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**A READY RECKONER DOCUMENTATION SHEET ON  
STANDARDIZATION STUDIES ON *CALYOPTERIS FLORIBUNDA*  
(ROXB.) LAM. – AN OVERVIEW**

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**ABSTRACT**

India has the potential to become a major country playing the lead role in the production of standardized, therapeutically efficient ayurvedic formulations. This can only be accomplished if the herbal products are tested and standardized by UV-visible, FTIR, TLC, HPLC, HPTLC, GC-MS, and other methods. The phytochemical profile of a plant is particularly important because it gives documented data on its medicinal potential. The World Health Organization (WHO) emphasizes the significance of qualitative and quantitative approaches for characterizing samples, evaluating biomarkers, chemical markers, and creating finger impression profiles for herbal formulations. The present overview document sheet covers the standardization parameters of the medicinal plant *Calycopteris floribunda*. This document sheet will aid as a ready reckoner to herbal formulation manufacturers.

**Keywords:** Standardization, WHO, Biomarkers, Herbal formulation, *Calycopteris floribunda*

**INTRODUCTION**

Traditional medicine is commonly used to treat a variety of human illnesses. The use of herbal medicine can also be traced back to the dawn of time. Traditional

medication has grown in popularity around the world due to its effectiveness and curative nature for ailments with less toxicity. Because of their medicinal

properties, all plants on this planet are deemed valuable [1, 2]. Medicinal plants have played an important part in global health. Medicinal plant formulations are of immense value in the Indian systems of medicine. The method of prescribing a series of standards or inherent features, constant parameters, definite qualitative and quantitative values that hold an assurance of consistency, effectiveness, purity, and reproducibility is known as standardisation of herbal medicine [3]. However, there is a paucity of documents of standardization protocols for herbal formulation to aid herbal formulation manufacturers, for preparing formulations containing standardized extracts and to increase the global market value and acceptability of herbal medicines [4, 5]. Hence, the present document sheet is

prepared for the plant *Calycopteris floribunda*.

*Calycopteris floribunda* Lam is an ethno medicinal plant that was commonly used to produce a variety of herbal medicines. It is a large climbing shrub that belongs to the Rangoon creeper *Combretaceae* family. The plant is said to have extensive therapeutic value, with medicinal properties mostly found in the stem and leaves [6]. The plant is referred as lifesaver for forest dwellers [7]. Standardization studies namely organoleptic evaluation, proximate analysis, phytochemical evaluation, quantitative evaluation and biological activity reported for *Calycopteris floribunda* are reviewed and documented in this paper. All reports of earlier work, including figures are duly cited with reference number in square bracket.



Figure 1: *Calycopteris floribunda* plant

### Rationale

Prior to any significant experimental examination of medicinal

plants, a rational approach would be to collect ethnopharmacological data that could be systematically processed to

provide a preliminary description. However, there is a shortage of standardised procedures for herbal formulation and the chemicals responsible for their therapeutic effects are yet unknown for the majority of them. Hence, we documented the medicinal plant *Calycopteris floribunda*.

### Fact Sheet

Fact Sheet is a concise account of a plant, its applications, and cultural

guidelines.

**Table 1** represents the detailed plant Profile of *Calycopteris floribunda*.

### STANDARDIZATION STRATEGIES

The chemical, physical, microbiological, therapeutic, and toxicological tests carried out on plant and plant extracts, can be valuable tool in standardization studies [28]. **Chart 1** represents the standardization protocols for herbal formulations of plants.

Table 1: Fact Sheet of *Calycopteris floribunda* [7-27]

Scientific Name	<i>Calycopteris floribunda</i> (Roxb.) Lam.
Taxonomy Id	134915
Subkingdom	Tracheophyta
Class	Magnoliopsida
Order	Myrtales
Family	Combretaceae
Genus	Calycopteris
Synonyms	<i>Calycopteris nutans</i> , <i>Combretum sericeum</i> , (Roxb.), <i>Calycopteris coccineum</i> Lam., <i>Calycopteris nutans</i> , <i>Combretum extensum</i> Roxb. and <i>Getonia floribunda</i> Roxb
Vernacular names	Tamil: Pullanji Valli, Minnarkoti, Eruzham English: Paperflower climber Hindi :Kokkare Marathi: Ukshi Telugu: Murugadutige, bandimurududu Kannada: Enjarigekubsa Sanskrit: Susavi, Toyavalli Malayalam: Pullani, Varavalli
Common names	Ukshi ,Minnarkodi, Guichalata
Special name	Life Saver – Forest Dwellers
Morphological Characteristics	Straggling shrubs, young pubescent. Leaves opposites, flowers I dense panicles at the ends of branches. Calyx lobes 5, white, showy, persistent, calyx tube elongate, 5-angled produced above the ovary. Petals 0. Stamens 10 in 2 series. Ovary inferior 1-celled, ovules 3. Fruits are brownish or yellow-tomentose with 5-angled, crowned by accrescent calyx, brownish or yellow-tomentose and solitary seeds. (www.greenpatio.com)
Flowering & Fruiting	January – April
Distribution	Indomalaysia
Adaptation	This plant is found extensively within the low-lying tropical evergreen forests of the Western Ghats, and infrequently in the Eastern Ghats of coastal Andhra. It is also found in “kavus” or the sacred Groes of Kerala.
Number of isolated chemical constituents	Twenty four [10-22]
Major Components	Flavonoids and Phenols
Biologically active components	Calycopterin [11], Pachypodol [17, 18], 3'-amino-5-hydroxy-3,6,7,8,4'-penta methoxy flavone [21], Calycopterones, isocalycopterone, 4-methyl calycopterone [22]
Biomarker	A potential anticancer drug lead compound– Pachypodol [18] Plant Anti-tumor Agents – Calycopterone, iso calycopterone, 4-methyl calycopterone [22]
Quantification of Biomarker and Protocol adopted	1.983 mg/g Pachypodol [18] Method : High Performance Liquid Chromatography

	<p>Column : Lichrospher C18  Solvent System : Acetonitrile and ultrapure water  Detector : UV detector  Wavelength : 365 nm  Injected Vol. : 1.0 mL min<sup>-1</sup>  Temperature : 35°C</p>
Therapeutic and other attributes	Used for worm infestations, anaemia, obstinate skin diseases and fever (therapeutic uses of leaf, stem and root)
Folk use	Leaves are bitter useful in intestinal worms, laxative, colic, astringent, antiseptic, wounds leprosy malaria fever, dysentery, ulcers, vomiting. The fruit is useful in treating jaundice, ulcers and skin diseases.
Medicinal Part	Leaves
Pharmacological potential	Antioxidant, anti-inflammatory, antifungal, antimicrobial, antibacterial, hepatoprotective, $\beta$ – glucuronidase inhibition activity
Dosage and safety aspects	Morbidity and mortality were observed in rats and rabbits. Calycopterin and its 4'-methyl ether derivative exhibited marked toxicity to fish.
Databases	<ul style="list-style-type: none"> <li>NAPRALERT™ database</li> </ul> National Resource Conservation Service-Plants Database of USDA (United States Department of Agriculture)
History	Protologue: Enchycl., suppl. 2:41 (1811) Family: <i>Combretaceae</i> Chromosome number; 2n=48 (Plant Resources of South-East Asia)

