International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 09-02-2021, Accepted: 17-03-2021, Published: 25-03-2021
www.botanyjournals.com
Volume 6; Issue 2; 2021; Page No. 312-318

Classical taxonomy, quantitative and qualitative phytochemical profile of *Embelia Tsjeriam-cottam* (Roem. & Schult.) A. dc- a threatened Indian traditional species

Ananth V[1*], Gideon V[2]
[1] Research Scholar, Department of Botany, Bishop Heber College, (Autonomous), (Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India
[2] Associate Professor and Head, Department of Botany, Bishop Heber College, (Autonomous), (Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India

Abstract
Embelia tsjeriam-cottam (Roem. & Schult.) A.DC. is used in Indian system of medicine. This article presents results of gross morphology; anatomy and phytochemical investigations of revealed the presence of pharmaceutically important secondary metabolites compounds presented in high concentrations. Many of bioactive compounds have been determined as potent Antioxidant, anticancerous and antimicrobial. The study revealed that the plant is promising source for the production of many drugs against antibacterial, antidiabetic, anti-inflammatory antihelmintic, antifertility, antitumor, analgesic, cardioprotective and antioxidant activity in cure several human diseases.

Keywords: *E. Tsjeriam-cottam*, microcope, FT-IR, HPLC

Introduction
The genus *Embelia* is mainly tropical and subtropical regions. The genus comprising of about 130 species all over the world of which 18 have been reported from India. *Embelia tsjeriam-cottam* (Roem. & Schult.) A.DC. belongs to family Primulaceae. (APG IV 2016). The Primulaceae are 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. The distribution range of this species extends from India to Sri Lanka, Myanmar and Malaysia. Within India it is seen in southern states of Tamil Nadu, Kerala, Karnataka, Andhra Pradesh and Maharashtra.

Materials and Methods
Collection and Authentication
Plant materials of *Embelia tsjeriam-cottam* (Roem. & Schult.) A. DC. (Leaves and Stem) were collected from the Western Ghats of Tamil Nadu and Kerala. They were authenticated Dr. S. John Britto SJ, Director and Head, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous) Tiruchirappalli, Tamil Nadu, S. India. (Voucher specimen no: RHT 68254).

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

Morphological 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.

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) [2] and Edeoga *et al*., (2005) [1].

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 milky cream 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 of 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-napthol 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 whatman 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 % $CuSO_4$ 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 (50 mg) was dissolved in 2 ml acetic anhydride. To this one or two drops of concentrated H_2SO_4 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 0.5N 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 gelation 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% NH_4OH 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.

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

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} 21. The powdered sample of the plant extract was treated for FT-IR spectroscopy. The result was analyzed based on peak obtained.

HPLC Analysis

The underlying principle of HPLC is that the sample mixture (mobile phase) is pumped at high pressure (up to 400 atm.) through a column with chromatographic packing material (stationary phase). All chromatographic separations, including HPLC operate under the same basic principle. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column. Sample retention time varies based on the interaction between the stationary phase, the molecules being analysed, and the solvent. As the sample passes through the column, it interacts between the two phases at different rate due to different polarities in the molecules. The molecules that have the least amount of interaction with the stationary phase will exit the column faster.

Specification and Procedure

For HPLC analysis those ethanolic extracts of the selected plant materials were used in Shimadzu HPLC System (Model SPD-20A UV-VIS Detector). The conditions and specifications were adopted from with a slight modification given below:

Communication Module: CBM-20A Shimadzu
Detector: SPD-20A Shimadzu at 254nm
Pump A: LC-8A Shimadzu pumps HPLC distilled water
Pump B: LC-8A Shimadzu pumps acetonitrile
Mobile Phase: Acetonitrile: Distilled Water (80:20
Injection Volume: 20µl
Flow Rate: 1ml/min
Column Temperature: 25℃
Column Pack: LC-18 column (25cm × 4.6mm)
Run Time: 20-30 minutes based on sample type
Application Software: LC Solution Version 1.24 SP1

