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Research

Pharmacognostical, Preliminary Phytochemical Screening, Pharmacobotany and DNA Barcoding Characterization of *Calliandra haematocephala* Leaves (Hassk)

A. Krishnaveni[*1], Manjula. B[2], Sandhiya. S[2], Vaishnavi. G[2], T.Venkata Rathina Kumar[3]

*[*1]Assistant professor, [2]II year M. Pharm, [3]Principal,*
Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai.
Affiliated to Tamil Nadu Dr. MGR Medical University, Chennai-600032.

*Author for Correspondence: Dr. A. Krishnaveni, M.Pharm, Ph. D
Email: akrishnaveni72@rediffmail.com

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Abstract

Background: *Calliandra haematocephala* Hassk commonly known as the red powder puff, is a small ornamental shrub belonging to the family Fabaceae. It is widely distributed in tropical and subtropical regions, including India, Southeast Asia, Africa, and Central America. Though popularly cultivated for its vibrant crimson flowers, the plant also holds considerable ethnomedicinal value. Traditionally, various parts of *Calliandra haematocephala* have been used in the treatment of inflammatory disorders, epilepsy, fever, bacterial infections, and as a natural blood purifier. Tribal and folk communities (Ogbomoso, Oyo State) have employed its extracts for their anti-inflammatory, anticonvulsant, immunomodulatory, and antibacterial properties.

Materials and methods: Leaves were collected from the Alagarkoil foot Hills, Madurai district, Tamil Nadu in the month of March 2025.It was authenticated by Dr. Stephen, Professor of Botany, The American College, Madurai-625002. Collected leaves were washed with water, shade dried powdered and aqueous extract were prepatred. The extract was concentrated and stored in airtight container for further use. These compounds are believed to contribute to its therapeutic potential. In the present study, fresh leaves of *Calliandra haematocephala* were collected, cleaned and crushed to juice using blender, the resulting juice was used for further analysis.

Results and discussion: The study was evaluated for the pharmacognostical characteristics, physicochemical parameters, qualitative and quantitative estimation of *Calliandra haematocephala* leaves along with evolution of phenetics analysis.

Keywords: Calliandra haematocephala, Fabaceae, Pharmacognostical, Phenetics, Physiochemical, Qualitative and Quantitative estimation and DNA Barcoding.

INTRODUCTION

Calliandra haematocephala, belongs to Fabaceae, is an ornamental shrub commonly known as Red Powder Puff plant. It is native to Bolivia, but widely cultivated in India[1]. In Southern India the plant traditionally used to treat pain, inflammatory conditions, and neurological disorders,[2] In Tamil Nadu (Kani, Paliyar)[3] leaf and flower preparations for used to treat skin infections, wounds, fever and inflammatory disorders. In some parts of northeastern India (Irula, Kurumba) indigenous users rely on aqueous leaf extracts for gastrointestinal issues and as a general tonic [4].In Central and South America, Nigeria, Colombia people used leaf decoction to treat diabetics and Yoruba, Nupe people used to treat malaria[5],[6]. In south western Nigeria Tude people used antioxidant and blood purifier[7]. In China, Jinjiang, Yunnan people leaf, flower and bark used by tranquilizing effect[8]. Phytochemical survey revealed the presence of flavanoids, terpenoids, saponins, phenolic compounds, glycosides and alkaloids[9]. It exhibited pharmacological activities such as, antimicrobial[10], antioxidant and anti-inflammatory[11], antidiabetic[12], hepatoprotective[13]. The objective of the study is to evaluate the pharmacognostical, physiochemical parameters, qualitative and quantitative estimation of *Calliandra haematocephala* leaves.

MATERIALS AND METHODS

Collection of leaves and authentication

Leaves were collected from the Alagarkoil foot Hills, Madurai district, Tamil Nadu in the month of March 2025.It was authenticated by Dr. Stephen, Professor of Botany, The American College, Madurai-625002. A herbarium of this specimen was deposited in the department for future reference.

Pharmacognostical evaluation

Fresh leaves were subjected to pharmacognostical studies. Organoleptic, macroscopy and microscopy of the leaves of *Calliandra haematocephala* were studied.

Organoleptic evaluation

Fresh leaves are collected and checked for their colour,odour and taste by sensory characters.

Macroscopical evaluation

It includes length, width, base, apex, arrangement and venation of the leaf was identified as per the standard procedure described in Gopinath S.M. (2013) [14,15](figure 1&2 and table 1).

Microscopy evaluation

Free handmade sections were taken, stained with routine staining reagents and were observed under microscope as per Wallis[16] . Sections were first observed in distilled water, then stained with safranin examined to assess different cellular structures and contents. The samples were observed under a compound microscope.[16,17,18] (figure 3&4).

Quantitative microscopy

The study was carried out using small square pieces of leaf taken from the area between the midrib and the margin. These samples were decolourised and examined under a microscope to assess vein islet number, vein termination, and palisade ratio. For stomatal index analysis, the upper and lower epidermal layers were carefully peeled from separate leaf pieces, mounted in glycerin water on a slide, and observed microscopically to determine stomatal index and stomatal type[19].

Preparation of leaves powder

The fresh matured leaves of *Calliandra haematocephala* were collected, washed with water and shade dried. The dried leaves were made into a fine powder.

Powder microscopy

A small amount of the powdered sample was mounted on a microscopic slide with routine reagents as per K.R. Khandelwal[20]. Photomicrographs of diagnostic characters were detected displayed and documented in fig 6.

Behaviour of powder with various chemical reagents

A small quantity of leaf powder was transferred to test tube and 1-2 drops of freshly prepared various solution was added and the colour was observed under visible UV-254 and 365 nm is presented in table 4[21]

Determination of Physico-chemical parameters

The leaf powder was analysed by various physio-chemical parameters such as foreign matters, loss on drying, total solids, ash values and extractive values were determined by using various solvents by Ayurvedic Pharmacopoeia and is given in table 5. The ash was subjected to inorganic elements identification as per procedure given in Atherden[22]

Preparation of fresh juice of *Calliandra haematocephala* leaves(*C.haematocephala*$_{frj}$)
Fresh leaves of 20g were weighed, washed with water and 20ml of water is added to make a 20 ml of *Calliandra haematocephala* fresh juice.

Preliminary phytochemical screening
Preliminary phytochemical screening were carried out by using different reagents for identification the presence of phytoconstituents as per standard procedures[23].

Limit of Detection (LOD) and Limit of Quantitation (LOQ) of *C.haematocephala*$_{frj}$
Procedure
The LOD of an individual analytical procedure is the lowest amount of an analyte in a sample that can be detected but not necessarily quantified. The LOQ of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected and quantified. *C.haematocephala*$_{frj}$ were prepared and scanned under UV range of (200-700nm). *C.haematocephala*$_{frj}$ were analyzed to identify the presence of peak and the corresponding wavelength was recorded. Subsequently, *C.haematocephala*$_{frj}$ at concentrations of (0-120µg/ml) were prepared and examined under UV spectroscopy at the wavelength identified from *C.haematocephala*$_{frj}$. The LOD and LOQ values were calculated based on the standard deviation of the response and the slope derived from the *C.haematocephala*$_{frj.}$[24,25]

Quantitative estimation of phytoconstituents
Determination of digoxin equivalent in *Calliandra haematocephala*$_L$
Procedure: The method was adopted as per Indian Pharmacopoeia 1985 [26]
Various concentrations (0 to 25 µg/ml) of *C.haematocephala*$_{frj}$ were prepared. Dissolving 0.5 ml of *C.haematocephala*$_{frj}$ add 5ml of a mixture of 65 volumes of chloroform and 35 volumes of methanol. Add 20ml of glacial acetic acid and shake continuously for one hour. Allow to stand and filter the supernatant liquid through a sintered glass filter (grade 4, maximum pore size, 5 to 15µ), rejecting the first few ml of filterate. Dilute 5 ml of the filterate to 25ml with glacial acetic acid containing 0.005%w/v of ferric chloride and 2%v/v of sulphuric acid and allow to stand for one and half an hour. From this 5- 25µg/ml is prepared and compared with Digoxin as standard under UV at 590nm.

Determination of gallic acid equivalent in *Calliandra haematocephala*$_L$
Procedure: The method was adopted as per Singleton V.L[27]
Different concentration 5-25µg/ml *of C.haematocephala* juice was taken. To each of this add 5ml of distilled water and 0.5ml of Folin- Ciocalteu's reagent is added, mixed and shaken. After 5 minutes, 1ml of 10% sodium carbonate solution is added and the volume is made upto 10ml with distilled water was allowed to incubate for 2 hours at room temperature, intense blue colour was developed whose absorbance was recorded at 725nm and compared with gallic acid as standard.

