



ANATOMICAL AND PHYTOCHEMICAL INVESTIGATION OF *EMBELIA TSJERIAM-COTTAM* (ROEM. & SCHULT.) A.DC.

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ABSTRACT

Embelia tsjeriam-cottam (Roem. & Schult.) A. DC. is a valuable medicinal plant, used in Indian system of medicine. This article presents results of gross morphology, anatomy and phytochemical investigations on the selected species.

KEY WORDS

E. tsjeriam-cottam, Microscope, FT-IR, Preliminary phytochemistry.

INTRODUCTION

India is one of the 12 mega-biodiversity countries in the world. It is well known for its varied flora thus reflecting diverse habitats. The habit wise distribution of medicinal plants of the country indicates that the climbers constitute only 15% of the total population, while erect or non-climbing forms (herbs, shrubs and trees) constitute 85%. Medicinal plants face a high degree of threat because of unsustainable harvesting methods and commercial exploitation.

Embelia tsjeriam-cottam (Roem. & Schult.) A. DC. belongs to Primulaceae. (APG IV 2016). The Primulaceae are a family of herbaceous and woody flowering plants with about 53 genera and 2790 species. It is commonly known as the primrose family and has been variously circumscribed but it is now accepted in the broad sense including the former families Myrsinaceae and Theoprastaceae because of recent molecular analysis and phylogenetic findings

Taxonomical Classification

Kingdom: Plantae

Phylum : Angiosperms

Order: Ericales

Family: Primulaceae

Genus: *Embelia*

Species: *tsjeriam-cottam*

Botanical Name

Embelia tsjeriam-cottam (Roem. & Schult.) A. DC.

Vernacular Name

Tamil: Vaivilangam, Malayalam: Basaal, Cheriyanattam, Kannada: Vaivalig, Choladhanna, Hindi: Babrang, Baibrang, Bayabrang, Marathi: Ambati, Kokla, Waiwaraung, Sanskrit: Bidanga, Krimighnam, Vellah, Vidanga.

Geography

The distribution range of this species extends from India to Sri Lanka, Myanmar and Malaysia. Within India it is seen in southern states of Tamilnadu, Kerala, Karnataka, Andhra Pradesh and Maharashtra.

MATERIALS AND METHODS

Collection and Authentication

Plant materials of *E. tsjeriam-cottam* (Roem. & Schult.) A. DC. (Leaves and Stem) were collected from the Western Ghats of Tamilnadu and Kerala. They were authenticated Dr. S. John Britto, Director, The Rapinat

Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous) Tiruchirappalli, Tamilnadu, S. India. Leaves and stems were cut into small pieces separately shade dried and powdered.

Preparation of Extract

The powdered plant parts (leaves and stem) 10 g of each were extracted in 100 ml of acetone, ethanol, methanol, aqueous with continuous shaking on mechanical shaker for 24/hrs at room temperature. The extracts were then filtered through Whatmann No.1 filter paper and stored airtight.

Macroscopic Studies

The macroscopic study of plants provides the morphological description of the plant and the simplest as well as quickest means to establish the identity and purity to ensure quality of a particular drug.

Microscopic Studies

Microscopic study involves anatomy and is done by taking appropriate section of the plant parts under study. Detailed microscopic characters were studied by taking free hand thin transverse sections of leaf, petiole and stem Surface preparation of the leaves done by peeling method to observe epidermal cells. The leaf was examined transversely through lamina and midrib. Microphotographs were taken in digital microscope attached with computer system.

Phytochemical Screening

Preliminary phytochemical screening involves the identification of the bioactive components present in the samples by using the standard method. The components then were separated from the co-extractives.

Qualitative Phytochemical tests were conducted to test the powdered form of the plant samples (methanol, ethanol, acetone and aqueous) using standard methods of Harborne (1978) and Edeoga *et al.*, (2005).