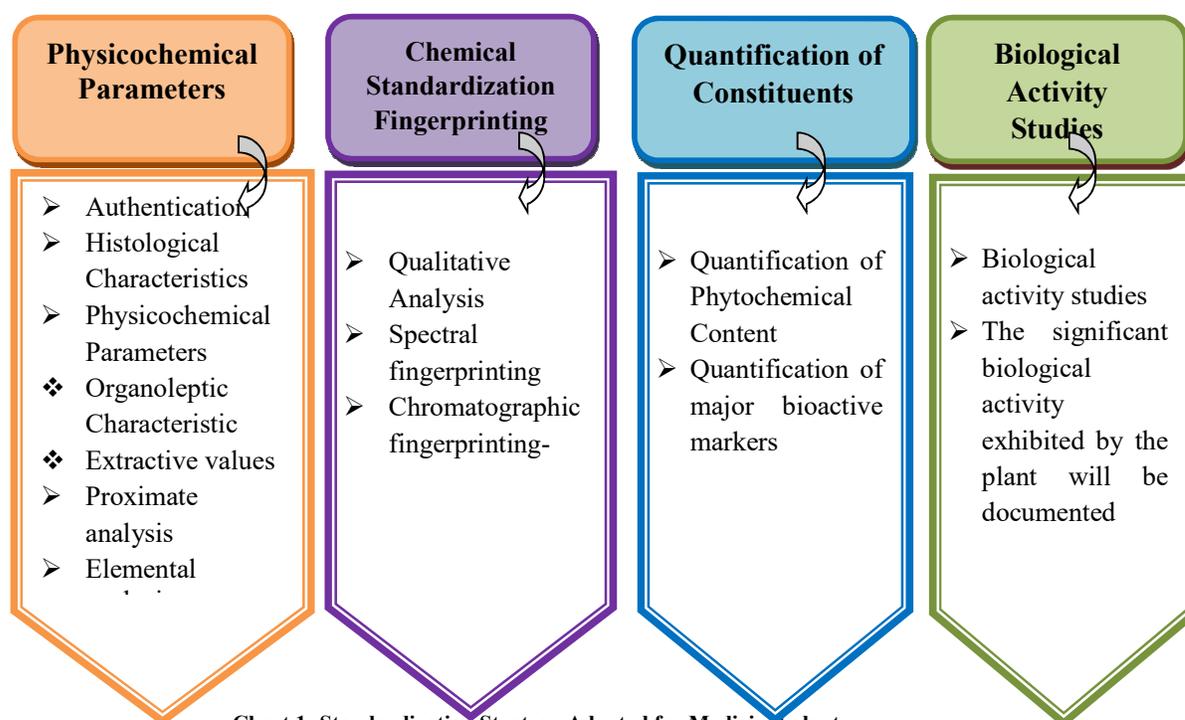


Chart 1: Standardization Strategy Adopted for Medicinal plants

### Histological Characteristics

The structure and growth of the phloem in the stems of *Calycopteris floribunda* plant were studied [29]. Cells in the centre of the cambial zone

differentiated into their descendants in tiny segments of the cambium, resulting in the cambial zone being divided into outer and inner segments. The outside segment connected with the existing cambial ring to

re-establish the cambial cylinder, whereas the inner segment was surrounded by differentiating xylem. This process is repeated multiple times, leading in the development of a number of phloem islands embedded in secondary xylem. **Figure 2** shows the transverse, tangential and radial longitudinal view of secondary xylem.

## PHYSICOCHEMICAL PARAMETERS

### Organoleptic Characteristics and Extraction efficacy

Soaking and refluxing methods were employed to find the extraction efficacy of solvent for extracting the plant material. The colour and yield of the petroleum ether, methanol, chloroform, hexane, ethyl acetate, hydroethanol extracts of *Calycopteris floribunda* are tabulated (**Table 2**).

### Proximate Analysis

Proximate parameters namely moisture content, ash content and water-soluble content were determined for air-dried powdered leaf material. *Calycopteris floribunda* contains 22.72% ash content, 3.97% moisture content and 24.55% water-soluble extract. The percentage of ash content and water soluble content is an indication of the content of polar molecules and glycosides.

### Elemental Analysis

Calcium, magnesium, sodium, potassium, zinc, copper, chromium, lithium and nickel content have been determined by EDXRF technique [26], ICP – OES method [34], and EDX metal content analysis method. **Table 3** shows the metal content values for flowers and leaves of *Calycopteris floribunda*.

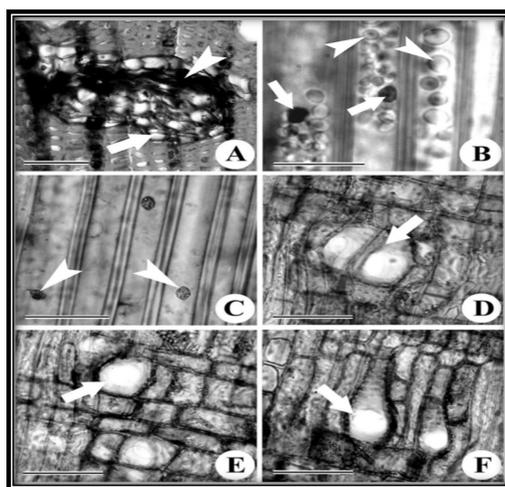


Figure 2: Transverse (A), tangential (B and C) and radial (D–F) longitudinal view of secondary xylem of *Calycopteris floribunda* [29]

Table 2: Organoleptic Properties and Extractive Value

S.No	Part	Method	Extracts	Colour	Yield (%)
1	Leaves [27]	Soxhlet	Pet Ether	Brown	2.78
2			Chloroform	Greenish Black	5.81
3			Methanol	Green	9.81
4			Aqueous	Brown	8.71
5	Flowers [30]	Soxhlet	Pet Ether	Golden Yellow	0.70
6			Chloroform	Dark Green	1.30
7			Methanol	Brownish Orange	6.70
8	Leaves (Present Work)	Soxhlet	Hexane	Greenish Brown	0.94
9			Ethyl Acetate	Dark Green	1.51
10			Hydroethanol	Dark Green	8.55
11		Reflux	Hexane	Greenish Brown	3.46
12			Hydroethanol	Dark Green	3.47

Table 3: Elemental Analysis of *Calycopteris floribunda*

S.No	Elements	Root – Concentration (mg kg <sup>-1</sup> ) [26]	Flowers (ppm) [34]	Leaves (%) (Present Work)
1.	Calcium	-	6977.93	6.39
2.	Magnesium	-	2638.79	0.81
3.	Sodium	-	103.30	0.88
4.	Potassium	-	8829.19	14.05
5.	Iron	-	378.86	-
6.	Manganese	3924.1	340.52	0.81
7.	Zinc	148.7	13.57	-
8.	Copper	-	12.59	-
9.	Lithium	-	5.39	-
10.	Chlorine	-	-	6.39
11.	Aluminium	-	-	0.75
12.	Silicon	-	-	0.29
13.	Carbon	-	-	30.28
14.	Oxygen	-	-	40.36
15.	Nitrogen	-	-	6.19
16.	Nickel	50.5	-	-

## CHEMICAL STANDARDIZATION OF EXTRACTS

### Qualitative Analysis

The preliminary phytochemical analysis was done by standard procedure and reported [7, 9, 14, 27, 31-34]. Table 4 represents the preliminary phytochemical screening for the various extracts of *Calycopteris floribunda*.