Determination of rutin equivalent in *Calliandra haematocephala*$_L$
Procedure: The method was adopted as per Boham A.B[28]
Different concentration 10-50µg/ml *of C.haematocephala* juice was taken. To each of these solutions add 4ml of water and 0.3ml of 5% sodium nitrite solution. After 5 minutes, 0.3ml of 10% aluminium chloride and 2ml of 1M sodium hydroxide is added. Finally, volume is made up to 10ml with distilled water and mix well. Orange yellowish colour is developed. The absorbance is measured at 510 nm using UV-visible spectrophotometer and compared with Rutin as standard.

Pharmacobotany
Numerical taxonomy of plant derived by using five characters such as leaf blade, venation, apex, base and margin[29].

DNA Barcoding
Genomic DNA Isolation
About 100 mg of plant tissue was ground with liquid nitrogen to make fine powder using mortar and pestle. Added 1 ml of preheated CTAB extraction buffer with 20µl of β-mercaptoethanol to the mortar and finely ground. The contents were transferred into a 2 ml centrifuge tube and incubated for 20 to 30 min at 65°C on a water bath. After centrifuging using Refrigerated Centrifuge (Eppendrof, 5418R) the tube at 12,000 rpm for 10 min the supernatant was transferred to a fresh centrifuge tube and added equal volumes of chloroform: isoamyl alcohol (24:1) mixture and mixed gently by inverting tubes till an emulsion was formed. The tubes were centrifuged at 13,000 rpm for 12 min. The clear aqueous phase was transferred to fresh centrifuge tubes and equal volumes of ice-cold isopropanol was added. The sample was incubated overnight at -20^o C and centrifuged at 12000 rpm for 3 min. The supernatant was discarded and the pellet was washed with 70% ethanol.

DNA pellets were then air-dried at room temperature by allowing evaporation of uncovered centrifuge tubes. The pellets were suspended in an appropriate volume (20-30 µl) of $T_{10}E_1$ buffer.

Qualitative and Quantitative estimation of DNA

The quality and concentration of genomic DNA were checked by running the DNA sample on 1% agarose gel. The DNA concentrations were rechecked by visual assessment of band intensity under UV-trans-illuminator (Biorad, GelDoc Go, USA). The quantity of 1 µl of isolated DNA was checked using Nanodrop (Thermoscientific, Nanodrop One, USA).

PCR Amplification

The DNA barcode candidate ITS was used for PCR amplification as the same resulted in amplification. The isolated DNA was used as a template for PCR reaction and carried out in a thermocycler (Applied Biosystem,Veriti™, USA). The PCR products were then loaded onto 1% agarose gel and the amplification was confirmed.

Sequence analysis

FASTA format of the nucleotides were obtained using Finch TV from the chromatogram. The FASTA was fed into Basic Local Alignment Search Tool (BLAST) algorithm of NCBI to identify the closest matching sequence in the nucleotide database of GenBank. The sequence was converted to Barcode using the software BioRad barcode generator.

NCBI submission

After confirmation of the species, the sequence was submitted to NCBI with the necessary details to obtain a GenBank ID [30]

Morphological study

Calliandra haematocephala leaves were simple, oblong or lanceolate and arranged in the opposite direction. They were dark green in ventral and pale green in dorsal view, with a slightly bitter taste and a characteristics odour. It has a short petiole, a symmetrical,round base, and an acute apex. Its entire edge features Smooth (glabrous) upper surface; slightly pubescent lower surface. (Tab.1& Fig.1)

Table 1: Morphological characters of leaves of *Calliandra haematocephala*

S.No.	Characters	Observation
1	Leaf Type	Bipinnately compound with one pair of pinnae, each with 7-10 pairs of elliptic to oblong-lanceolate sessile leaflets.
2	Arrangement	Alternate
3	Leaflet Shape	Oblong to lanceolate
4	Leaflet Size	0.5-3.7 cm long and 0.3-1.3 cm width
5	Leaf Margin	Entire
6	Leaf Apex	Obtuse to mucronate
7	Leaf Base	Obliquely rounded to subcordate
8	Venation	Pinnate-reticulate
9	Texture	Smooth (glabrous) upper surface; slightly pubescent lower surface
10	Color (Fresh Leaf)	Upper surface dark green; lower surface pale green
11	Odor	Characteristic (mildly earthy or green)
12	Taste	Slightly bitter
13	Petiole	Short, green, and hairy
14	Stipules	Present, small and leaf-like at the base of the petiole
15	Midrib	Prominently raised on the lower surface

Fig 1: Bipinnately compound leaflet

Microscopy

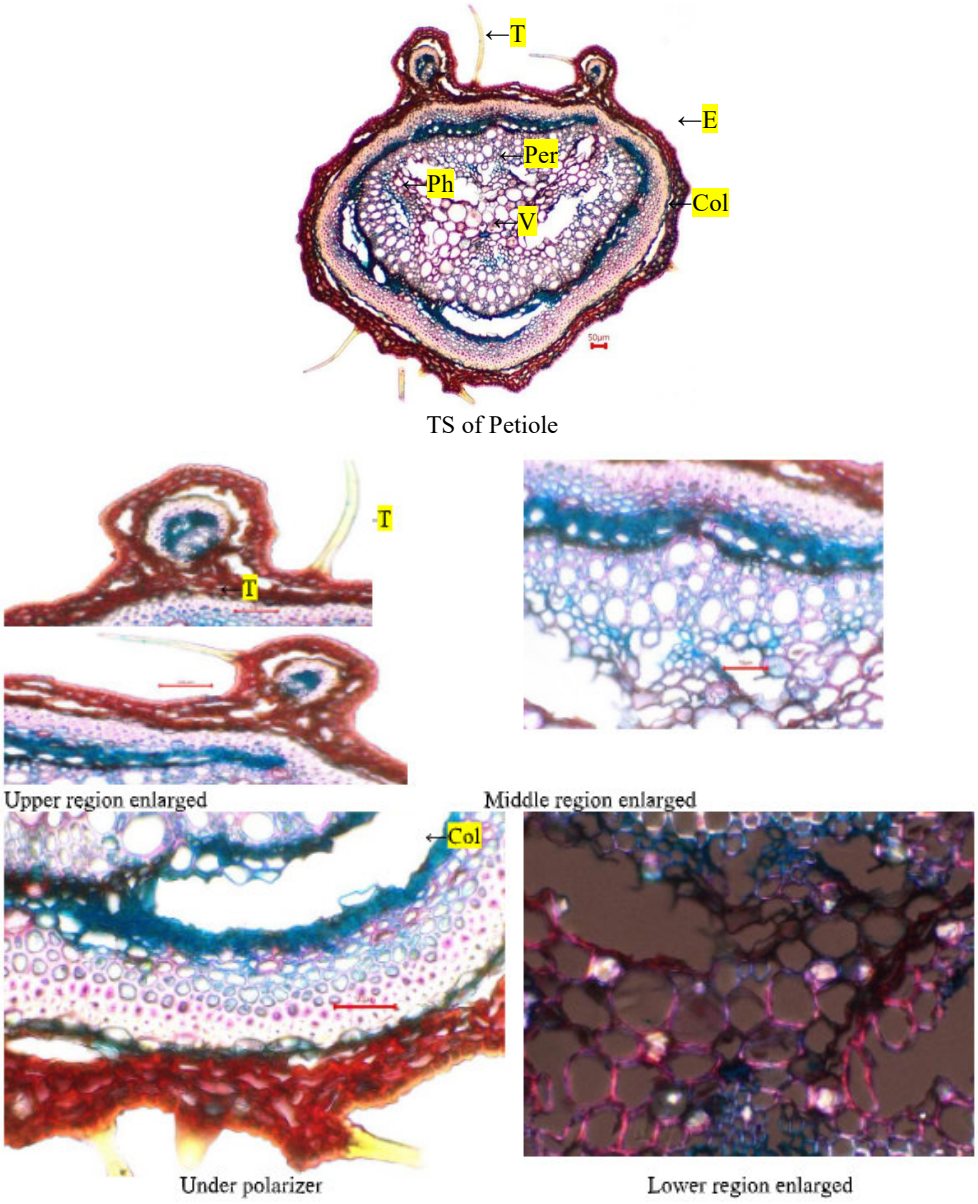
Epidermis
Three rows of long, closely spaced parenchyma palisade cells made comprised the top epidermis and stomata encircled by many guard cells were visible in the lower epidermis.

Stomata type
The lower epidermis contains stomata has two semicircular guard cells which are parallel and bigger subsidiary cells surrounds stomata and it is known as paracytic stomata.

Vascular bundles
Bicollateral hollow vascular cylinder made up of long, tubular cells. The cells are round, with a broad lumen and thick, lignified walls. Short radial lines are used to arrange the elements.