Result and Discussion

Botanical Description

Straggler to 8 (12) m. Leaves broadly obovate to oblong, (5) 9-14 x 3.5-5.5 cm; petiole 2 cm. Racemes axillary, to 4 cm, usually 4-merous, rarely 5- or 6-merous in the same branch. Calyx –lobes 4(5), coriaceous, 1 mm. Corolla orange, 3.5 mm wide; lobes 4(5), each 3 mm. Stamens 4(5), exserted; filaments 4 mm, yellowish; anthers 1 mm. Ovary 1 mm; style 0.3 mm. Drupe 8 mm wide, crowned with persistent style. Extensive strggler, sometimes with branches hanging over. Stems with white, elongate lenticels. Flowers in peak during November-January (short and precise flowering time). Fruits globose, ripening red, easily falling off, February onwards. Hills above 900m, on thickest forest border.

Phytochemicals studies

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.

Table 1: Preliminary phytochemical analysis of *E. tsj* stem extract

	Test	Acetone	Ethanol	Methanol	Aqueous
Alkaloids	Wager's	++	++	++	++
	Hager's	+++	++	+++	+++
	Mayer's	+	+	++	+
Flavonoids	Pew's	+	-	+	-
	Shinoda	++	+	++	+
	NaoH	+	++	++	+
	Con.H_2so_4	++	+	+	+
Phenol & tannin	$Fecl_3$	++	++	+++	+
	$K_2cr_2o_7$	-	-	+	-
	Lead Acetate	+	+	-	+
	Braymers	++	++	++	-
Saponins	Foam	++	++	++	-
	$NaHco_3$	-	-	-	+
Glycosides	Keller kiiani	++	++	++	+
	Glycosides	+	++	++	+++
	Liberman	+	+	++	-
Carbohydrates	Molish	++	+	++	+
	Benedicts	-	-	-	-
	Emodins	++	-	++	+
Anthocyanin	Born tragers	-	-	-	-
	Quinones	++	++	++	+s
Sterols	Salkowskis	++	+	+	+
	Triterphenoids	-	-	-	+
Protein	Biuret	++	+	++	++
	Con. H_2so_4	+	++	++	+
	Xanthoprotein	-	+	+	-
	Terphenoids	++	++	++	+

FT-IR Results and Interpretation

This stem powder exhibited 13 characteristics bands. The highest band occurred at 3403.29cm^{-1} indicating the presence of functional groups like alcohols and alkyl halide having O-H stretch and C-F stretch groups, 2975.12 cm^{-1} indicating the presence of alkane (C-H Stretch), 1735.00 cm^{-1} indicating the presence of carbonyl (C=O stretch), 1569.34 cm$_{-1}$ indicating the presence of amide (N-H stretch), 1251.77 cm^{-1} indicating the presence of acid (C-O stretch), 1111.32 cm^{-1} indicating the presence of ether (C-O

stretch), 778.39 cm^{-1} indicating the presence of alkene (=C-H bending), 620.72 cm^{-1} indicating of the presence of alkyl halide (C-C1 stretch).