FT-IR Spectrometric Analysis

Fixed amount of plant specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as KBr pellets on a Thermo scientific Nicot iS5 iD1 transmission, between 4000-400 cm^{-1} . The powdered sample of the plant extract was treated for FT-IR spectroscopy. The result was analyzed based on peak obtained.

Preliminary Phytochemical Analysis

Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids and terpenoids were

carried out for all the extracts by the method described by Mukherjee (2002).

Detection of Alkaloids

Solvent free extract 50 mg was stirred with few ml of dil. HCl and filtered. The filtrate was tested carefully with various alkaloidal reagents as given below.

a) Mayer's Test

To a few ml of filtrate, one or two drops of Mayer's reagent were added by the side of the test tube. A white creamy precipitate indicated the test as positive.

b) Wagner's Test

To a few ml of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish-brown precipitate confirmed the test as positive.

c) Hager's Test

To a few ml of filtrate 1 or 2 ml of Hager's reagent (Saturated aqueous Solution of picric acid) was added. A prominent yellow precipitate indicated the test as positive.

Detection of Carbohydrates and Glycosides

The extract (100mg) was dissolved in 5 ml water and filtered. The filtrate was subjected to the following tests:

a) Molish's Test

To 2 ml of filtrate two drops of alcoholic solution of α -naphthol was added, the mixture was shaken well and 1 ml of con H_2SO_4 was added slowly along the sides of the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.

b) Benedict's Test

To 0.5 ml of filtrate, 1 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 mins. A characteristic coloured precipitate indicated the presence of sugar.

Detection of Glycosides

50 mg of extract was hydrolysed with concentrated HCl for 2 hours on a water bath, filtered and the hydrolysate was subjected to the following tests:

a) Borntrage's Test

To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicated the presence of glycosides.

b) Legal's Test

50 mg of the extract was dissolved in pyridine. Sodium nitro prusside solution was added and made alkaline using 10% NaOH. Presence of glycosides was indicated by pink colour.

Detection of Saponins

To 1ml of extract, add 2ml of distilled water and shaken vigorously and allowed to stand for 10 min. There is the development of foam on the surface of the mixture. Then shake for 10 minutes, it indicates the presence of saponins.

Detection of Proteins and Amino Acids

The extract (100 mg) was dissolved in 10 ml of distilled water and filtered through whatmann no: 1 filter paper and filtrate was subjected to tests for proteins and amino acids.

a) Millon's Test

To 2 ml filtrate, few drops of millon's reagent were added. A white precipitate indicated the presence of proteins.

b) Biuret Test

An aliquot of 2 ml of filtrate was heated with 1 drop of 2 % CuSO₄ solution. To this 1 ml of ethanol (95%) was added, followed by excess of KOH Pellets. Pink colour in the ethanolic layers indicated the presence of proteins.

c) Ninhydrin Test

2 drops of Ninhydrin solution (10 mg of Ninhydrin in 200 ml of acetone) was added to 2 ml of aqueous filtrate. A characteristics purple colour indicated the presence of amino acids.

Detection of Phytosterols

a) Libermann – Burchard's Test

The extract (50mg) was dissolved in 2ml acetic anhydride. To these one or two drops of concentrated H₂SO₄ were added slowly along the sides of the test tube. An array of colour change showed the presence of phytosterols.

Detection of Fixed Oils and Fats

a) Spot Test

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

b) Saponification Test

A few drops of 0.5 N alcoholic KOH solution were added to a small quantity of extract along with a drop of phenolphthalein. The mixture was heated on water bath for 2 hours. Formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

Detection of Phenolic Compounds and Tannins

a) Ferric Chloride Test

The extract (50 mg) was dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride

solution was added. A dark green colour indicated the presence of phenolic compounds.

b) Lead Acetate Test

The extract (50 mg) was dissolved in distilled water and to this 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds.

c) Gelatin Test

The extract (50 mg) was dissolved in 50 ml of distilled water 2 ml of 1% solution of gelatin containing 10% sodium chloride was added to it. White precipitate indicated the presence of phenolic compounds.

d) Alkaline Reagent Test

An aqueous solution of the extract was heated with 10% NH₄OH solution. Yellow fluorescence indicated the presence of flavonoids.

e) Magnesium and Hydrochloric Test

The extract (50 mg) was dissolved in 5 ml of alcohol and few fragments of magnesium ribbon and concentrated HCl were added (dropioire). If any pink to crimson developed, presence of flavanol glycoside was inferred.