Fibres
Fibers are cells of the sclerenchyma, there is a elongated cell is present that is brachysclereid.

Petiole
TS is broadly oval or obovate in outline with two prominent on the upper side; outermost single-layered epidermis consists of small, compact, barrel-shaped cells with thick cutinized walls; few multicellular hairs are seen; collenchymatous hypodermis of 3 to 4 layers is seen, particularly under the ridges compared to the lower surface; cortex made up of thin-walled, round to oval, parenchymatous cells; vascular bundles are arranged in a crescent-shaped arc, forming a semicircular vascular ring; each vascular bundle is conjoint, collateral, and open, composed of xylem located on the inner side with prominent large thick-walled vessels, and phloem is present toward the outer side; cambium is thin, seen between xylem and phloem; pericycle is multiyared , sclerenchymatous and formed as a bundle sheath surrounding the vascular bundles; a large parenchymatous pith occupies the central region composed of large, loosely arranged thin-walled cells with intercellular spaces; accessory vascular bundles are present inside the adaxial ridges; numerous prismatic crystals are seen in the cortical parenchyma cells (Fig. 2).

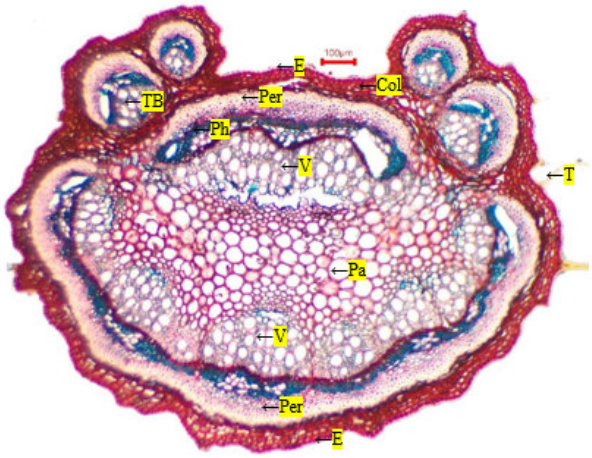


Col - collenchyma; **E** - epidermis; **Pa** - parenchyma; **PCr -** prismatic crystal; **Ph** - phloem; **T** - trichome; **TB** - trace bundle; **VB** - vascular bundle; **V** - vessel

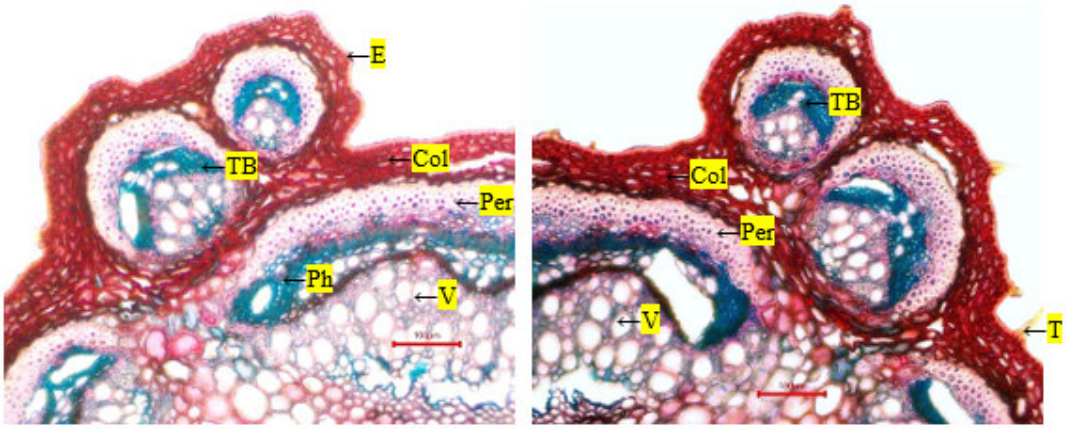
Fig 2: Microscopy of *Calliandra haematocephala* petiole

Petiolule

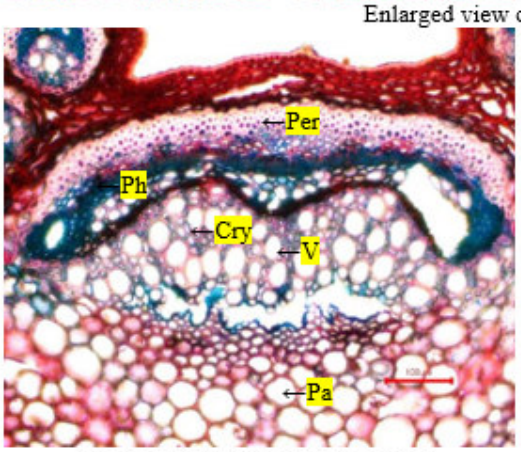
The TS is broadly oval to circular, deeply lobed with multiple prominent projections or ridges extending outward from the main body; the lobes are vascularized, each containing smaller vascular bundles and well-developed collenchyma; outermost single layer of epidermal cells compactly arranged and covered by a thick cuticle; few covering trichomes are seen arising from the epidermis; cortex is differentiated into outer 2 to 3 collenchymatous layers made up of thick-walled cells, it is pronounced at the ridges; parenchymatous inner cortex composed of thin-walled, rounded or oval parenchymatous cells that appear loosely arranged with intercellular spaces; vascular system is arranged in a concentric pattern with multiple vascular bundles each containing centrally located xylem made up of large thin walled vessels and peripherally arranged phloem; pericycle is multiyared, sclerenchymatous and has formed as a bundle sheath surrounding the vascular bundles and ground tissue; central pith region composed of large thin walled parenchymatous cells; few prismatic crystals are seen scattered in the outer cortical region (Fig3)



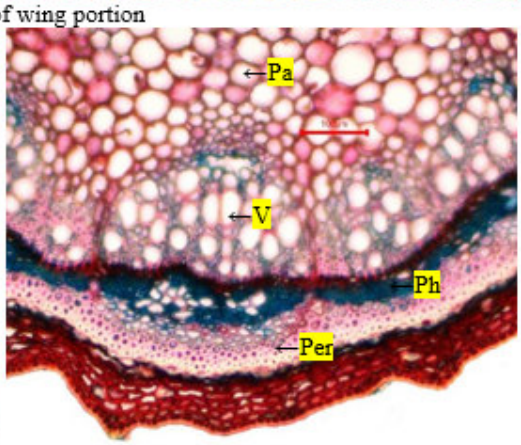
TS of Petiolule



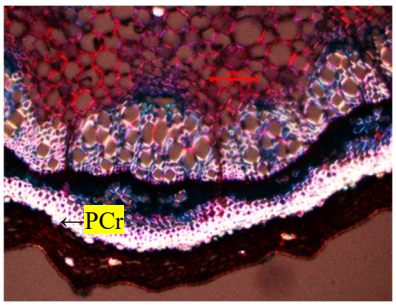
Enlarged view of wing portion



Enlarged view of the upper portion



Enlarged view of the lower portion



Col - collenchyma; **E** - epidermis; **Pa** - parenchyma; **PCr -** prismatic crystal; **Ph** - phloem; **T** - trichome; **TB** - trace bundle; **VB** - vascular bundle; **V** - vessel

Fig 3: Microscopy of *Calliandra haematocephala* petiolule

Leaflet

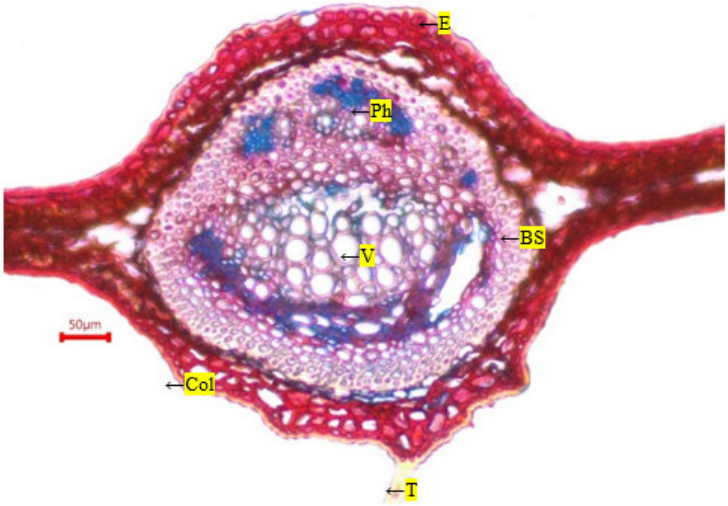
Transverse sectiob is biconvex in outline and shows isobilateral structure with continuous upper palisade layers; the lower palisade is interrupted in the midrib region by a mass of subepidermal collenchymatous cells.