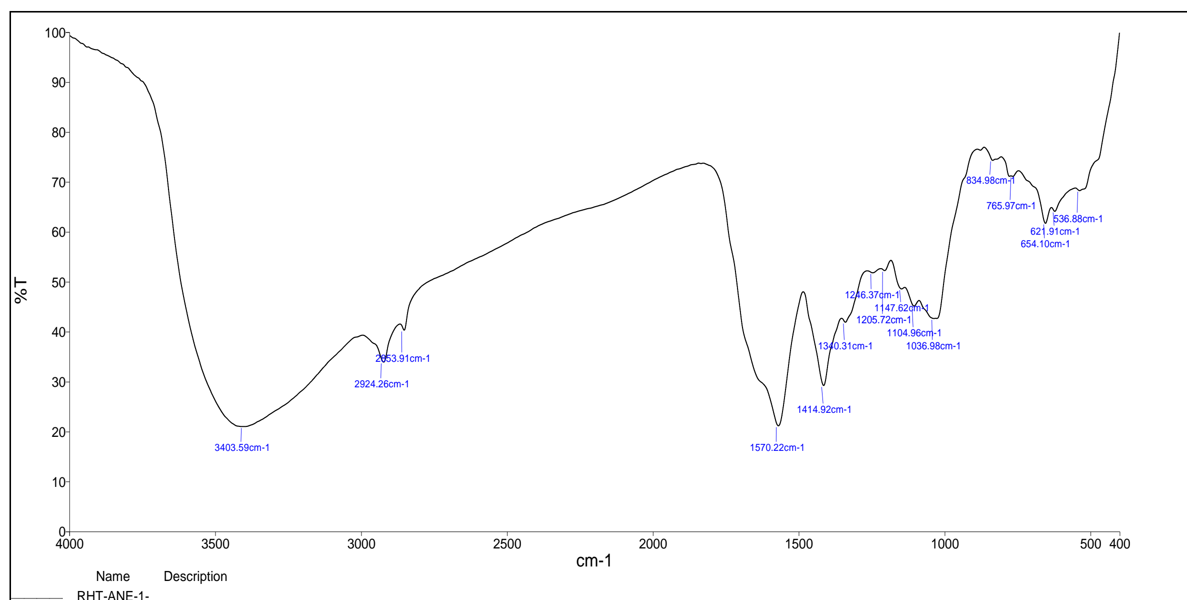

Fig 1: FT-IR Spectrum of *E. tsj* Stem powder

Table 2: FT-IR Spectrum of *E. tsj* Stem powder

Si No	Frequency (cm-1)	Type of vibration	Functional group	Intensity
1	620.72	C-C1stretch	Alkyl Halide	Strong
2	654.95	C-CBr stretch	Alkyl Halide	Strong
3	778.39	=C-H bending	Alkene	Strong
4	1045.94	C-F stretch	Alkyl Halide	Strong
5	1111.32	C-O stretch	Ether	Strong
6	1154.28	C-N stretch	Amine	Medium
7	1251.77	C-O stretch	Acid	Strong
8	1416.48	C=C stretch	Aromatic	Medium
9	1569.34	N-H bending	Amide	Strong
10	1735.00	C=O stretch	Carbonyl	Strong
11	2932.62	C-H stretch	Alkane	Strong
12	2975.12	C-H stretch	Alkane	Strong
13	3403.29	O-H stretch	Alcohol	Strong

UV-Vis Results and Interpretations

The ethanolic stem extract showed the peaks at 212.7, 275.5, 403.7 and 647.3 nm with the absorption of 3.276, 1.325, 0.761, and 0.013 respectively. Methanolic stem extract showed three peaks at 227.3, 276.2, 663.2 nm with absorption of 4.000, 3.468 and 0.024 respectively.