Detection of Gum and Mucilages

The extract (100 mg) was dissolved in 10ml of distilled water and to this 25 ml of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of gums mucilages.

Detection of Volatile Oil

In volatile oil estimation apparatus, 50 g of powdered material (crude drug) was taken and subjected to hydro distillation. The distillate was collected in graduated tube of the assembly wherein the aqueous portion automatically separated out from the volatile oil.

Test for Steroids

10 ml of all extract of the test plant was evaporated to a dry mass and the mass dissolved in 0.5 ml of chloroform. Acetic anhydride (0.5 ml) and 2 ml of concentrated sulphuric acid were added. A blue or green colour or a mixture of these two shades shows the presence of steroidal compounds.

Test for Starch

To mix 3 ml of the extract was added a few drops of dilute iodine solution. Blue colour indicated the presence starch. Colour disappears on boiling and reappears on cooling.

Test for flavonoids

a) Shinoda Test

To 2 ml of the extract and a few fragments of magnesium ribbon were added and to it con. Sulphuric acid was added drop wise. Pink scarlet or crimson red appeared.

b) Zinc Chloride Reduction Test

To 2 ml of the extract a mixture of zinc dust and con. HCl were added. A red colour was obtained after few minutes.

c) Alkaline Reagent Test

To 2 ml of the extract sodium hydroxide solution was added to give a yellow or red colour.

RESULT AND DISCUSSION

Macroscopic Studies

It is a large woody rambling shrub with glabrous branchelets. The dark green colour of the leaves on the

above and purplish green below with prominent lateral nerves and reticulations are notable characters to locate and identify the species in wild. Fruit if longitudinally striated and smooth with a pointed structure, consists of single round seed.

Microscopic Studies

Leaves – T. S of leaves shows a epidermis, cuticle, adaxial vascular stand, sclerenchyma, mesophyll, palisade tissue, xylem, phloem was presented,

Stem – T. S of stem shows a circular outline, single layer of epidermis covered with a thin cuticle, lenticels, epidermis, chlorophyll, endodermis, collenchymatous cortical tissue is present. The cells along with Compound Starch grains, oil glands and crystals are identified in different parts. Fibers are towards vascular bundles, cambium, medullary rays and pith is seen.

