Hplc And Gc-Ms Analysis Of Bioactive Compounds In Embelia Tsjeriam-Cottam (Roem. & Schult.) A. Dc-A Threatened Species.

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Abstract: This study investigates the HPLC and GC-MS revealed the presence of pharmaceutically important compounds. Such as eicosane, tetradecanoic acid, isopropyl myrsitate, mortenol, neophytadiene, β-sitosterol, squalene. Several bioactive compounds have been determined as potent antioxidant, anticancerous, antimicrobial, anti blood cholesterol. The study revealed that the plant is promising source for the production of many drugs against several human diseases.

Key words:*Embelia tsjeriam-cottam*, HPLC, GC-MS,Western Ghats.

1 INTRODUCTION

The Soil is a dynamic body on earth which provides nutrients for growth of the plants. Medicinal plants have been the mainstay of traditional herbal medicine amongst rural inhabitants worldwide since ancient time. Humans used plants for a variety of purposes. It is generally estimated that over 7000 plants in India is in usage in various traditional, folk and herbal medicines. This mode of usage amounts to nearly 88 percent of the world's inhabitants, who rely mainly on traditional medicine for their primary health care. Natural therapeutics which is derived from plants are most significant because of lesser side effects, cost effective and ecofriendly as compared to chemically synthesized medicines. The concentration of phytochemicals is different in different plants and also in different parts of the same plant. Embelia tsjeriam-cottam (Roem. & Schult.) A.DC. takes for investigation belongs to Primulaceae. (APG IV 2016). The Primulaceae are a family now accepted in the broad sense including the families Myrsinaceae and Theoprastaceae because of recent molecular analysis and phylogenetic findings

2 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 Tamil Nadu and Kerala. They were authenticated Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous) Tiruchirappalli, Tamil Nadu, S.

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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.

HPLC Analysis

High Performance Liquid Chromatography (HPLC) is widely used for the separations and purifications of compounds in a variety of areas such as pharmaceuticals, environmental, biotechnology, polymer and food industries (Skoog *et al*., 1998). HPLC is used to determine the amount of a specific compound in a solution. The different solutes in the sample solution will interact with the stationary phase. The different interaction with the column helps separate different sample components from each other (Kupiec, 2004).

Principle

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 the methanolic 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°C
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

The extracts were filtered through sartorius regenerated cellulose membrane syringe filter (0.2µ). Then, 20µl of filtrate was injected into the HPLC. Chromatography was performed using Shimadzu HPLC and super coiled LC-18 column with mobile phase consisting of acetonitrile and HPLC grade water (80:20) Flow rate was maintained at 1ml/minute with a back pressure of 300psi. The chromatograms were recorded at 254nm with LC Solution software. The total run time varied between 20 to 30 minutes based on the type of sample being analyzed.

GC-MS Analysis
Gas Chromatography-Mass Spectrometry (GC-MS) is an analytical method that combines the features of Gas-Chromatography (GC) and Mass Spectrometry (MS). GC-MS, with the use of internal standards, provides a multidimensional compound identification and quantification of the sample (Skoog *et al*., 2007). Gas Chromatography (GC) is a type of chromatography in which the mobile phase is usually an inert gas such as helium or un-reactive gas such as nitrogen, and the stationary phase is a microscopic layer of liquid or polymer inside glass or metal tube, called as a column. Mass Spectrometry (MS) is the detector for the GC. GC can separate volatile and semi-volatile compounds with great resolution, but it cannot identify them. MS can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them.

Principle
GC-MS is used to analyse complex organic and biochemical mixtures. The GC-MS instrument consists of two main components. The gas chromatography portion separates different compounds in the sample into pulses of pure chemicals based on their volatility by flowing an inert gas (mobile phase), which carries the sample, through a stationary phase fixed in the column (Skoog *et al*., 2007). Spectra of compounds are collected by the mass spectrometer as they exit a chromatographic column, which identifies and quantifies the compounds according their mass-to charge ratio (m/z). These spectra can then be stored on the computer and analysed (Hussain and Maqbool, 2014).