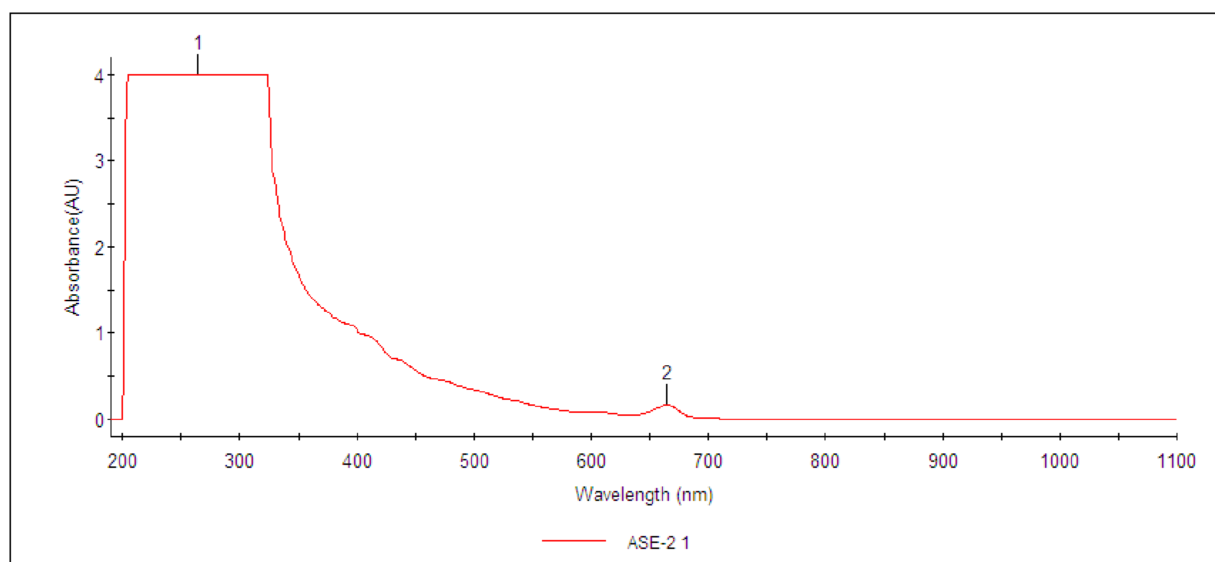

Fig 2: UV- VIS Spectrum of *E. tsj* ethanol stem

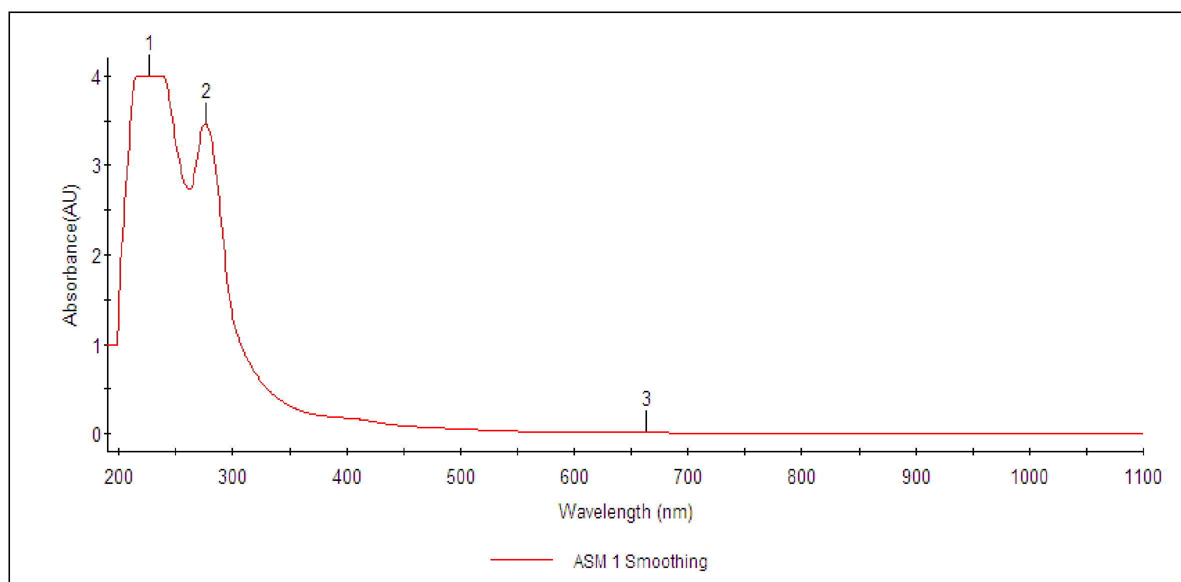

Fig 3: UV- VIS Spectrum of *E. tsj* methanol stem

Table 3: UV- VIS Spectrum of *E. tsj* ethanol and methanol stem extract

S.No	Ethanol Stem Extract		Methanol Stem Extract	
	Nanometer	Absorption	Nanometer	Absorption
1	212.7	3.276	227.3	4.000
2	275.5	1.325	276.2	3.468
3	403.7	0.761	663.2	0.024
4	647.3	0.013		

HPLC Results and Interpretations

The qualitative HPLC profiles of the selected medicinal plants were detected at a wavelength of 254nm producing sharp peaks at proper baseline. The results of HPLC analysis are presented in (table no: 4&5) the data consist of following order-number of peak obtained, peak number, retention time, area percentage and height percentage.