Figure - 1: *E. tsjeriam-cottam* (Roem. & Schult.) A. DC. - T. S. of Leaf

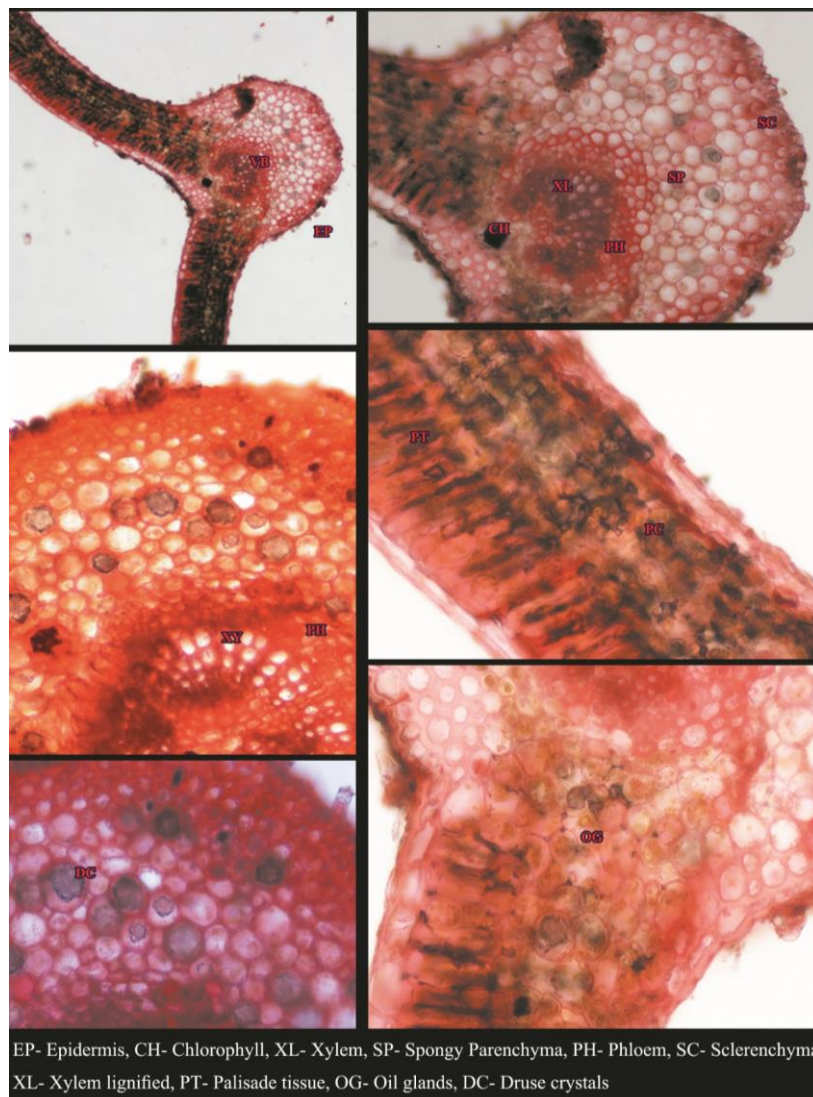
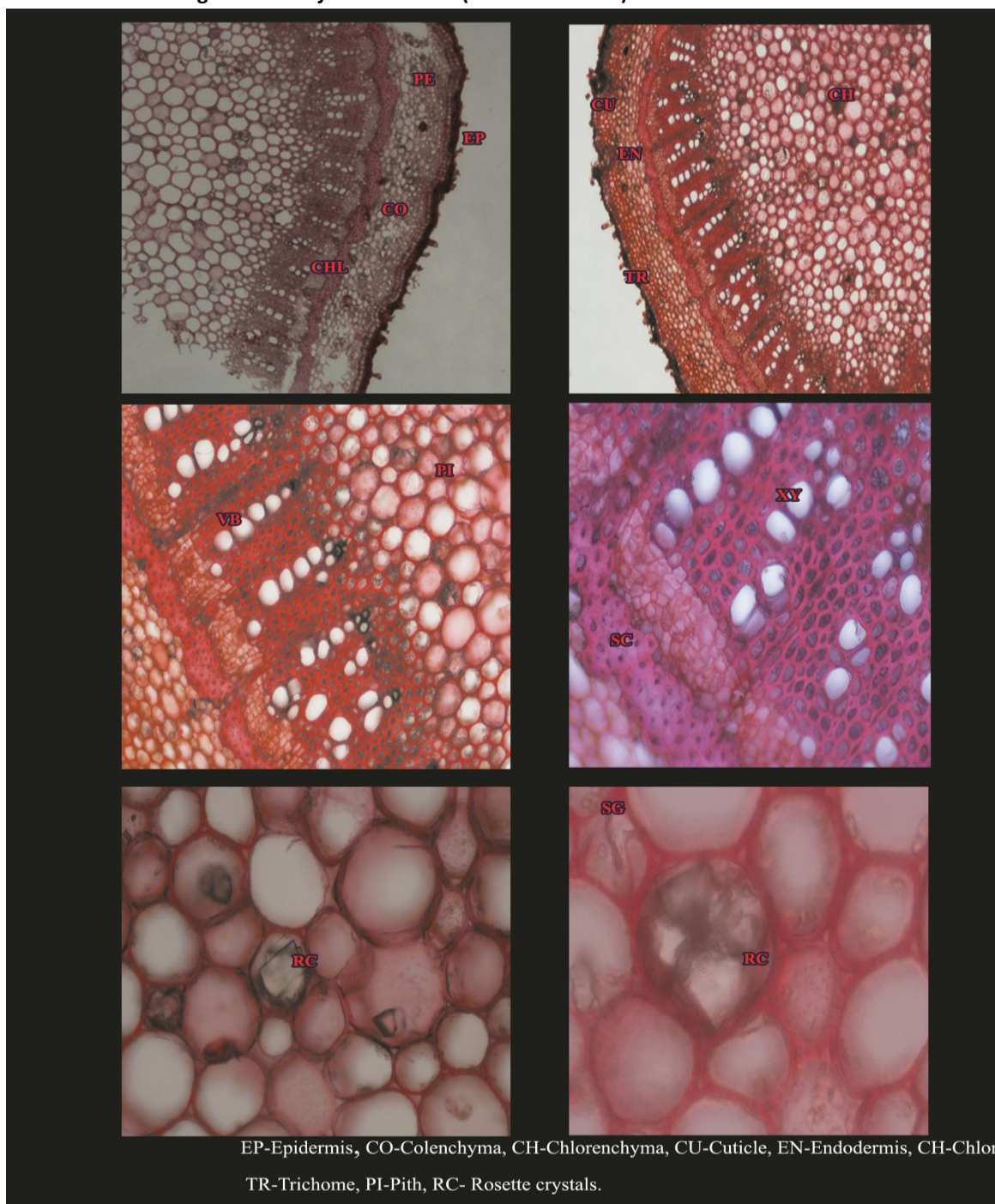