Specification and Procedure
For the GC-MS analysis, the concentrated methanolic extracts of the selected plant materials were used. The conditions and specifications of Mohan *et al*. (2011) were adopted with a small modification in temperature. They were as follows:

GC-MS Model : Agilent Technologies-7890 GC System, 5975C inert MSD
Column : Agilent 190913-433; 325°C
Capillary : 30m x 250µm x 0.25µm
Carrier Gas : Helium(67)
Fuel Gas : Hydrogen
Flow Rate : 1.1 mL/min
Oven Temperature : 100°C-250°C
Temperature Rate : Increase of 10°C/min up to 200°C
: 5°C/min up to 250°C
: 250°C maintained till end
Split Ratio : 10:1
Injector Temperature : 250°C
Ion-source Temperature : 250°C
Injection Volume : 1µl
Detector : 5975C Inert Mass Selective Detector
Total Run Time : 45 minutes
Application Software : ChemStation Softwar

GC-MS analysis was carried out on an Agilent-7890 GC-MS System comprising an AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing given conditions. The oven temperature was programmed from 100°C (isothermal for 2 min), with an increase of 10°C/min up to 200°C, then 5°C/min up to 250°C, ending with a 9 min isothermal at 250°C. The samples were diluted to 1/10 with ethanol and 10µl of the diluted sample was injected using automatic injector (Agilent). Mass spectra of the samples were taken with GC/MSD ChemStation Software at 70eV with a scan interval of 0.5 seconds and fragments from 40 to 550 Da.

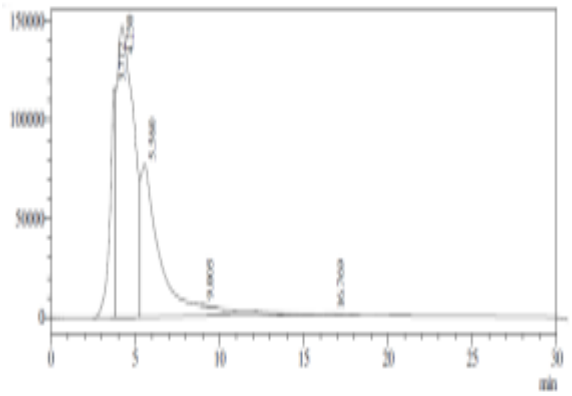
Identification of compounds
Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) which consists of more than 62,000 patterns. The spectrum of the unknown compound was compared with the spectrum of the known compound stored in the NIST library. The names, molecular formula, molecular weight and molecular structures of the compounds of the test extracts were ascertained.

3 RESULTS AND DISCUSSION

HPLC analysis
The qualitative HPLC profiles of the selected species were detected at a wavelength of 254nm producing sharp peaks at proper baseline. The results of HPLC analysis are presented in **Table 1.** The tabulated data consists of following order: number of peaks obtained, peak number, retention time, area percentage and height percentage. The highest number of peaks (5) was exhibited by HPLC chromatograms. Analytical HPLC has gained popularity in fingerprinting study as well as in characterization and quantification of secondary metabolites. This technique indicates the number of secondary metabolites present in high concentration, which can be isolated and purified (Boligon and Athayde, 2014). Moreover, HPLC fingerprinting of the ethnomedicinal plants provided a quick

analysis of the compound present in crude drug. The chromatograms can be used to identify the unknown compounds by comparison with the chromatograms of known compounds reported in literature (Pandey *et al*., 2013). These chromatographs will help as standard chromatogram in future studies. These chromatograms also helped in identification of important bioactive compounds through GC-MS analysis.