Midrib

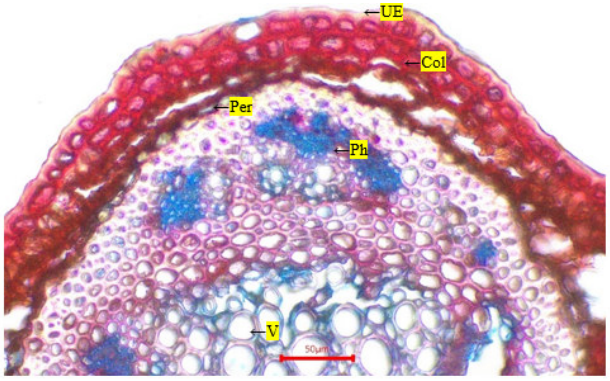
Upper and lower epidermis consists of one row of square to sub-rectangular cells covered by distinct cuticle, a few unicellular non-glandular trichomes are seen in the lower epidermis; the upper and lower surface is broad and curved, the midrib region shows a central vascular bundle surrounded by distinct sclerenchymatous bundle sheath; midrib region reveals the presence of 2 to 3 layers of compactly arranged cells of collenchyma just between the two layers of epidermii; 4 to 6 layers of sclerenchymatous bundle sheath is seen surrounding the centrally placed large conjoint, collateral, and closed vascular bundle; xylem is seen towards the inner side with prominent metaxylem vessels, parenchyma and fibre; phloem is located externally to xylem and contains parenchyma, companion cells and sieve elements (Fig. 4)

Lamina

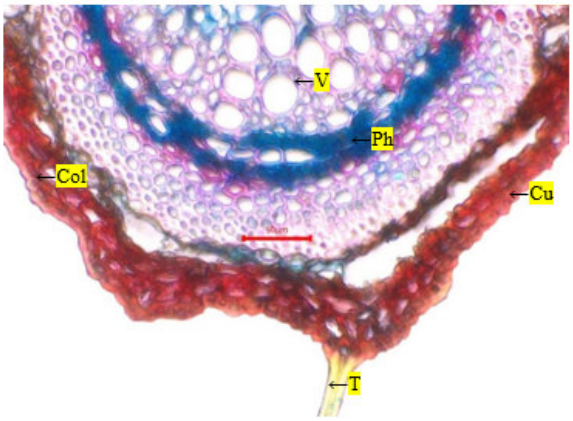
TS of lamina is dorsiventral and shows outer single-layered, compactly arranged epidermal cells which are cutinized; mesophyll tissue is composed of outer one to two layers of elongated columnar palisade cells closely packed followed by spongy cells consisting of loosely arranged, irregularly shaped cells; veins or traces of vascular bundles surrounded by bundle sheath cells are present; lower epidermis composed of single layer of slightly smaller and more flattened cells than those of the upper epidermis; few prismatic crystals are observed in the mesophyll region (Fig. 4).



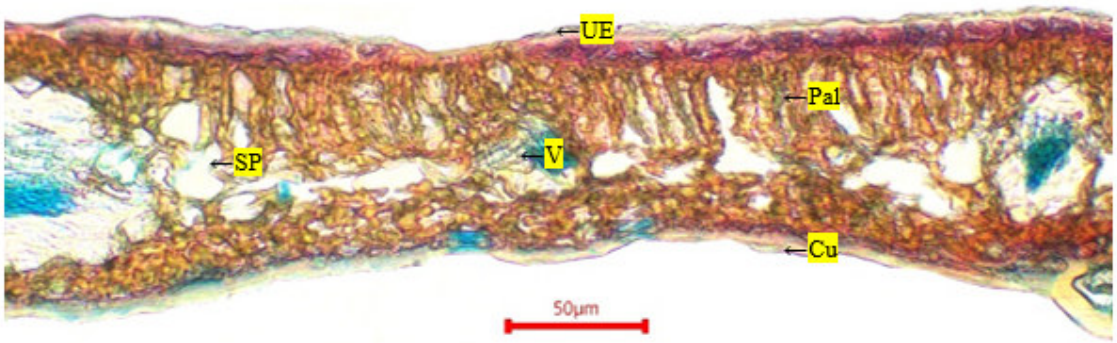
TS of midrib



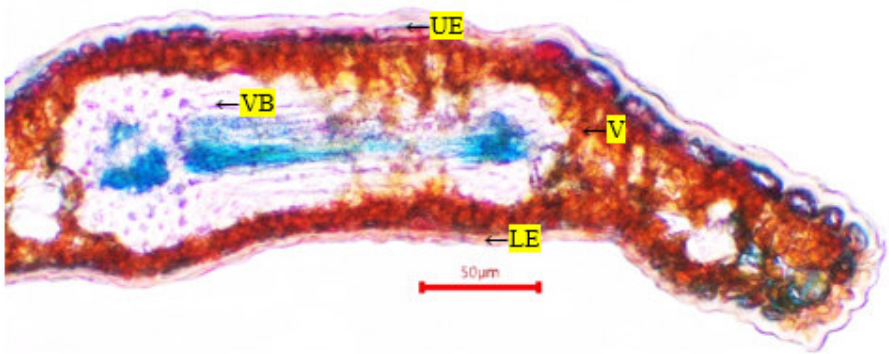
Upper region enlarged



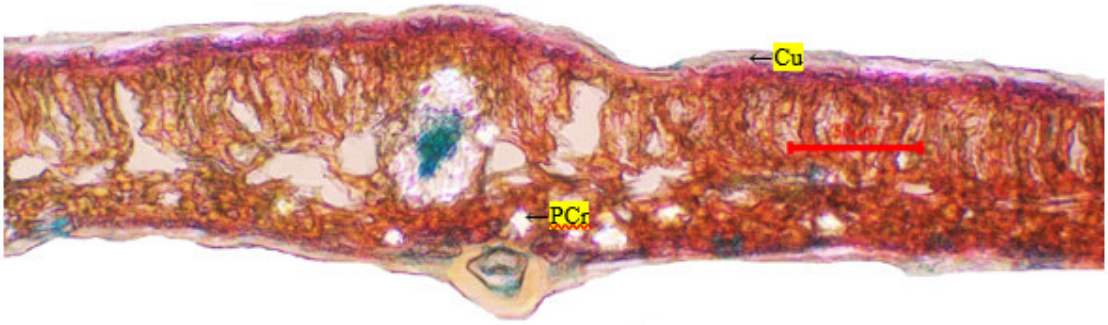
Lower region enlarged



TS of lamina



Lamina margin enlarged



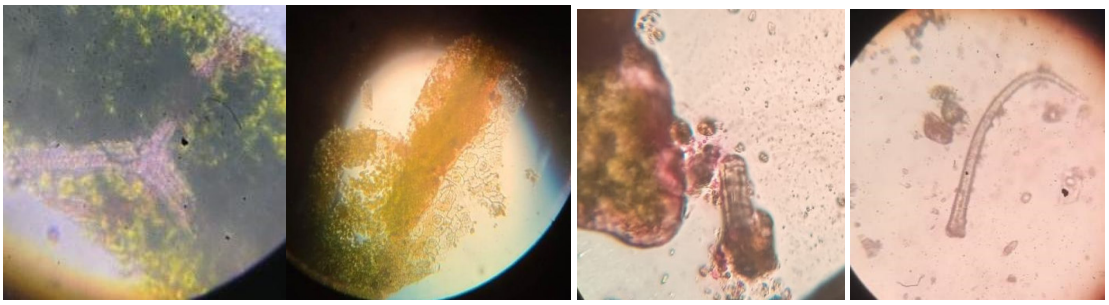
TS of lamina under polarizer

BS - bundle sheath; **Col** - collenchyma; **Cu** - cuticle; **LE** - lower epidermis; **Pa** - parenchyma; **Pal** - palisade cells; **Ph** - phloem; **SP** - spongy parenchyma; **T** - trichome; **UE** - upper epidermis; **V** - vessel; **VB** - vascular bundle

Fig 4: TS of the leaflet passing through the midrib

Table 2: Histochemical studies of *Calliandra haematocephala* leaf

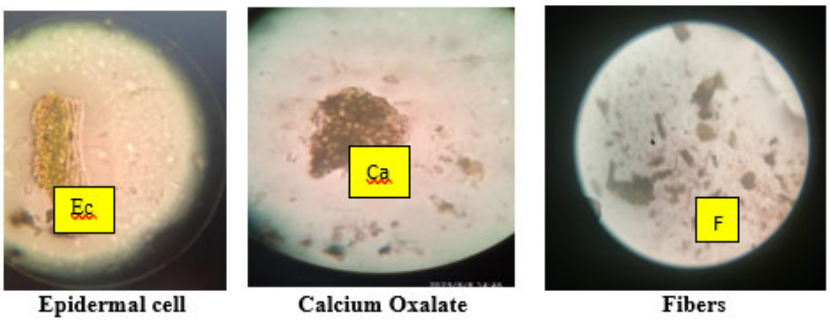
S.No.	Reagent	Test	Observation	Identification
1.	Phloroglucinol + HCL	Lignin	Red	Xylem Vessels
2.	Iodine solution followed by Sulphuric acid	Cellulose	Yellow	Parenchyma cells
3.	Heating with KOH	Suberin	Yellow	Trichomes, stomata
4.	Picric acid	Alkaloids	Red	Fibres



Xylem vessel **Stomata** **Parenchyma** **Trichomes**

Powder microscopy

The shade dried, powdered plant material was used for powder microscopic analysis. The organoleptic characters were observed and to identify the different characteristic features various staining reagents are used. It showed epidermal cells, stomata of paracytic type, parenchyma cells.