Analytical HPLC has gained popularity in fingerprinting study as well as in characterization and quantification of secondary metabolites. The number of peaks produced in this technique indicates the number of secondary metabolites present in high concentration. Moreover, HPLC fingerprinting of the medicinal plants provided a quick analysis of the compound present in crude drug. These chromatographs will help as standard chromatogram in future studies and also helped in identification of important bioactive compounds through GC-MS analysis.

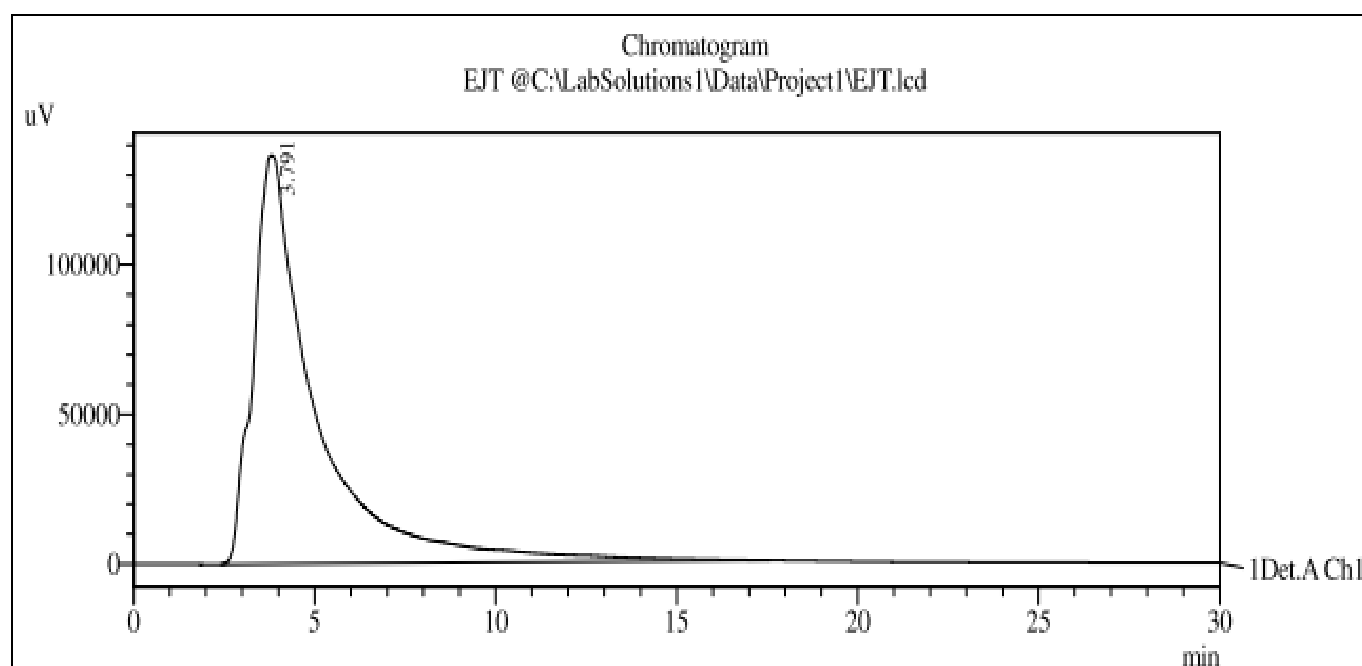

Fig 4: HPLC analysis of *E. tsj* ethanol stem extract

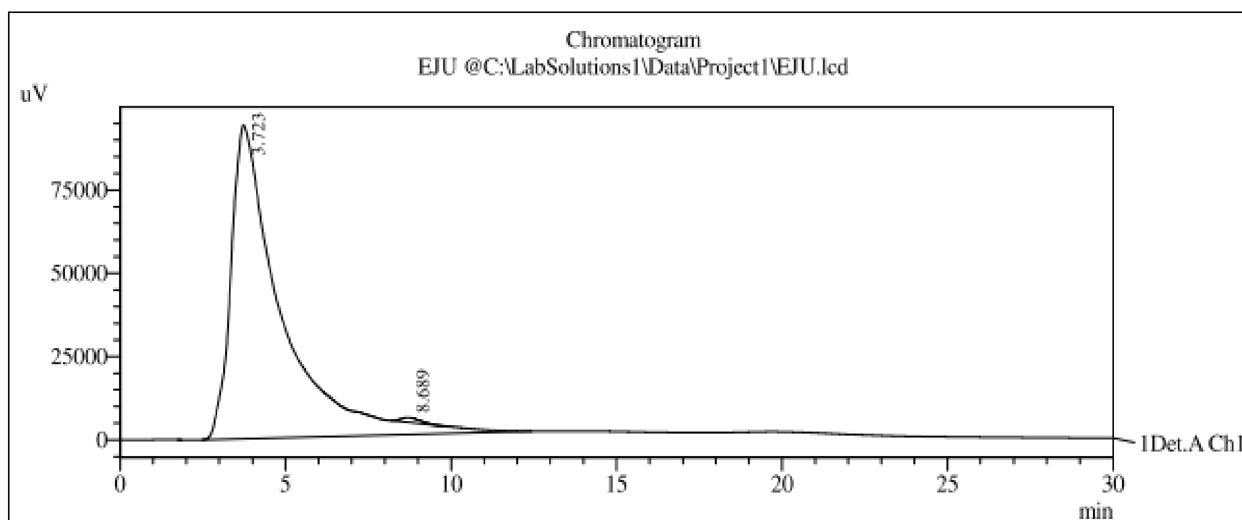

Fig 5: HPLC analysis of *E. tsj* methanol stem extract

Table 4: HPLC analysis of *E. TSJ* ethanol stem extract

No. of Peak	RetentionTime	Area	Height	Area%	Height%
1	3.791	16319315	136387	100.000	100.000
Total		16319315	136387	100.000	100.000

Table 5: HPLC analysis of *E. TSJ* methanol stem extract

No. of Peak	Retention Time	Area	Height	Area%	Height%
1	3.723	9946330	94239	99.388	98.483
2	8.689	61267	1452	0.612	1.517
Total		10007597	95691	100.000	100.000

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. HPLC have shown very satisfactory results proving that these species contain chemical properties for curing the related ailments in humans. *Embelia tsjeriam-cottam* (Roem. & Schult.) A. DC. Researcher include the preliminary phytochemical studies on its leaf and stem using triphytochemcial screenings (aqueous, ethanol and methanol), HPLC and GC-MS studies.

Conclusion