## SPECTRAL FINGERPRINTING OF EXTRACTS

### UV-Visible Spectral Fingerprinting

UV-Visible spectrophotometric analysis was done in UV-Systronics AR 2701 double beam spectrophotometer.

Figures 3 and 4 represent the UV spectral fingerprinting of the non-polar CAFL (H) and polar CAFL (HE) extracts respectively.

Figure 5, represents the overlay spectra showing a shift in wavelength of the longer wavelength band. A bathochromic shift was observed at 375 nm for the hydroethanol extract with the shift reagent sodium ethoxide. This shift indicates the presence of flavonoidal hydroxyl groups in *Calycopteris floribunda* leaf extract.

Table 5 represents the UV absorption  $\lambda_{max}$  for various chemical

compounds isolated from *Calycopteris floribunda*.

### FT-IR Spectral Fingerprinting

FT-IR spectrometer Shimadzu-Miracle 10 make was used for recording the IR spectrum for *Calycopteris floribunda* extracts. **Figures 6 and 7** represent the FT-IR fingerprints of the non-polar and polar extracts respectively. The IR spectrum of CAFL (H) indicates the presence of non-polar constituents mostly while the CAFL (HE) indicates the presence of polar groups.

**Table 6** provides the IR absorptions of various chemical compounds isolated from *Calycopteris floribunda*.

### NMR Spectral Fingerprinting

$^1\text{H}$  NMR spectral fingerprinting of the extracts CAFL (H) and CAFL (HE) were recorded in Bruker 300 MHz Proton Nuclear Magnetic Resonance under room temperature. The extracts were dissolved d6-DMSO. The proton NMR spectral fingerprints for CAFL (H) and CAFL (HE) are represented by **Figures 8 and 9**.

## CHROMATOGRAPHIC FINGERPRINTING OF EXTRACTS

### Thin Layer Chromatography

TLC analysis was obtained for non-polar and polar extracts of *Calycopteris floribunda*. The optimized TLC developing solvent system for the extracts is given in following **Table 7**.

Table 4: Qualitative Analysis of *Calycopteris floribunda* [7, 9, 14, 27, 31-34]

Phytochemicals	Extracts					
	Pet Ether	Chloroform	Ethyl Acetate	Methanol	Ethanol	Aqueous
Carbohydrates	-	-	-	+	-	+
Glycosides	-	-	+	+	-	+
Proteins	-	-	-	+	-	+
Alkaloids	-	+	+	-	+	-
Steroids	+	+	+	+	-	+
Terpenoids	-	+	+	+	-	+
Flavonoids	-	+	+	+	+	+
Saponins	-	-	+	+	-	+
Phenolics	-	-	+	+	-	+

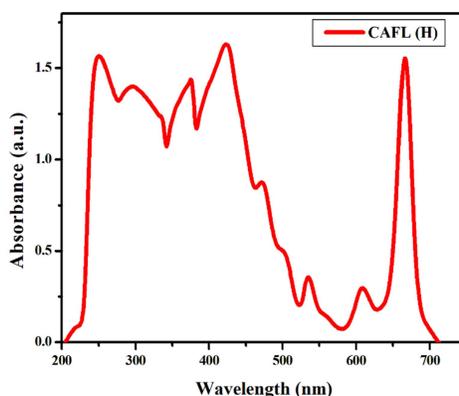


Figure 3: UV fingerprints of CAFL (H)

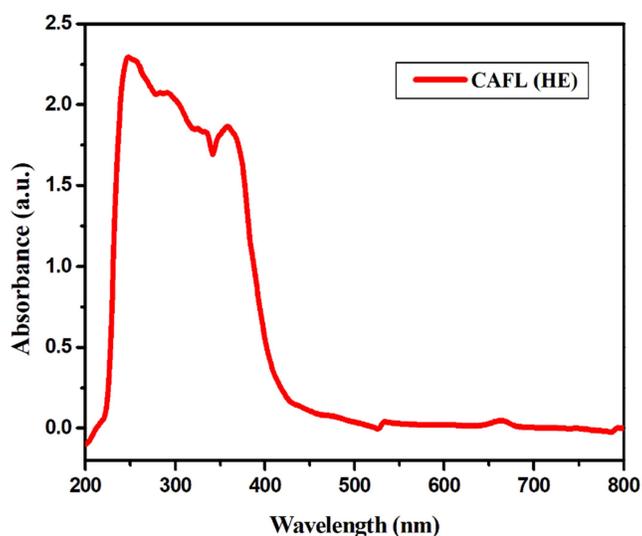


Figure 4: UV fingerprints of CAFL (HE)

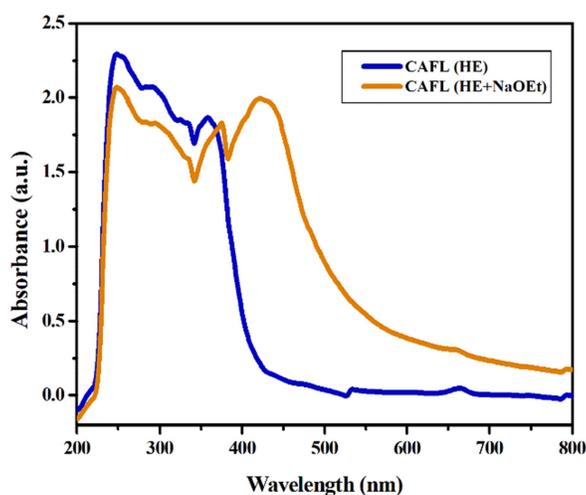


Figure 5: Overlay of UV Spectrum of CAFL (HE) with UV Spectrum of CAFL (HE+NaOEt)

Table 5: UV absorption  $\lambda_{max}$  for *Calycopteris floribunda* Compounds

S.No	Extracts /Compounds	Solvents and Shift reagents	Absorption $\lambda_{max}$ (nm)
1	Pachypodol [17]	MeOH	357, 270
		MeOH + NaOMe	404, 357, 266
		MeOH + AlCl <sub>3</sub>	398, 362, 268
		MeOH + AlCl <sub>3</sub> /HCl	396, 360, 266
		MeOH + NaOAc	360, 255
		MeOH + NaOAc/H <sub>3</sub> BO <sub>3</sub>	357, 254
2	Calyflorenone A [19]	EtOH	292,258
3	Calyflorenone B [19]	EtOH	296, 259, 212
		EtOH + NaOMe	296, 258, 211
4	Neocalycopterone [19]	EtOH	288
5	Neocalycopterone-4-methyl ether [19]	EtOH	288, 209
6	6''-Demethoxy neocalycopterone [20]	EtOH	295, 257, 212
7	Calyflorenone C [20]	EtOH	294, 258, 213
8	6''-Epi-calyflorenone B [20]	EtOH	294, 257, 212
9	6''-Epi-calyflorenone C [20]	EtOH	295, 257,215
10	Calyflorenone D [20]	EtOH	295, 257, 211
11	Calycopterone [22]	MeOH	289
12	Isocalycopterone [22]	MeOH	277

13	4-Demethylcalycopterone [22]	MeOH	288
14	4',5-Dihydroxy-3,3',6,7-tetramethoxyflavone [22]	MeOH	350, 270, 256
15	CAFL (H) Extract (Present Work)	Hexane	666, 609, 535, 423, 375, 297, 251
16	CAFL (HE) Extract (Present Work)	EtOH	664, 359, 248
		EtOH +NaOEt	423, 375, 249