Quantitative microscopy of *Calliandra haematocephala*

Table 3: Quantitative microscopy of *Calliandra haematocephala*

S.No.	Parameters	Cells /mm2
1.	Epidermal number	71.5
2.	Vein islets number	6.25
3.	Vein termination	5
4.	Stomatal number	36
5.	Stomatal Index	33
6.	Palisade ratio	7-9
7.	Stomata type	Paracytic

Behaviour of powder with various chemical reagents

Powdered drug of *Calliandra haematocephala* Hassk plant gave different fluorescence under visible and ultraviolet (UV) radiation. (254nm and 365 nm), when treated with various reagents. Therefore, fluorescence evaluation is used for the identification of powdered drug.

Table 4: Behaviour of powder with various chemical reagents

Sample + Reagent	Visible (<400 nm)	Short Wave (254 nm)	Long Wave (365 nm)
Powder + HCl	Green	Black	Green
Powder + HCl + H_2O	Green	Black	Green
Powder +H_2SO_4	Brown	Dark Green	Green
Powder +H_2SO_4 + H20	Brown	Dark Green	Green
Powder + HNO_3	Yellow	Black	Green
Powder + HNO_3+ H_2O	Yellow	Dark Green	Green
Powder + 20% NaOH	Light Green	Brown	Fluorescent Green
Powder + Alcoholic NaOH	Light Green	Yellow	Fluorescent Green
Powder + Acetic acid	Yellow	Brown	Green
Powder + Fe_3Cl_4	Dark Green	Black	Green

Powder + Iodine	Red	Brown	Green
Powder + Picric Acid	Yellow	Dark Green	Fluorescent Green
Powder+ammonia	Dark green	Brown	Green
Powder+water	Dark Green	Black	Green

Determination of Physiochemical parameters of *Calliandra haematocephala*

The ash values of the plant were estimated using standard procedures which showed a total ash of 16.43±1.263% w/w, water soluble ash 0.64±0.037 %w/w and acid insoluble ash of 0.73±0.37 w/w. Loss on drying and total solid value of the powder was determined as 129.36±0.945 & 29.36± 0.945%w/w respectively. Petroleum ether extractive, Ethyl acetate extractive, Ethanol extractive, Chloroform extractive,water extractive the percentage yield of the extractive was found to be 0.113±0.033%, 0.18±0.0163%, 0.31±0.0203%, 0.133±0.0094%, 21.1±0.0294% w/w respectively. To the ash of *Calliandra haematocephala* Hassk., leaves was treated with 50%v/v hydrochloric acid and kept for 1 hour. It was filtered, filtrate was used for inorganic and heavy metal analysis using various reagents.

Table 5: Determination of Physiochemical parameters of *Calliandra haematocephala*

S.No	Physio-chemical parameters	Results
1	Foreign matter	Nil
2	Loss on drying	129.36±0.945%w/w
3	Total solid	29.36±0.945%w/w
4	Petroleum ether extractive	0.113±0.033%w/w
5	Ethyl acetate extractive	0.18±0.0163%w/w
6	Ethanol extractive	0.31±0.0203%w/w
7	Chloroform extractive	0.133±0.0094%w/w
10	Water extractive	21.1±0.0294%w/w
12	Total Ash	16.43±1.263%w/w
13	Water soluble ash	0.64±0.037%w/w
14	Acid insoluble ash	0.73±0.0374%w/w
15	Inorganic mineral analysis	Presence of chloride, sulphate
16	Heavy metals - Lead(I.P.1985)	Absence

Qualitative phytochemical analysis

The extract were subjected to preliminary phytochemical screening to determine the presence of various phytoconstituents. Its showed the presence of alkaloid, carbohydrates, glycosides, phytosterol, phenol,saponins, flavonoid and steroids.

Table 6: Qualitative phytochemical analysis of *Calliandra haematocephala* Hassk

S.No.	Phytochemical analysis	Observation
1.	Test for Alkaloids	+
2.	Test for Pyrolidine alkaloid	+
3.	Test for Glycosides	+
4.	Test for Cardiac glycosides	+
5.	Test for Tannins	-
6.	Test for Saponins	+
7.	Test for Flavonoids	+
8.	Test for Triterpenoids	-
9.	Test for Proteins	-
10.	Test for Coumarin	-
11.	Test for Amino acids	-
12.	Test for Starch	-
13.	Test for Carbohydrates	+
14.	Test for Steroids	+
15.	Test for Anthroquinone glycosides	-
16.	Test for Phytosterols	+
17.	Test for Phenolic compound	+

18.	Test for Gums & mucilage	-
19.	Test for Anthocyanin	-
20.	Test for Quinone	-

+ Presence, - absence

Limit of Detection (LOD) and Limit of Quantitation (LOQ) of *C.haematocephala*$_{FRJ}$

Various concentration (0.1-1ml) of *C.haematocephalaFRJ* leaf were prepared as blank, observedunder UV at 222nm. Absorbance was observed and dispaced table 7

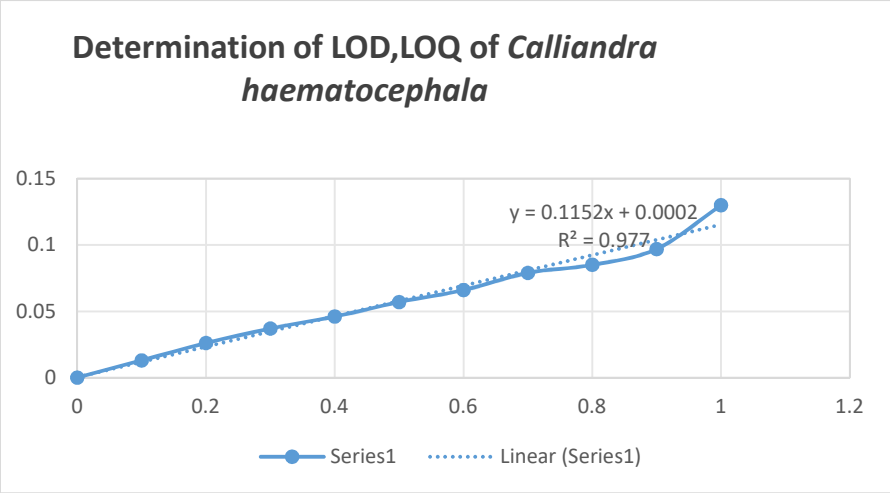


Fig 6. Calibration curve of *Calliandra haematocephala*

Table :7. Determination of LOD, LOQ in *C.haematocephala*

S.No.	Concentrations(ml)	Absorbance
1	0	0
2	0.1	0.0125
3	0.2	0.0256
4	0.3	0.0365
5	0.4	0.0458
6	0.5	0.0569
7	0.6	0.0658
8	0.7	0.0785
9	0.8	0.0854
10	0.9	0.0965
11	1	0.125

Quantitative estimation of phytoconstituents

Quantitative analysis such as total cardiac glycoside equivalent, total phenolic equivalent, total flavonoid equivalent were estimated for the *Calliandra haematocephala*

Determination of digoxin equivalent in *Calliandra haematocephala*$_{L}$

Various concentration(0-25µg/ml) of *C.haematocephala*$_{FRJ}$ were treated with containing 0.005%w/v of ferric chloride and 2%v/v of sulphuric acid. which produces reddish brown colour is observed and displayed in table.8and Fig.7

