* a rare threatened climber of South India. Int J Pharmacogn Phytochem Res 7:991–994

Mayer R (1999) Calycopterones and Calyflorenones, Novel Biflavonoids from *Calycopteris floribunda*. J Nat Prod 62(9):1274–1278. https://doi.org/10.1021/np990182e

Nagannawar AG, Jayaraj M (2020) GC-MS analysis of bioactive compounds from ethanolic extract of whole plant of *Mollugo oppositifolia* Linn and their pharmacological activities. IJPSR 11(5):2504–2509. https://doi.org/10.13040/IJPSR.0975-8232.11(5).2504-09

Nair RV, Jayasree DV, Biju PG, Baby S (2020) Anti-inflammatory and anticancer activities of erythrodiol-3-acetate and 2,4-di-tert-butylphenol isolated from *Humboldtia unijuga* Bedd. Nat Prod Res 34(16):2319–2322. https://doi.org/10.1080/14786419.2018.1531406

Nissar S, Raja WY, Majid N, Nawchoo IA, Bhat ZA (2021) Pharmacognostic characterization and development of quality control standards for *Dictamnus albus*: a comparative study of different parts. Adv Trad Med. https://doi.org/10.1007/s13596-021-00559-6

Papiya B, Sourabh V, Alok S, Shekhar SC (2014) Pharmacologic and Physico-chemical standardization of *Macrotyloma uniflorum* (Lam.) verdc edible seed. Pharmacog Commun. https://doi.org/10.5530/pc.2014.1.4

Rashed K (2020) Beta-sitosterol medicinal properties: a review article. IJSIT 9(4):208–212

Ratnagiriswaran AN, Sehra KB, Venkataraman K (1934) The anthelminthic constituent of the leaves of *Calycopteris floribunda*. Biochem J 28(6):1964. https://doi.org/10.1042/bj0281964

Reddy GJ, Reddy KB, Reddy GS (2020) GC-MS analysis and in-vitro anti-diabetic activity of bioactive fractions of *Feronia elephantum* fruit. Int J Pharm Sci Res 11(5):1000–1010. https://doi.org/10.13040/IJPSR.0975-8232.11(5).2415-24

Safari M, Ahmady-Asbchin S (2019) Evaluation of antioxidant and antibacterial activities of methanolic extract of medlar (*Mespilus germanica* L.) leaves. Biotechnol Biotechnol Equip 33(1):372–378. https://doi.org/10.1080/13102818.2019.1577701

Santharam E, Ganesh P, Soranam R, Divya VV (2015) Evaluation of in vitro free radical scavenging potential of various extracts of whole plant of *Calycopteris floribunda* Lam. J Chem Pharm Res 7(1):860–864

Selvaraju R, Sakuntala P, Jaleeli KA (2021) GC–MS and FTIR Analysis of Chemical Compounds in *Ocimum gratissimum* Plant. Biophysics 66(3):401–408. https://doi.org/10.1134/S0006350921030167

Shivanna MB, Rajakumar N (2011) Traditional medico-botanical knowledge of local communities in Hosanagara Taluk of Shimoga District in Karnataka, India. J Herbs Spices Med Plants 17(3):291–317. https://doi.org/10.1080/10496475.2011.602617

Silva da Costa R, Pinheiro WBDS, Arruda MSP, Costa CEF, Converti A, Ribeiro Costa RM, Silva Júnior JOC (2022) Thermoanalytical and phytochemical study of the cupuassu (*Theobroma grandiflorum* Schum.) seed by-product in different processing stages. J Therm Anal Calorim 147(1):275–284. https://doi.org/10.1007/s10973-020-10347-0

Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol 299:152–178. https://doi.org/10.1016/S0076-6879(99)99017-1

Srinivasan S, Priya V (2019) Phytochemical screening and GC-MS analysis of *Cyperus dubius*, Rottb.(Cyperaceae). J Med Plants 7(2):89–98

Tyagi T, Agarwal M (2017) Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms. J Pharmacog Phytochem 6(1):195–206

Vantamuri AB, Sounshi YB, Kotresh O, Dodamani SS (2018) A preliminary study on total phenolic content and antioxidant activities of *Calycopteris floribunda* root extracts. Int J Pharm Sci Res 9(7):3020–3023. https://doi.org/10.13040/IJPSR.0975-8232.9(7).3020-23

Vasudha Udupa A, Gowda B, Kumarswammy BE, Shivanna MB (2021) The antimicrobial and antioxidant property, GC–MS analysis of non-edible oil-seed cakes of Neem, *Madhuca and Simarouba*. Bull Natl Res Centre 45(1):1–14. https://doi.org/10.1186/s42269-021-00498-x

Vats S, Gupta T (2017) Evaluation of bioactive compounds and antioxidant potential of hydroethanolic extract of *Moringa oleifera* Lam.

from Rajasthan India. Physiol Mol Biol Plants 23(1):239–248. https://doi.org/10.1007/s12298-016-0407-6

Vinayaka KS, Kekuda TP, Shivakumar B, Shravanakumara S (2009) Anti-dermatophyte activity of methanol and aqueous extracts of *Calycopteris floribunda* Lam leaves. Nat Prod 5:215–219

Zaheer J, Najam-Us-Saqib Q, Anwar T, Khan FS, Akram M, Munir N, Thiruvengadam M (2021) Phytochemical profile of rock Jasmine (*Androsace foliosa* Duby ex Decne) by using HPLC and GC–MS analyses. Arab J Sci Eng 46(6):5385–5392. https://doi.org/10.1007/s13369-020-05241-8

Zimila HE, Matsinhe AL, Malayika E, Sulemane ÁI, Saete VN, Rugunate SC, Munyemana F (2020) Phytochemical analysis and in vitro antioxidant and antimicrobial activities of hydroalcoholic extracts of the leaves of *Salacia kraussii*. Biocatal Agric Biotechnol 30:101862. https://doi.org/10.1016/j.bcab.2020.101862