\*EtOH – Ethanol; MeOH – Methanol; NaOMe – Sodium Methoxide; NaOAc- Sodium Acetate; NaOEt – Sodium Ethoxide

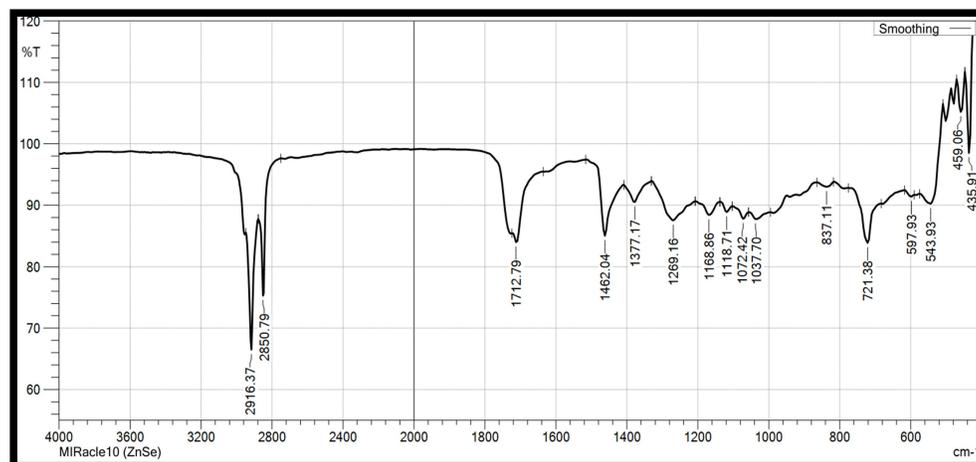


Figure 6: FTIR fingerprint of CAFL (H)

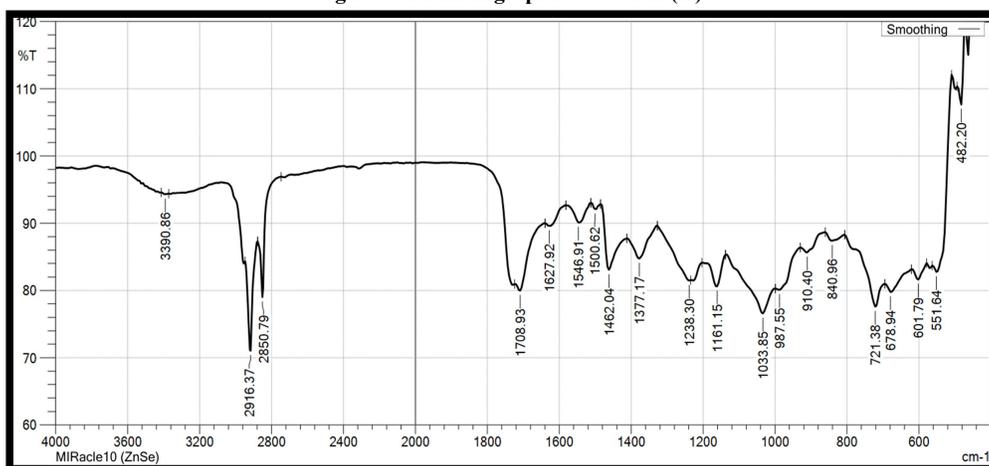


Figure 7: FTIR fingerprint of CAFL (HE)

Table 6: IR Absorptions of Compounds isolated from *Calycopteris floribunda*

S.No	Extracts /Compounds	Absorption Frequency (cm <sup>-1</sup> )
1	Calyflorenone A [19]	3440, 2930, 2840, 1665, 1630-1600, 1450, 1250, 1195, 1170, 1135, 1085, 1030, 700
2	Calyflorenone B [19]	3430, 3075, 3040, 2940, 2840, 1665, 1635, 1600, 1450, 1195, 1170, 1090, 1020, 765, 700
3	Neocalycopterone [19]	3470, 3100, 3070, 3040, 2940, 2840, 1670, 1630, 1600, 1450, 1330, 1025, 925, 760, 700
4	Neocalycopterone-4-methyl ether [19]	3450, 3060, 3040, 2940, 2830, 1670, 1630, 1595, 1450, 1325, 1095, 1025, 920, 700
5	6''-Demethoxy neocalycopterone [20]	3394, 2930, 1669, 1630, 1602, 1456, 1234, 1170, 1068, 1045, 913, 765, 700.
6	Calyflorenone C [20]	3424, 2933, 2835, 1662, 1635, 1602, 1457, 1260, 1194, 1163, 1126, 1090, 1020, 801, 699
7	6''-Epi-calyflorenone B [20]	2928, 2829, 1675, 1619, 1609, 1457, 1330, 1289, 1248, 1195, 1161, 1092, 1027, 761, 700
8	6''-Epi-calyflorenone C [20]	2930, 2852, 1675, 1616, 1457, 1252, 1193, 1161, 1094, 1041,

		1061, 1020, 870, 760, 700
9	Calyflorenone D [20]	3372, 2925, 2851, 1653, 1457, 1327, 1288, 1194, 1162, 1089, 1023, 869, 760, 700
10	Calycopterone [22]	3485, 3400, 2915, 1660, 1620, 1595, 1448, 1317, 1157
11	Isocalycopterone [22]	3480, 2910, 1661, 1631, 1591, 1444, 1420, 1317, 1096, 1030, 933
12	4-Demethylcalycopterone [22]	3480, 2815, 1660, 1628, 1601, 1492, 1446, 1315, 1156, 1034, 918
13	CAFL (H) Extract (Present Work)	2916, 2850, 1713, 1462, 1377, 1169, 721
14	CAFL (HE) Extract (Present Work)	2916, 2851, 1709, 1547, 1462, 1377, 1238, 1161, 1034, 721

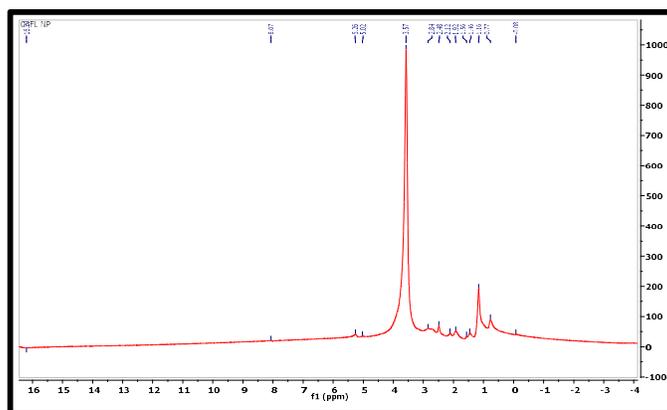
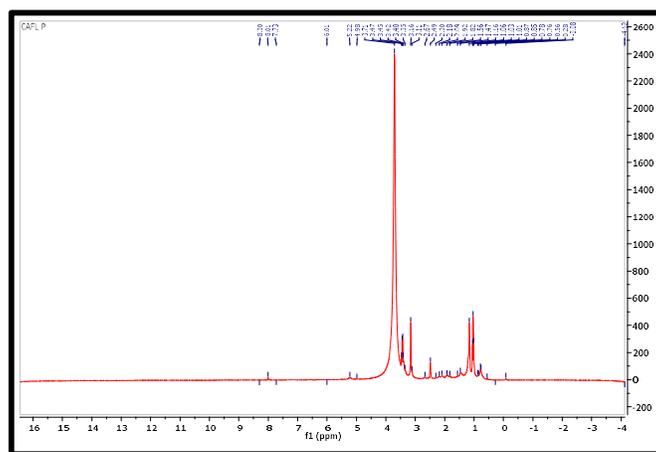
Figure 8:  $^1\text{H}$  NMR Spectral fingerprint of CAFL (H)Figure 9:  $^1\text{H}$  NMR Spectral fingerprint of CAFL (HE)

Table 7: Optimized TLC Solvent System

Extracts	Solvent System
CAFL (H)	Hexane : Ethyl acetate 7:3
CAFL (HE)	Hexane : Ethyl acetate 6:4

### GC-MS Fingerprinting

Clarus 680 GC, Clarus 600(EI) Mass spectrometer and Agilent (GC) JEOL GC MATE II (MS) was used for recording

the non-polar extract and polar *Calycopteris floribunda* extracts respectively.