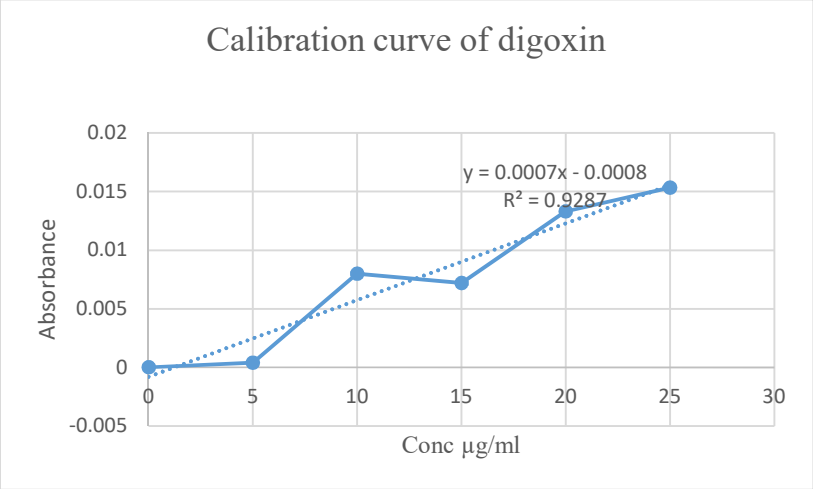


Fig 7: Calibration curve of digoxin

Table 8: Determination of digoxin equivalent in *Calliandra haematocephala*$_L$

S.No.	Concentration (µg/ml)	Absorbance of digoxin	Absorbance of *C.haematocephala*
1.	0	0	0
2.	5	0.0004	0.014
3.	10	0.008	0.017
4.	15	0.0072±0.000125	0.020±0.0004714
5.	20	0.0133±0.000272	0.032±0.000816
6.	25	0.01533±0.000272	0.036
		DE	**0.00376mg/g**

Quantitative estimation of cardiac glycoside was done by colorimetric method using digoxin as standard. The total digoxin equivalent in *C.haematocephala* was found to be 0.00376 DE/g (Fig 7&Tab 8).

Determination of gallic acid equivalent in *Calliandra haematocephala*$_L$

Various concentration(0-25µg/ml) of *C.haematocephala*$_{FRJ}$ were treated with Folin-ciocalteau reagent which produces blue colour is observed and displayed in table.9 and Fig.8

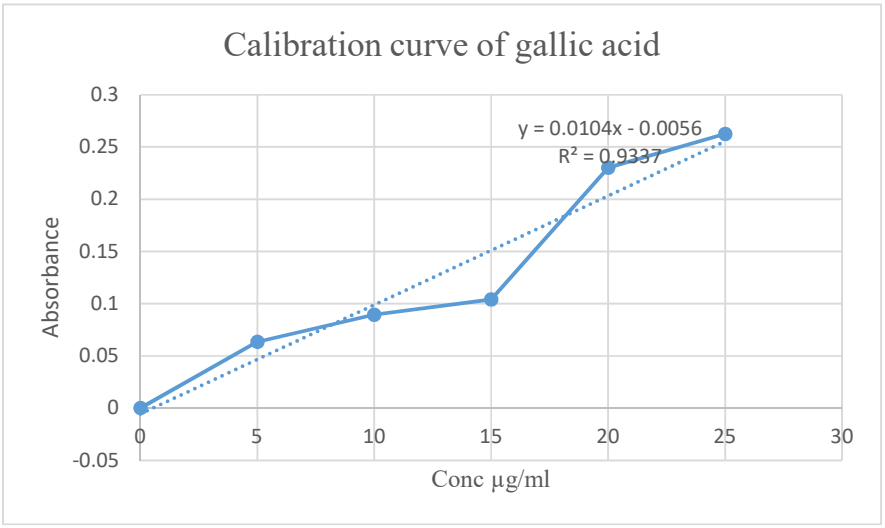


Fig 8: Calibration curve of gallicacid

Table 9: Determination of gallic acid equivalent in *Calliandra haematocephala*$_L$

S.No.	Concentration (µg/ml)	Absorbance of gallic acid	Absorbance of *C.haematocephala*
1.	0	0	0
2.	5	0.063±0.0002	0.027±0.001699723
3.	10	0.089±0.0010	0.087666667±0.00306724
4.	15	0.154±0.0006	0.102333333±0.000272174
5.	20	0.230±0.0001	0.126±0.001633041
6.	25	0.262±0.0002	0.22666667±0.000544347
		GE	**0.0213mg/g**

Quantitative estimation of phenolic was done by Folin- cio calteu method using gallic acid as standard. The total gallic acid equivalent in *C.haematocephala* was found to be 0.0213GE/g (Fig 8 &Tab 9).

Determination of rutin equivalent in *Calliandra haematocephala*$_L$

Various concentration(0-10µg/ml) of *C.haematocephala*$_{FRJ}$ were treated with aluminium chloride which produces yellow colour is observed and displayed in table.11 and Fig.10

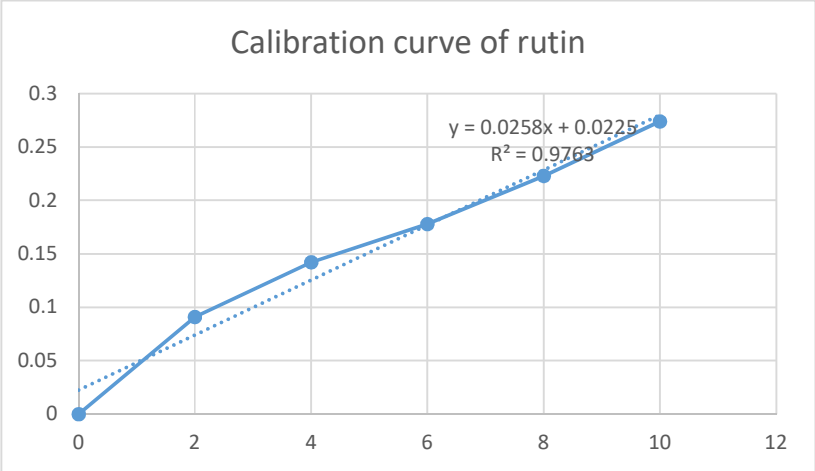


Fig 9: Calibration curve of rutin

Table 10: Determination of rutin equivalent in *Calliandra haematocephala*$_L$

S.No.	Concentration (µg/ml)	Absorbance of rutin	Absorbance of *C.haematocephala*
1.	0	0	0
2.	2	0.0944±0.0001	0.122±0.0002
3.	4	0.1873±0.0001	0.176±0.0004
4.	6	0.2523±0.001	0.230±0.0002
5.	8	0.408±0.002	0.258±0.000
6.	10	0.5163±0.00092	0.275±0.0002
		RE	**0.0033mg/g**

Quantitative estimation of flavonoid was done by using rutin as standard.The total flavonoid content in the extract is expressed as milligrams of Rutin equivalent per gram of extract 0.0033(RE/g) (Fig 9 &Tab 10)

Pharmacobotany of *Calliandra haematocephala*

The genus *Calliandra* (Family: Fabaceae) includes around **140–150 species** distributed in tropical and subtropical regions, with many used traditionally for their medicinal and ornamental value. Among these, ***Calliandra haematocephala*** is a widely cultivated and recognized species, especially in tropical Asia and South America.

Calliandra haematocephala
Calliandra surinamensis
Calliandra calothyrsus
Calliandra eriophylla
Calliandra tweediei

Table 11: Table of similar *Calliandra haematocephala* characters with other four species of *Calliandra*

S.No	Species	Blade	Venation	Apex	Base	Margin	Shape
1	*C.haematocephala*	1	1	1	1	1	1
2	*C.surinamensis*	1	1	1	1	1	1
3	*C. calothyrsus*	1	1	1	1	1	0
4	*C. eriophylla*	1	1	0	1	1	0
5	*C. tweediei*	1	1	1	1	1	1

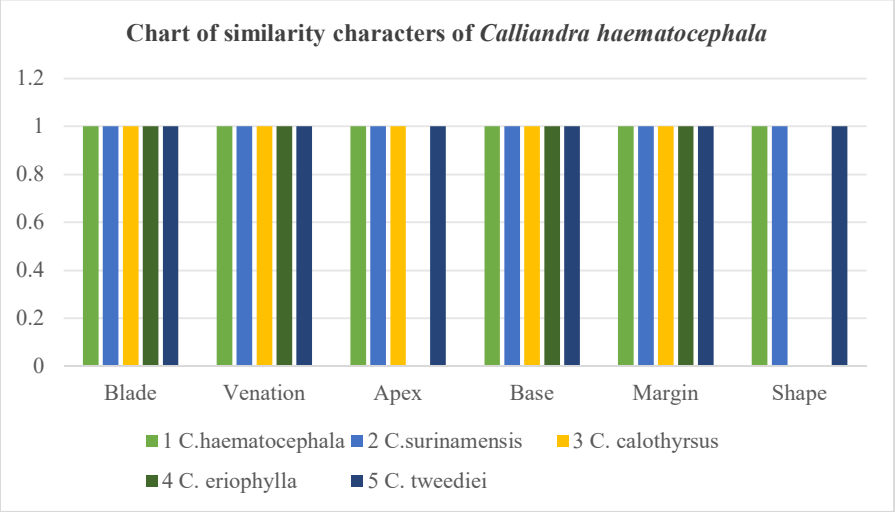


Fig 10: Chart of similarity characters of *Calliandra haematocephala Hassk* Leaves