Fig. 1: HPLC analysis of Methanolic Leaf extract of ***Embelia tsjeriam-cottam***



C-MS analysis

The GC-MS study of *E. tsjeriam-cottam* (Roem. &Schult.) A. DC has shown the presence of several phytochemicals which contribute to the medicinal activity of these plants. On comparison of the spectra of the sampled compounds with the spectra of known compounds of the NIST library, the biomolecules were identified as per the number of peaks shown. The results are presented in Table 2inthe following order: number of peaks and the identified 25 bioactive phytochemical compounds such as phenol, dodecanoic acid, cyclohexanone, diphenylamine, tetradecanol, eicosane, tetra decanoic acid, hexadecanal, isopropyl myristate, mortenol and other compounds. These 25 compounds are responsible for antimicrobial, antifungal, sedative, antitumor, antioxidant and anti-inflammatory and insecticidal in this plant.

Table. 1: HPLC analysis of Methanolic Leaf extract of ***Embelia tsjeriam-cottam***

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.772	2787895	116287	14.519	34.061
2	4.230	9867516	147354	51.389	43.160
3	5.560	6521079	77256	33.961	22.628
4	9.005	12189	285	0.063	0.083
5	16.769	13008	228	0.068	0.067
Total		19201687	341410	100.000	100.000

Fig. 2: GC-MS analysis of Methanolic Leaf extract of ***Embelia tsjeriam-cottam***

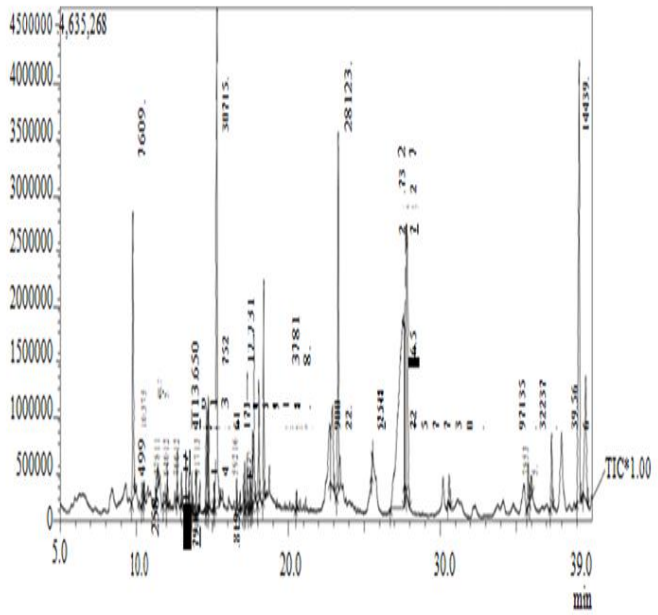
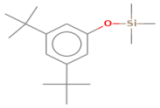
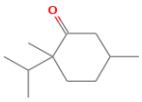
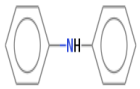
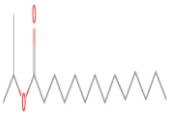
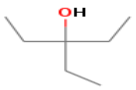
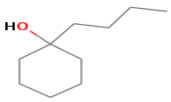
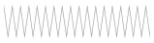
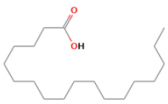
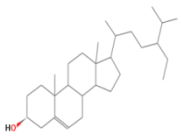
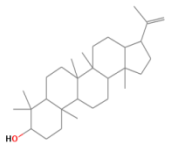


Table. 2**: GC-MS analysis of Methanolic Leaf extract of **Embelia tsjeriam-cottam