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**GC-MS Protocol for CAFL (H) (SIF-VIT, Vellore)****CAFL (H)**

- Oven temperature : Initial temperature 60° C for 2 minutes, ramp 10° C/min 300° C hold 6 minutes.
- Total run time : 32 minutes
- Auto injector run : 260° C
- Sample volume : 1 µl
- Split ratio : 10:1
- Flow rate : 1 mL/min
- Carrier gas : Helium
- Solvent delay : 2 minutes
- Ion source temperature : 230° C
- Scan : 50 to 600Da

**GC-MS Protocol for CAFL (HE) (SAIF-IIT, Chennai)****CAFL (HE)**

- Column – HP 5 MS
- Oven temperature : Initial temperature 50° C for 2 minutes, ramp 10° C/min 250° C hold 6 minutes.
- Total run time : 40 minutes
- Auto injector run : 260° C
- Sample volume : 1 µl
- Flow rate : 1 mL/min
- Carrier gas : Helium
- Ion source temperature : 250° C
- Scan : 50 to 600 amu

Figures 10 and 11 represents the GC-MS fingerprint for CAFL (H) and CAFL (HE) respectively. The prominent peaks with an area greater than 10% in the total ion chromatogram were noted. The probable compounds predicted based on NIST library search are listed in Table 8.

**HPLC Fingerprinting**

HPLC analysis was performed to assess the chemical profile of *Calycopteris floribunda* leaf extracts produced by supercritical fluid extraction (SFE) and

Carbon dioxide-ethanol (CE) technique [18]. In Figure 12 [18] shown below, curve 1 represents the HPLC fingerprints of crude flavonoid fraction of leaves obtained by supercritical fluid extraction and curve 2 represents the HPLC fingerprints of crude flavonoid fraction of leaves obtained by CE technique. The peak at retention time near 4.5 identified as pachypodol.

**Quantitative Analysis**

The flavonoid and phenolic content of dried leaves was determined by standard

procedure and the reported results were tabulated (Table 9).

### Chemical Constituents isolated from *Calycopteris floribunda*

The first isolation of chemical constituents from *Calycopteris floribunda* was reported in 1934. Table 10 provides the details of the chemical constituents isolated from the various parts of *Calycopteris floribunda*.

The structures of the chemical constituents isolated from this plant are represented in Chart 2.

### Quantification of Biomarker Pachypodol [18]

So far, around 24 chemical constituents of *Calycopteris floribunda* have been isolated. Among these, the

compounds calycopterin, calycopterones, isocalycopterone, 4-methyl calycopterone, 3'-amino-5-hydroxy-3,6,7,8,4'-pentamethoxy flavone and pachypodol have been reported as flavonoid markers. Among these markers, pachypodol was quantified from the leaf extracts of *Calycopteris floribunda* by an optimized HPLC method.

Figure 13 shows the HPLC results obtained for extracts of *Calycopteris floribunda* and the standard Pachypodol. Curve 1 represents the HPLC chromatogram of the compound Pachypodol.

The HPLC quantification revealed the presence of **1.983 mg/g** pachypodol in the leaf extract of *Calycopteris floribunda*.

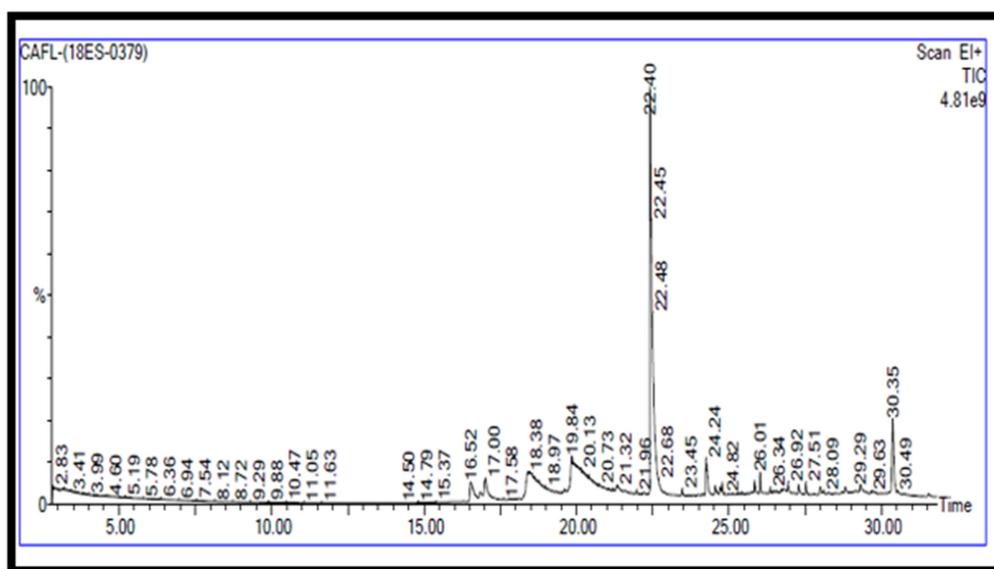


Figure 10: Gas Chromatogram of CAFL (H)