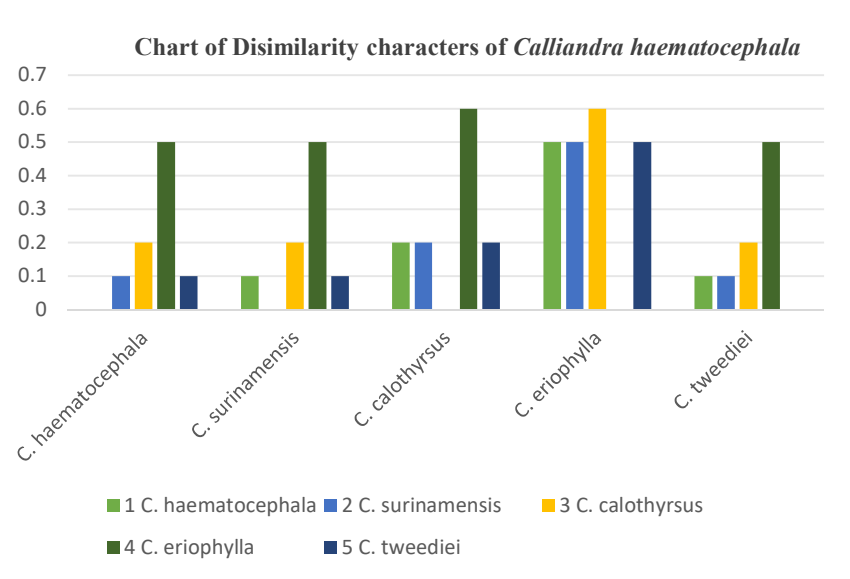


Fig 11: Chart of Disimilarity characters of *Calliandra haematocephala Hassk* Leaves

Table 12: Table of dissimilar *Calliandra haematocephala* characters with other four species of *Calliandra*

S.No	Species	C. haematocephala	C. surinamensis	C. calothyrsus	C. eriophylla	C. tweediei
1	*C. haematocephala*	0	0.1	0.2	0.5	0.1
2	*C. surinamensis*	0.1	0	0.2	0.5	0.1
3	*C. calothyrsus*	0.2	0.2	0	0.6	0.2
4	*C. eriophylla*	0.5	0.5	0.6	0	0.5
5	*C. tweediei*	0.1	0.1	0.2	0.5	0

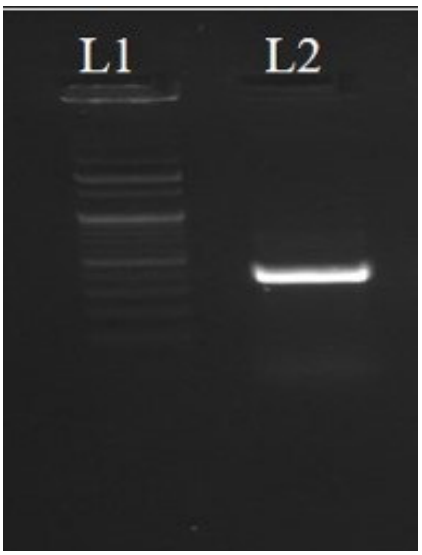
Sample matching coefficient
SSM= NS/NS+ND*100 Where,
NS=Number of similarity characters - 19
ND=Number of dissimilarity characters - 1
N=Number of samples
N=19/19+1*100
The matching coefficient of *Calliandra haematocephala* with respect to other species was found to be 95%.

DNA Barcoding

Genomic DNA was isolated from the authenticated sample, and its quality was assessed spectrophotometrically, followed by agarose gel electrophoresis (Table 13). The genomic DNA concentration and absorbance (A260/A280) ratio are depicted in Table 13. The PCR-amplified products after electrophoresis were subjected to gel documentation using a 100 bp DNA ladder (Fig. 12).The sequence was obtained using ITS as the marker (Fig. 13), the BLAST hit is shown in fig. 14; the nucleotide sequences obtained were converted to a barcode (Table 14), and submitted to GenBank (Table 15).

Table 13: Quality check and quantification of DNA

Sample code	Concentration in ng/µl	$A_{260/280}$	$A_{230/260}$
C14052501H	495.2	2.00	1.25



L1: Ladder; L2: Sample

Fig 12: Gel image of PCR amplified product

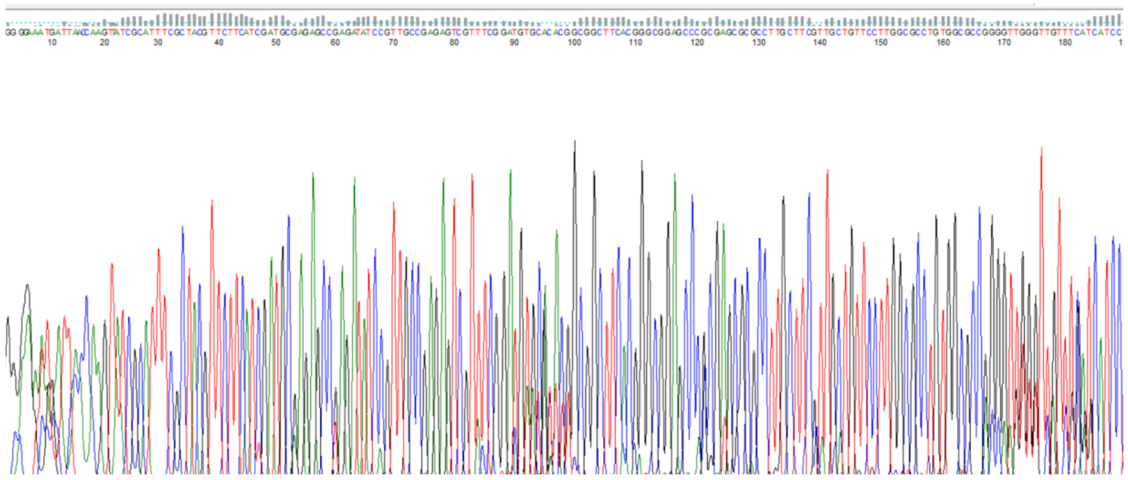


Fig 13: Chromatograms of *Calliandra haematocephala*

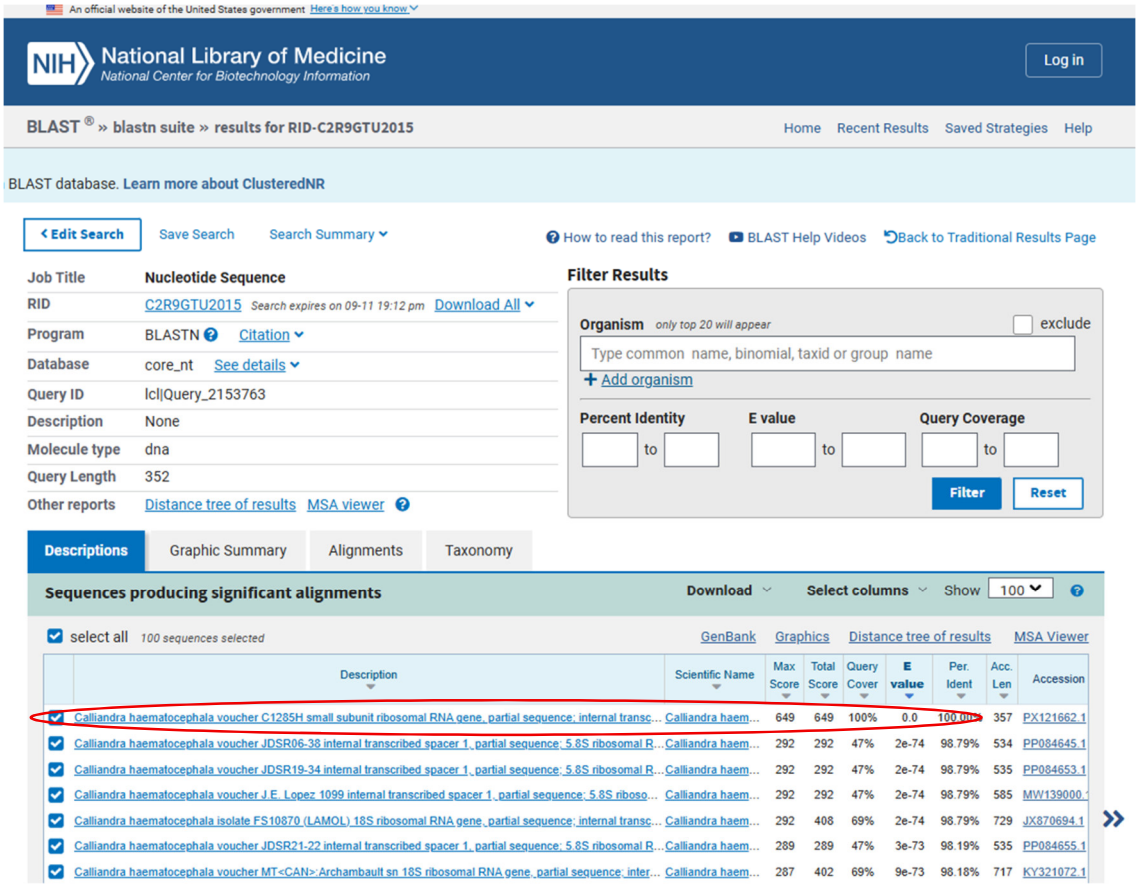
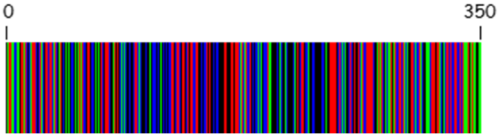