In this morphological investigation of plants external morphological and internal structure are presented in various levels. The phytochemical studies are having proved the presence of secondary metabolites. Based on these results it may be concluded that the components of further studies carried out to the future.

Acknowledgement

The author is thankful to Dr. V. Anand Gideon, Associate Professor and Head and also staff of Department of Botany, Bishop Heber College (Autonomous), Tiruchirappalli. He is also indebted to Director and Head, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph’s College (Autonomous) Tiruchirappalli, for providing necessary facilities.

Reference

1. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants, African Journal Biotechnology,2005:4(7):685-688.
2. Harborne JB. *Phytochemical Methods*. Chapman and Hall, London. 3rd Ed, 1978, 135-203.
3. Jain, S. K., Raghavendra Rao, R., Field Techniques and Herbarium Methods, Today and Tommorow’s Printers and Publishers, New Delhi, 1978.
4. Yathav NP, Dixit VK, Recent Approaches in Herbal Drug Standardization, International Journal of Integrative Biology,2008:2(3):195-203.
5. Matthew KM. A pocket flora of the Sirumalai hills, TN, South India, 2001.
6. Pub Chem Structure Search, managed by National Center for Biotechnology Information (NCBI),
7. US National Library of Medicine, https://pubchem. ncbi.nlm.nih.gov/.
8. Komal JK, Prasad AGD. Fourier Transform Infrared Spectroscopy an Advanced Technique for Identification of Biomolecules. Drug Invention Today,2012:4(12):616-618.
9. Kokate CK, Purohit AP, Gokhale SB. Carbohydrate and derived Products, drugs containing glycosides, drugs

containing tannins, lipids and protein alkaloids. Text book of Pharmacognosy, 7, edition, 2001, 133-166, 167- 254, 255-2 69, 272- 310, 428-523.