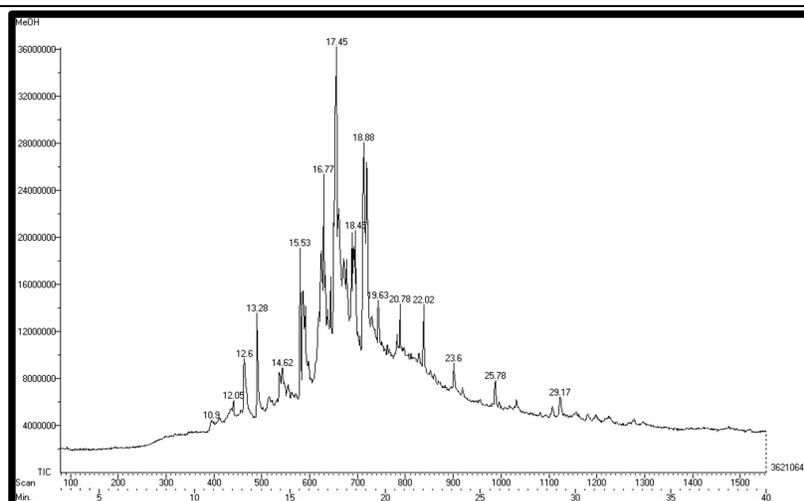
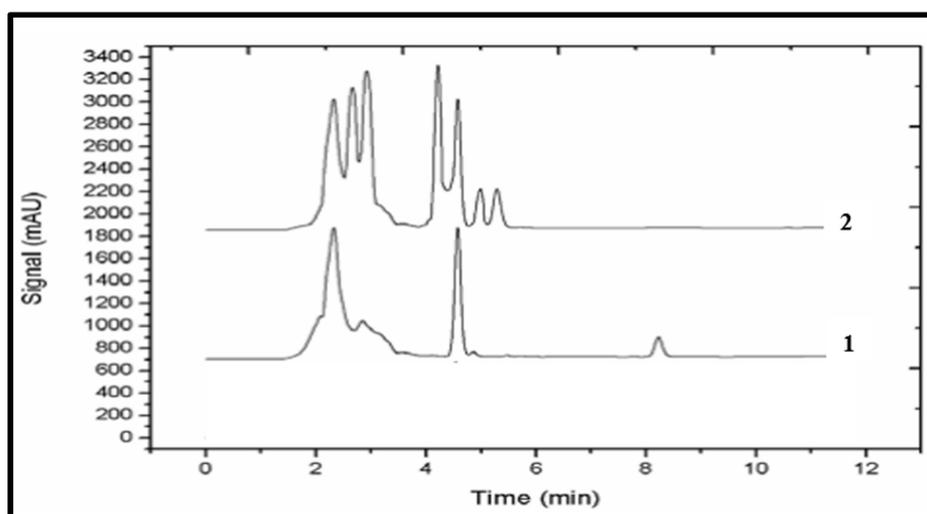


Figure 11: Gas Chromatogram of CAFL (HE)

Table 8: Compounds Predicted from NIST Database for CAFL extracts

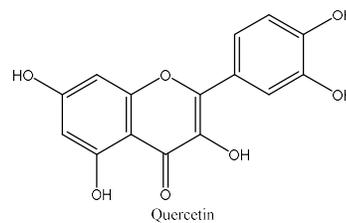
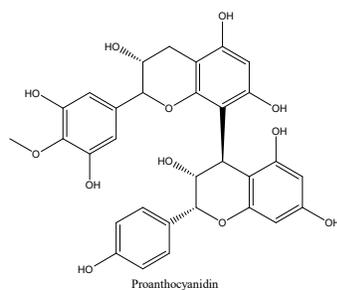
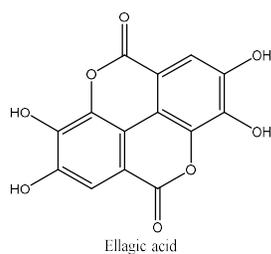
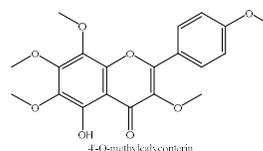
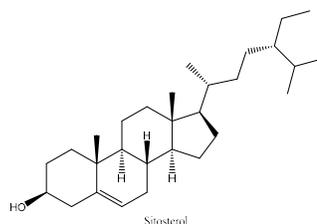
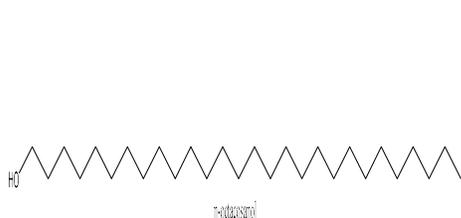
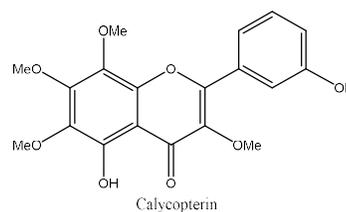
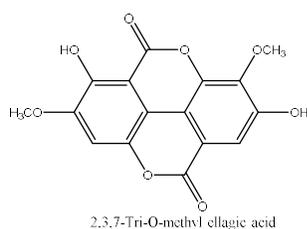
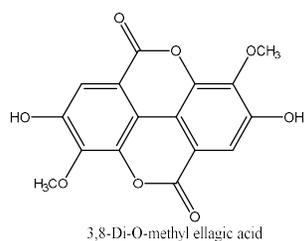
CAFL (H)		CAFL (HE)	
Retention Time (min.)	Probable Compounds	Retention Time (min.)	Probable Compounds
16.52	3,7,11,15-tetramethyl-2-hexadecen-1-ol	12.66	4-hydroxy-3-methoxybenzyl alcohol
17.01	Phytol	15.53	$\alpha$ -caryophyllene
18.47	N-hexadecanoic acid	17.45	n-hexadecanoic acid
19.84	(Z)-14-tricosenyl formate	18.88	Oleic acid
20.40	1,2-15,16-diepoxyhexadecane	19.63	9,15-octadecadienoic acid, methyl ester
22.40	Di-N-octyl phthalate	20.78	Phytol
30.35	3,7,11,15-tetramethyl-2-hexadecen-1-ol	23.60	1-tetradecene,2-decyl-

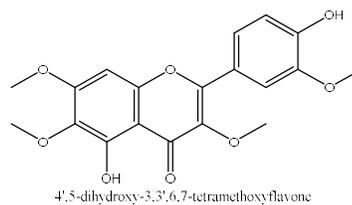
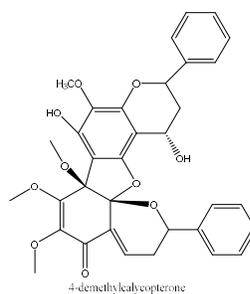
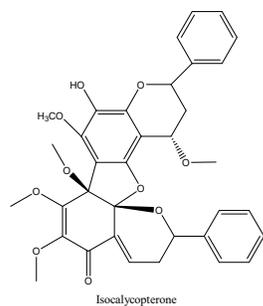
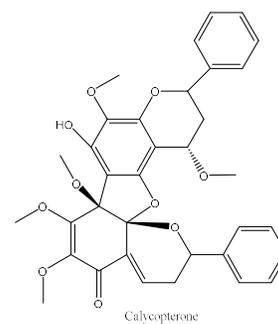
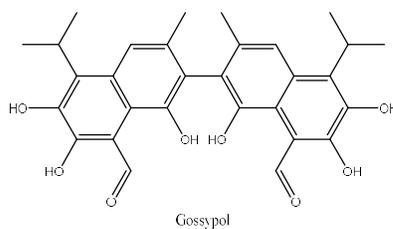
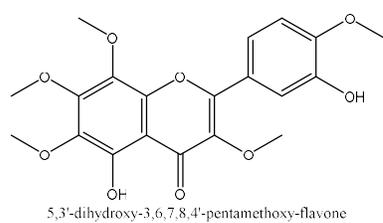
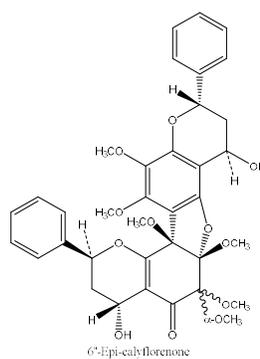
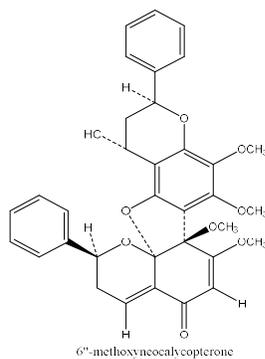
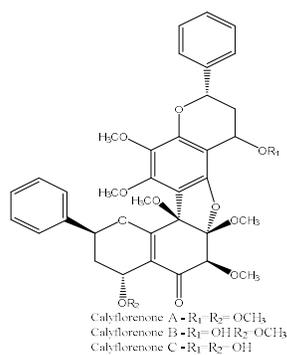
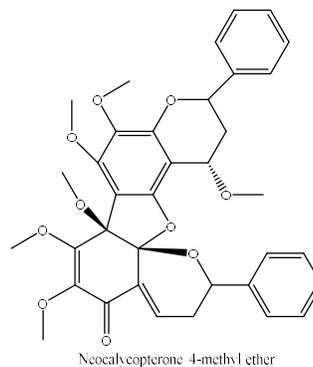
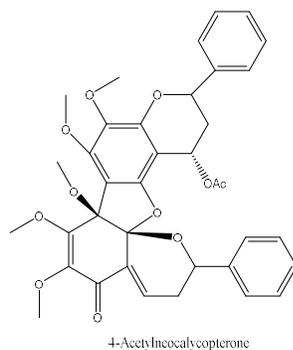
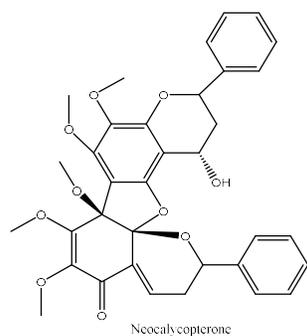
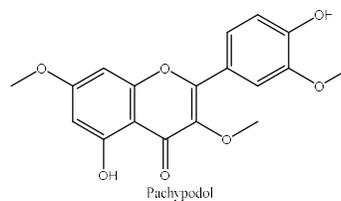
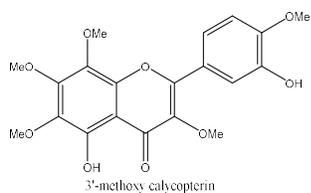
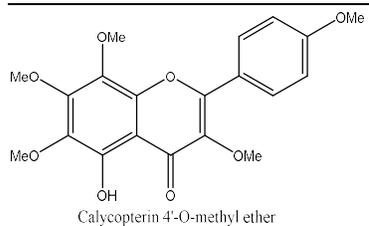
Figure 12: HPLC Fingerprints of crude flavonoid fractions of *Calycotris floribunda* [18]Table 9: Quantitative Analysis of *Calycotris floribunda* [8, 30]