Fig 14: BLAST Hit

Table 14: Sequence obtained

Sample code	Sequence with Barcode
>SCRIPCOG	gattaaccaagttatcgcatttcgctacgttcttcatcgatgcgagagccgagatatccgttgccgagagtcgtttcggatgtgc acacggcggcttcacgggcggagcccgcgagcgcgccttgcttcgttgctgttccttggcgcctgtggcgccggggttggg ttgtttcatcatcctgcgccacagccctaaggggcgacggacggggcagtgcttgccaggccgggggccgaggtttttgct

83BG cacaggctcgtggtcatccttttcggatatcgacaatgatccttctacaggttcacctacggaaactttgttacgacttctccttc
ctctaaatgataaggaa



The genomic DNA of *Calliandra haematocephala* was successfully isolated with good yield and purity, as confirmed by spectrophotometric analysis and agarose gel electrophoresis. PCR amplification of the ITS region produced distinct bands, which were further sequenced to generate high-quality chromatograms. BLAST analysis of the obtained sequence confirmed species identity, and the sequence was successfully converted into a DNA barcode and submitted to GenBank. These findings validate the use of ITS markers for authenticating *Calliandra haematocephala* and provide a reliable molecular reference for future pharmacognostical and phylogenetic studies.

CONCLUSION

The Pharmacobotanical, Pharmacognostical,Physicochemical,Phytochemical and DNA Barcoding evaluations of *Calliandra haematocephala* leaves have provided reliable diagnostic parameters for their accurate identification and standardization. These comprehensive investigations offer valuable reference data for quality control and lay a strong foundation for future pharmacological and therapeutic studies.

REFERENCES

1. Rao, K. S., & Sreeramulu, K. Phytochemical and pharmacological potential of *Calliandra haematocephala* – A review. International Journal of Pharmacognosy and Phytochemical Research, (2017) 9(5), 658–662.
2. Pushpangadan, P., & Atal, C. K. Ethnomedico-botanical investigations in Kerala: Some primitive tribes of Western Ghats and their herbal medicine. Journal of Ethnopharmacology, (1984) 11(1), 59–77.
3. Vedavathy, S., Rao, K. N., & Narasimha, B. L. Ethnobotany of Eastern Ghats in Andhra Pradesh, India. Economic Botany, (1997) 51(4), 305–312.
4. Devi, Y. S., & Singh, P. K. Ethnobotanical uses of medicinal plants in Manipur Valley. Indian Journal of Traditional Knowledge, (2013) 12(3), 434–442.
5. Akinmoladun, F. O., Akinrinlola, B. L., & Komolafe, A. O. Ethnomedicinal uses and pharmacological potential of Calliandra species in Africa. Journal of Herbal Medicine, (2018) 14, 100226.
6. Gbolade, A. A. Ethnobotanical study of plants used in treating malaria in southwestern Nigeria. African Journal of Traditional, Complementary and Alternative Medicines, (2009) 6(3), 287–293.
7. Ogudemi, T. Outlines and Pictures of Medicinal Plants from Nigeria. 1st ed. Lagos: University of Lagos Press; 2006. p. 95–156.
8. Zhang, Q., Wang, Y., & Yang, L. Ethnobotanical survey of medicinal plants used by Yunnan ethnic groups. Journal of Ethnopharmacology, (2012) 140(3), 606–618.
9. Mahesh, S., & Satish, S. Antimicrobial activity of some important medicinal plants against plant and human pathogens. World Journal of Agricultural Sciences, (2008) 4(5), 839–843.
10. Ríos, J. L., & Recio, M. C. Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology, (2005) 100(1–2), 80–84.
11. Eze, S. O., et al. Antioxidant and anti-inflammatory activities of plant extracts in traditional medicine. Phytotherapy Research, (2019) 33(6), 1568–1578.
12. Punnagai, K., et al. Alpha amylase and alpha glucosidase inhibitory effects of *Calliandra haematocephala*. Asian Journal of Pharmaceutical and Clinical Research, (2018) 11(12), 429.
13. Pereira, D. F., et al. Phytochemical and pharmacological investigations of *Calliandra* species. Natural Product Research, (2010) 24(9), 842–849.
14. Kirtikar, K. R., & Basu, B. D. (2006). Indian Medicinal Plants, Vol. 1. International Book Distributors, Dehradun, India. pp. 813–814.
15. Gopinath, S. M., & Jagadeesh, R. Morphological and Anatomical Studies of Some Medicinal Plants of Fabaceae. Int. J. Pharm. Bio. Sci., (2013) 4(3), 320–325. (Contains morphological characteristics including leaf structure of (*Calliandra haematocephala).*
16. Wallis T.E. Analytical Microscopy: Its aims and methods in relation to foods, water, spices and drugs. 3rd ed. Boston: Little, Brown and Company; 1955. p. 56–70.

17. Fahn A. Plant Anatomy. 3rd ed. Oxford: Pergamon Press; 1980. p. 95–120.
18. El-Emary N, Makboul M, Abdel-Hafiz M, Magdy M. Macro- and micromorphology of the stem and leaf of Calliandra haematocephala (Hassk.) cultivated in Egypt. Bulletin of Pharmaceutical Sciences, Assiut University. 2003;26(2):153–170.
19. Anonymous. Quality Control Methods for Medicinal Plant Materials. Geneva: World Health Organization; 1998. p. 9–20, 116.
20. K.R. Khandelwal. Practical Pharmacognosy. 9th ed. Pune: Nirali Prakashan; 2008. p. 30–37.
21. Kokoski C.J, Kokoski R.J, Sharma P.J. Fluorescence of powdered vegetable drugs under UV radiation. Journal of the American Pharmaceutical Association. 1958;47:715–717.
22. T.E. Wallis. Textbook of Pharmacognosy. 5th ed. New Delhi: CBS Publishers; 2005. p. 93–107.
23. J.B. Harborne. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman and Hall; 1998. p. 120–138.
24. Reference for (LOD, LOQ) Seo, Ji-Hye, Jung-Eun Kim, Jung-Hyun Shim, Goo Yoon, Mi-Ae Bang, Chun-Sik Bae, Kyung-Jin Lee, Dae-Hun Park, and Seung-Sik Cho.HPLC Analysis, Optimization of Extraction Conditions and Biological Evaluation of Corylopsis coreana Uyeki Flos.Molecules.2016;21(1):
25. [(accessed on 21 December 2020)]; Available online: https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf.
26. Government of India. The Pharmacopoeia of India. 3rd ed. New Delhi: Controller of Publications; 1985. p. 245.
27. O.Singleton V.Lrthofer R, Lamuela-Raventos R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods in Enzymology. 1999;299:152–178.
28. Boham A.B, Kocipai A.C. Flavonoids and condensed tannins from leaves of Hawaiian Vaccinium vaticulatum and V. calycinum. Pacific Science. 1974;48:458–463
29. Ashwinkumar and Baidyanath kumar.,Biosystemic Studies in *Helitropium indicum, Trichodesma indicum* and *T.zeylanicum* of Boraginaceae. Int.J.Curr.Microbiol.App.Sci.5(4).2016,p.72
30. De Vere N, Rich TC, Trinder SA, Long C. DNA barcoding for plants. Plant Genotyping: Methods and Protocols. 2015:101-18.