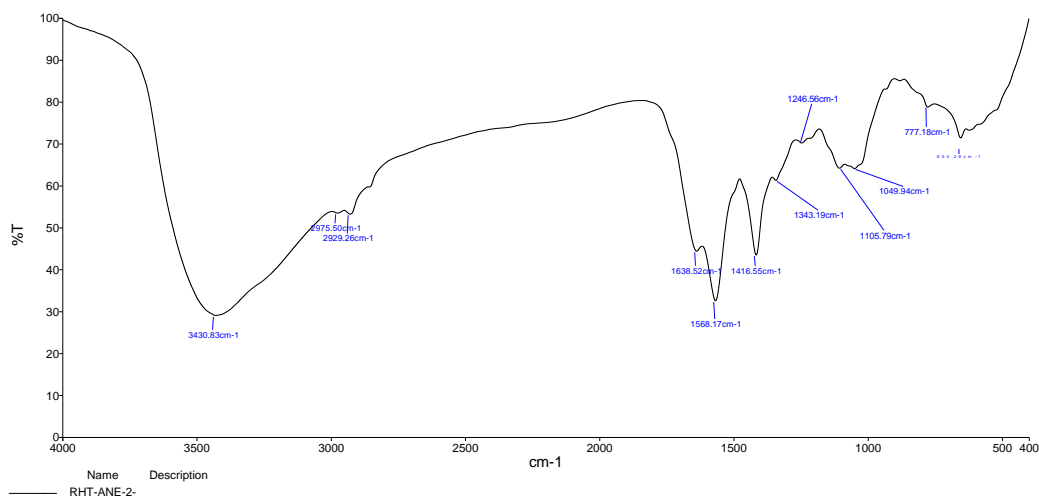
Figure - 2: *E. tsjeriam-cottam* (Roem. & Schult.) A. DC. - T. S. of Stem



FT-IR Spectrometric Analysis

The leaf powder of *E. tsjeriam-cottam* exhibited 12 characteristic bands. The highest band occurred at 3480.83 cm^{-1} indicating the presence of functional groups like alcohols and phenols having O-H stretch and H-bonded groups, 2975.50 cm^{-1} indicating the presence of alkane (C-H stretch) group, 1638.52 cm^{-1} indicating

the presence of aromatic (C=C stretch), 1416.55 cm^{-1} indicating the presence of amide (N-H bending), 1343.19 cm^{-1} indicating the presence of nitro compounds (N-O symmetric stretch), 1049.94 cm^{-1} indicating the presence of aliphatic amine (C-N stretch).