Extracts	Total Flavonoid Content	Total Phenolic Content
Petroleum ether	0.038 $\pm$ 0.076 mg/g	--
Ethyl acetate	3.545 $\pm$ 0.023 mg/g	--
Methanol	1.489 $\pm$ 0.043 mg/g	--
Petroleum ether	25.68 $\mu$ g CE /mg	25.68 $\mu$ g GAE /mg
Chloroform	55.85 $\mu$ g CE /mg	55.85 GAE /mg
Methanol	48.50 $\mu$ g CE /mg	280.31 $\mu$ g GAE /mg

Table 10: Isolation of Chemical Constituents from *Calycopteris floribunda* [8]

S.No	Plant Part	Solvents	Compound
1	Leaves	Dichloro methane methanol	3, 8-di-O-methyl ellagic acid [10]
2		Dichloro methane- methanol	2,3,7-tri-O-methyl ellagic acid [10]
3		Acetone, Benzene, Methanol	Calycopterin [11-15]
4		Petroleum ether	n-octacosanol [14]
5		Petroleum ether	Sitosterol [14]
6		Petroleum ether	4'-O-methylcalycopterin [14]
7		Petroleum ether	Ellagic acid [14]
8		Petroleum ether	Proanthocyanidin [14]
9		Petroleum ether, Methanol	Quercetin [14-15]
10		---	Calycopterin-4'-methyl ether [16]
11		---	3'-methoxy Calycopterin [16]
12		Dichloro methane-methanol, Ethanol	Pachypodol [17-18]
13		Ethanol	Neo calycopterone [19]
14		Ethanol	4-Acetyl neocalycopterone [19]
15		Ethanol	Neocalycopterone 4- methyl ether [19]
16		Ethanol, Ethyl acetate	Calyflorenone A-C [19-20]
17		Ethyl acetate	6''-demethoxy neocalycopterone [20]
18		Ethyl acetate	6''-epi-calyflorenone [20]
19		Dichloro methane	5,3' -dihydroxy-3,6,7,8,4'-pentamethoxy-flavone [21]
20	Flowers	Methanol	Gossypol [15]
21		50% Methanol/ Chloroform	Calycopterone [22]
22		50% Methanol/ Chloroform	Isocalycopterone [22]
23		50% Methanol/ Chloroform	4-demethyl calycopterone [22]
24		50% Methanol/ Chloroform	4', 5-dihydroxy-3,3',6, 7-tetra methoxy flavone [22]





**The optimized protocol [18]**

Method	: High Performance Liquid Chromatography
Column	: Lichrospher C18
Mobile phase	: Acetonitrile and ultrapure water (3:1)
Detector	: UV detector
Wavelength	: 365 nm
Injected Vol.	: 1.0 mL min <sup>-1</sup>
Temperature	: 35°C

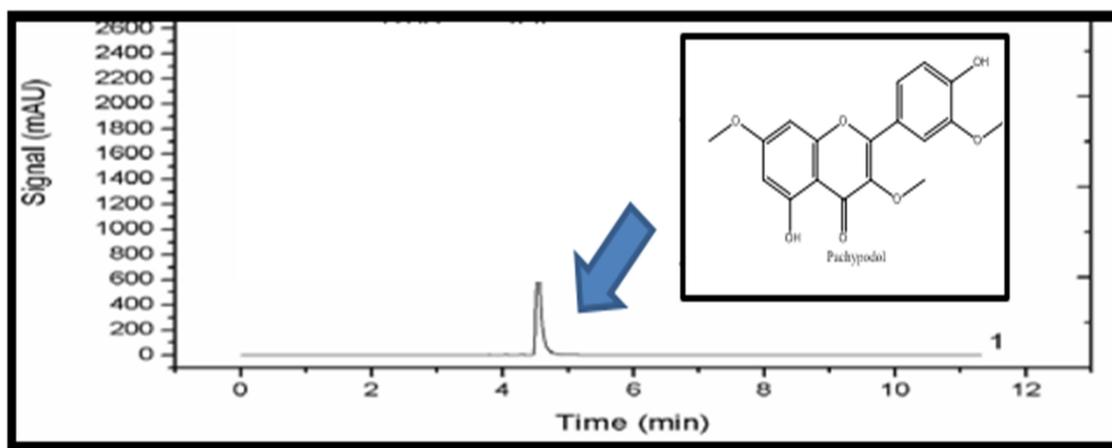


Figure 13: HPLC Quantification of Pachypodol (Arrowhead) [18]

**BIOLOGICAL STANDARDIZATION**

The biological potential of *Calycopteris floribunda* plant is widely established. The plant *Calycopteris floribunda* is host to a large number of flavonoids. There are numerous reports on the antimicrobial and antioxidant activity studies for the plant extracts.

**Antimicrobial Activity**

The various solvent extracts of *Calycopteris floribunda* earlier screened for antibacterial activity are reported to exhibit good inhibition of test bacteria. The earlier reports and present work on antibacterial and antifungal activity are summarised in **Tables 11 and 12**.