Si No	RT	Name Of The Compound	Molecular formula	Molecular Weight	Peak Area %	Structure	Activity
1	9.760	Phenol, 2,4-Bis(1,1-Dimethylethyl)-	$C_{17}H_{30}OSi$	278.5050	5.67		Antioxidant
2	10.375	Dodecanoic Acid	$C_{12}H_{24}O_2$	200.3178	0.36		Acne treatment, increases high-density lipoprotein
3	10.499	Cyclohexanone, 2-Isopropyl-2,5-Dimethyl-	$C_{11}H_{20}O$	168.2759	0.19		As a flavor component of cognac and in grapefruit peel oil, star fruit, corn mint oil and spearmint oil.
4	11.258	Diphenylamine	$C_{12}H_{11}N$	169.2224	0.25		Industrial antioxidant, dye mordant and reagent and is also employed in agriculture as a fungicide and antihelmintic.
5	11.775	1-Tetradecanol	$C_{14}H_{30}O$	214.3874	0.18		organic synthesis, plasticizers, antifoaming agent, intermediate, perfume fixative for soaps and cosmetics
6	12.040	Eicosane	$C_{20}H_{42}$	282.5475	0.36		candles and paraffin waxes with solar energy storage capacity
7	12.700	Tetra Decanoic Acid	$C_{14}H_{28}O_2$	228.3709	0.40		Soaps & Cosmetics; Surfactant; Cleansing Agent; Emulsifier; lubricant
8	13.262	3-Heptadecanol	$C_{17}H_{36}O$	256.4671	0.49		NR
9	13.417	Hexadecanal	$C_{16}H_{34}O$	242.4406	0.17		Emulsifier in cosmetics and pharmaceuticals
10	13.463	Isopropyl Myristate	$C_{17}H_{34}O_2$	270.4507	0.33		Flavouring Agents in food additives, mouth wash, solvent in perfume, flea and tick products for pets.
11	13.650	Neophytadiene	$C_{20}H_{38}$	278.5157	0.92		antidiabetic, anti-inflammatory, antiarthritic and anticancer activities

12	13.917	Pentadecanoic Acid	$C_{15}H_{30}O_2$	242.3975	0.46		Adhesives and sealant, lubricants and lubricant additives
13	14.079	Dodecane, 1,1-Dimethoxy-	$C_{14}H_{30}O_2$	230.3868	0.09		NR
14	15.110	3-Pentanol, 3-Ethyl-	$C_7H_{16}O$	116.2013	0.21		NR
15	15.307	Hexadecanoic Acid	$C_{16}H_{32}O_2$	256.4241	10.78		Soaps, cosmetics, food additives, antioxidant, hypocholesterolemic
16	16.592	Heptadecanoic Acid	$C_{17}H_{34}O_2$	270.4507	0.31		Surfactant, adhesives, sealant lubricants and lubricant additives
17	16.819	Cyclohexanol, 1-Butyl-	$C_{10}H_{20}O$	156.2652	0.17		NR
18	17.141	Methyl Linolenate	$C_{19}H_{32}O_2$	292.4562	0.47		skin whitening agent with anti-melanogenesis activity.
19	17.303	Phytol	$C_{20}H_{40}O$	296.5310	1.40		antinociceptive and antioxidant activities as well as anti-inflammatory and antiallergic effects
20	17.474	Hexatriacontane	$C_{36}H_{74}$	506.9728	0.33		Antioxidant, antineoplastic, antimicrobial
21	17.517	Octadecanoic Acid, Methyl Ester	$C_{19}H_{32}O_2$	292.4562	0.26		NR
22	18.059	Octadecanoic Acid	$C_{18}H_{36}O_2$	284.4772	1.51		Flavouring, soaps, cosmetics, detergents, lubricants, insecticide, herbicide
23	22.900	Beta.-Sitosterol	$C_{29}H_{50}O$	414.7067	3.25		Reduces prostatic hyperplasia and blood cholesterol
24	27.817	Moretenol	$C_{30}H_{50}O$	426.7174	6.65		Painkiller
25	30.577	Squalene	$C_{30}H_{50}$	410.7180	0.55		Antioxidant, wound healing

4 DISCUSSION

The phytochemical studies of *E. tsjeriam-cottam* (Roem. & Schult.) A. DC. By HPLC and GC-MS have shown very satisfactory results proving that these species contain chemical properties for curing the related ailments in humans. *E. 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.

5 CONCLUSION

Based on these results it may be concluded that the components of further studies carried out to the future.

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