Figure - 3: FT-IR Spectrometric Analysis of Leaves


Preliminary Phytochemical Screening

Qualitative Phytochemical tests were conducted to test the powdered form of the plant samples (methanol, ethanol, acetone and aqueous) using extracts and they

revealed the presence of alkaloids, flavonoids, tannins, amino acids, carbohydrates, proteins. Saponins and xanthoprotein were absent.

Table - 1: FT-IR Spectrometric Analysis of Leaves

Frequency (cm-1)	Type of Vibration	Functional Group	Intensity
3480.83	O-H stretch, H-bonded	Alcohol	Strong, broad
2975.50	C-H stretch	Alkane	Strong
2929.26	C-H stretch	Alkane	Strong
1638.52	C=C stretch	Aromatic	Medium-weak
1568.17	N-H bending	Amide	Strong
1416.55	C-C stretch	Aromatic	Medium
1343.19	N-O symmetric stretch	Nitro compounds	Medium
1246.56	C-O stretch	Alcohol,	Strong
1105.75	C-O stretch	Alcohol,	Strong
1049.94	C-N stretch	Aliphatic amine	Medium
777.18	C-H "loop"	Aromatic	Strong
654.28	C-C1 stretch	Alkyl Halide	Medium

Table - 2: Preliminary Phytochemical Analysis of Leaves

	Test	Acetone	Ethanol	Methanol	Aqueous
Alkaloids	Wager's	+	+++	+++	+++
	Hager's	+++	+++	+++	+++
	Mayer's	++	+	+++	-
Flavonoids	Pew's	-	-	-	-
	Shinoda	+	+	++	+
	NaOH	-	-	-	+++
	Con.H ₂ SO ₄	++	+	++	-
Phenols & Tannins	FeCl ₃	+	+	+++	+++
	K ₂ Cr ₂ O ₇	-	-	-	-
	Lead Acetate	+	+	-	+
	Braymers	++	++	+++	-

Saponins	Foam	++	++	+++	-
	NaHCO ₃	-	-	-	+
Glycosides	Keller-Kiiani	++	++	++	-
	Glycosides	+	+	+	+++
	Libermann	-	+	+	-
Carbohydrates	Molish	+	+	+	-
	Benedict's	-	-	-	-
	Emodins	-	-	-	-
Anthocyanin	Borntrager's	-	-	-	-
	Quinones	-	-	-	-
Sterols	Salkowski's	++	+	+	+
	Triterpenoids	-	+	-	+
Protein	Biuret	+	+	+	+
	Con. H ₂ SO ₄	+	+	++	+
	Xanthoprotein	-	-	-	-
	Terpenoids	++	+	+	-

DISCUSSION

Both macroscopic and microscopic analyses enable the pharmacologist to authenticate the taxonomic identification of the plant species. Plant morphology and anatomy play very important role to identify plants. The distinct presence of secondary metabolites and phytochemical compounds such as alkaloids, flavonoids, terphenoids, proteins, glycosides and phenols, as revealed in cells are indication of the presence of the above classes of compounds.

FT-IR confirmed various phytoconstituents with distinct peaks in the spectrum. The highest peak indicated the presence of alcohol and phenol groups which means that the plant contained larger measure of such compounds. Similarly, other functional groups like alkanes, alkynes, primary amines, aromatics, carboxylic acid and alkyl halides are evenly presented.

CONCLUSION

In this macroscopic and microscopic investigation of plants external morphological and internal structure are presented in various levels. The phytochemical studies such as FT-IR and preliminary phytochemicals studies are studied have proved the presence of alkaloids, flavonoids, tannins, protein, glycosides etc.

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