Table 11: Antibacterial Activity of Solvent Extracts of *Calycopteris floribunda* [7, 9, 10, 23, 30, 35]

S. No	Crude / Extracts	Bacterial Strains with Zone of Inhibition (mm)												
		a	b	c	d	e	f	g	h	i	j	k	l	m
1.	Petroleum ether	6	--	21	21	--	--	--	--	--	21	20	1	2
2.	Hexane (Present Work)	--	--	5	18	--	7	--	--	--	--	10	--	--
3.	Chloroform	4	--	24	22	--	--	--	--	--	23	19	2	1
4.	Ethyl acetate	6	--	--	--	--	--	--	--	--	4	--	4	1
5.	Diethyl ether – Methanol	18	--	21	--	--	--	--	--	--	--	19	--	--
6.	Pet ether-Butanol	14	--	16	--	--	--	--	--	--	--	16	--	--
7.	CH <sub>2</sub> Cl <sub>2</sub> : MeOH	21	19	19	14	14	19	--	--	--	--	--	--	--
8.	1-Butanol	15	16	16	16	15	14	--	--	--	--	--	--	--
9.	Methanol	8	--	16	14	--	--	10	11	14	09	18	4	2
10.	Aqueous 90% MeOH	18	18	23	16	16	23	--	--	--	--	13	--	--
11.	Hydroethanol (90% Ethanol) (Present Work)	--	--	22	20	--	20	--	--	--	--	10	--	--
12.	Aqueous	--	--	15	12	--	--	09	11	14	08	--	--	--

a- *Bacillus subtilis*; b-*Streptococcus pyogen*; c-*Staphylococcus aureus*; d-*Escherichia coli*; e-*Pseudomonas aerogenes*; f-*Salmonella typhi*; g-*Salmonella typhimurium*; h-*Klebsiella pneumoniae*; i-*Clostridium perfringens*; j-*Pseudomonas aeruginosa*; k-*Bacillus cereus*; l-*Actinomycetes sp.*; m-*Serratia sp*

Table 12: Antifungal activity of *Calycopteris floribunda*

S. No	Extracts	Zone of Inhibition (mm) [31]					
		<i>Microsporium gypsiu</i>	<i>Chrysosporium keratinophilum</i>	<i>Trichophytum rubrum</i>	<i>Chrysosporium indicum</i>	<i>Fusarium oxysporum</i>	<i>Pythium aphanidermatum</i>
1	Methanol	10	12	14	11	--	--
2	Aqueous	12	14	12	10	22	21

### Antioxidant Activity

The antioxidant activity was studied by various assays. The results of DPPH free radical scavenging assay, total antioxidant activity (phosphomolybdc acid method), ferric reducing antioxidant potential (FRAP) assay, superoxide anion radical scavenging, hydroxyl radical and lipid peroxidation assay are reported in **Table 13**.

### Other Activity Studies

There are numerous reports validating the antimicrobial, antioxidant potential of the various parts of *Calycopteris floribunda*. However, there are very few reports on other activity studies. Much of the studies on pharmacological potential of *Calycopteris floribunda* is done with leaf extracts. The leaf acetone extract is reported to possess anthelmintic activity [11]. The butanol extract of the leaves is found to inhibit  $\beta$ -glucuronidase (80% inhibition) [10]. The leaf extract has been also tested for hypoglycemic effect [37]. The stem extract of this plant is reported to exhibit anti-inflammatory [36] and hepatoprotective potential [38].

Apart from the extracts of this plant, some of the compounds isolated are also tested for medicinal potential. **Table 14** lists the compounds tested and their medicinal potential.

### Toxicity Studies

So far, there are two reports of toxicity studies on this plant *Calycopteris floribunda*. The fresh leaves of *Calycopteris floribunda* were found to be hepatotoxic, nephrotoxic, and cardiotoxic in calves; its methanol extract was found to be toxic at 400 mg/g body weights of rats and rabbits [27]. This study reveals that the plants and its parts might be toxic for oral intake. However, its immense medicinal potential indicates that this plant may be recommended as an ingredient of topical ointments and thailams. Acute toxicity studies and dermal toxicity studies have not been reported. Yet another report of general toxicity studies reveal that the flavonoid pachypodol isolated from the leaves has LD<sub>50</sub> of 435.8 M [17].

**Table 13: Antioxidant Activity Studies on *Calycopteris floribunda* [8, 10, 23,]**

S. No.	Assay	Name of extract	Scavenging Activity (%) or (% $\pm$ SM)
1	DPPH radical scavenging assay	Diethyl ether – Methanol	56
		Petroleum ether– Butanol	88
		DCM-MeOH	66
		Aq. 90%MeOH	89
		1-Butanol	92
		Chloroform	65.33 $\pm$ 1.38
		Methanol	72.5 $\pm$ 1.17
2	FRAP assay	Petroleum ether	37.52 $\pm$ 0.041
		Ethyl acetate	78.82 $\pm$ 0.013
		Methanol	67.56 $\pm$ 0.086
3	Total antioxidant activity	Petroleum ether	50.79 $\pm$ 0.021
		Ethyl acetate	76.53 $\pm$ 0.040
		Methanol	69.67 $\pm$ 0.034
4	Superoxide anion radical scavenging assay	Chloroform	52.4 $\pm$ 1.7
		Methanol	70.8 $\pm$ 0.95
5	Hydroxyl radical scavenging assay	Chloroform	53.83 $\pm$ 1.29
		Methanol	60.9 $\pm$ 0.50
6	Lipid peroxidation scavenging assay	Chloroform	58.06 $\pm$ 0.63
		Methanol	52.20 $\pm$ 1.04

Table 14: Bioactive Substances

Compound	Source	Medicinal Potential
Calycopterin [11]	Leaves	Anthelmintic activity
3'-amino-5-hydroxy-3,6,7,8,4'-pentamethoxy flavone [21]	Leaves	Antiproliferative activity
Calycopterone [22]	Flowers	Anti-cancer activity against Frederick-NCI human tumour cancer cell lines
Isocalycopterone [22]		
4-demethylcalycopterone [22]		
Pachypodol [17]	Leaves	Anti-cancer activity against CaCo-2 colon cancer cell lines (IC <sub>50</sub> = 185.6 µM)

## CONCLUSION

This review consolidates all the available scientific data on *Calycopteris floribunda* which is an important ayurvedic medicinal plant. It finds use as one of the constituents of the Ayurvedic formulation named “Jathyadi Thailam” used as a wound healing medicine in Ayurveda treatment. The plant is found to be a rich source of flavonoidal compounds. Of all the compounds isolated, only one compound named pachypodol has been quantified from leaf extracts. The highly medicinal flavonoid quercetin is also reported from this plant. However, this compound has not been quantified. The rich antioxidant potential of the plant may be bestowed upon it by the presence of its flavonoidal constituents. There are recent reports on the use of the crushed roots of *Calycopteris floribunda* as an oral antidote for snake bite [39]. Also the juice from the leaves are taken internally to cure dysentery [40]. An interesting observation reports use of an oral medication of leaves to cure manganese deficiency [26]. Deficiency of manganese leads to poor bone growth or skeletal defects. Hence, the plant may be considered as a source for manganese supplementation.

All available data on the plant *Calycopteris floribunda*, as well as the findings of this study, are documented as a ready reckoner sheet, which will assist herbal manufacturers who use this plant in developing standardized formulations. This will aid in increasing the validity of the product labels, thereby increasing the global market value of formulations containing *Calycopteris floribunda*.

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## CONFLICT OF INTEREST

The authors declare no Conflict of Interest.

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