

GAS CHROMATOGRAPHY - MASS SPECTROMETRY ANALYSIS OF ESSENTIAL OIL COMPOSITION OF *CLERODENDRUM SERRATUM* L.: A TRADITIONAL PLANT OF INDIA

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ABSTRACT

Objective: *Clerodendrum serratum* (L.) belonging to family Verbenaceae, commonly known as Bharangi, is a popular plant across various communities of India. Gas chromatography-mass spectrometry (GC-MS) is a unique technique to prospect various volatile principles and semivolatile compounds.

Materials and Methods: The phytoconstituents present in the root of n-hexane extract were evaluated by GC-MS using Clarus 500 mass spectrometer.

Results and Discussions: A total of 15 constituents were identified and characterized from the roots of *C. serratum* L. All the 15 compounds were tabulated with their retention time, percentage peak area, molecular formula, molecular weight, and Chemical Abstracts Service number. Out of all the compounds, 60% comprises the oxygenated compounds. Compound hexadecanoic acid, methyl ester, and benzene, 1,3-bis(1,1-dimethylethyl) showed highest percentage about 6.5 and 74.9, respectively.

Conclusion: This study showed the presence of 15 compounds ranging from alkane, alkene as longest chain along with ester, aromatic and non-aromatic hydrocarbons.

Keywords: *Clerodendrum serratum*, Gas chromatography-mass spectrometry technique, Phytoconstituents, Volatile principles.

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INTRODUCTION

Plant has been a big reservoir for humankind as well as other species for various fulfillments related to food and ailments, carrying huge amount of primary and secondary metabolites. The WHO clearly appraises the practice of traditional medicine, accounting for about 80% for primary health care [1]. Nature provides large degree of variation regarding the phytoconstituents present in it. Due to this, there has been large development in the area of extraction such as cold extraction, hot extraction, Soxhlet, and microwave-assisted extraction [2]. However, the extraction of essential oil and aromatic content has been done through various distillation procedures such as steam distillation, hydrodistillation, or combination of both [3,4]. Currently, gas chromatography-mass spectrometry (GC-MS) interface has been an advanced technique for the separation of aromatic content due to its sensitivity and specificity.

Clerodendrum serratum (L.) belonging to the family Verbenaceae, commonly known as Bharangi, is a popular indigenous plant to India. The plant is widely distributed across the globe ranging through various continents from Asia to Africa [2]. In Ayurveda, the plant is well known as Bharangi (Sanskrit) and commonly known as blue glory (English). As per the traditional claims, the roots are the potential source of drug for ailments such as asthma, body ache, bronchitis, cholera, dropsy, eye diseases, fever, inflammations, malaria, ophthalmia, snakebite, tuberculosis, ulcers, and wounds [5]. Traditionally, Bharangi has been reported for fever by the tribals of Bengal and Andhra Pradesh. It is also used in snakebite by the various communities of Orissa. In Chhattisgarh, Bharangi is used for malarial fever and liver problems. Literature supports its use in respiratory disorders such as asthma in various states of India such as Maharashtra, Chhattisgarh, Andhra Pradesh, and Madhya Pradesh [6-11]. Literature revealed the pharmacological activity of Bharangi such as anti-inflammatory activity, antinociceptive activity, and hepatoprotective activity [12-14].

Phytochemical studies reveal the presence of secondary metabolites such as glycosides, steroids, alkaloids, and phenolic class of compounds. It has been found to contain ursolic acid, spinasterol, spinasteryl-β-D-glucopyranoside, β-sitosterol, sucrose (disaccharide), bauer-9-en-3-one, and 5-hydroxy-7,4-dimethoxy flavones in the stem part [15]. Aerial part of the plant possesses bis(2-ethylhexyl) phthalate, hispulidin, serratumin A, acteoside, martynoside, serratumoside-A, and myricoside, whereas root was found to contain D-mannitol, stigmasterol, oleanolic acid, ferulic acid, lupeol, and ursolic acid [16]. Essential oil is supposed to have large market potential in today's herbal market and GC-MS is one of the most advanced techniques for its isolation and characterization [17]. GC-MS is one of the advanced techniques for identification of various metabolites such as long-chain hydrocarbons, alkanes, volatile, and semivolatile components [18].

Our objective of the study is to identify and characterize the volatile principles present in the root of Bharangi, which could be used as novel compounds for new drug development and quality control of plant.

METHODS

Collection and authentication of plant material

Roots of *C. serratum* L. was purchased from Faizabad (Uttar Pradesh). The taxonomical identification was done from Department of Agronomy, Acharya Narendra Dev Agriculture Technical University, District Faizabad, Uttar Pradesh.

Chemicals

Chemicals were procured from Sigma-Aldrich Co., St. Louis, USA. All other chemicals/solvent adopted was of analytical grade.

Extraction and isolation of essential oil

Root part of *C. serratum* L was cut into small pieces, shade dried at room temperature for 15 days and subjected to grinding mill. Coarse powder

obtained was further passed through sieve. 44. 10 g of root powder was macerated with 30 ml of hexane overnight followed by sonication for 15 minutes. Further, hexane extract was subjected to reflux for 2 hrs. Centrifugation was done for the refluxed sample for 10 minutes and kept in a sealed vial in the refrigerator (4°C) until further use.

Preparation of sample for GC/MS analysis

A volume of 5 ml of n-hexane plant extract was vortexed properly, centrifuge for 5 minutes and filtered through 0.22 mm syringe filter (Millipore Corp., Bedford, MA, USA). 1 ml aliquot of each sample solution was injected into the GC/MS system for the requisite analysis.

Instrumentation and chromatographic conditions

The phytoconstituents present in the n-hexane root extract was evaluated by GC-MS Clarus 500 mass spectrometer. Temperature programmed with a ramp 8°C/min up to 240°C and ramp 6°C/min up to 280°C hold for 15 minutes while the injector temperature kept at 280°C at injection volume of 1 µL with a syringe size of 10 µL. Flow rate was maintained at 1.2 ml/minutes. Oven was maintained for 70 minutes having transfer line temperature for about 280°C. Scanning was done in

the range of 35-500 amu. Identification of phytoconstituents was made by comparison of their mass spectra with NIST library mass spectra.

RESULTS AND DISCUSSION

GC/MS chromatograms of n-hexane root extract of *C. serratum* L. showed several peaks indicating the presence of a wide range of volatile principles. A total of 15 constituents were identified and characterized from the root of *C. serratum* L. Table 1 listed all the identified chemicals along with their peak percentage, retention time, molecular weight, molecular formula, and Chemical Abstracts Service number. The structures of all the identified compounds are shown in Fig. 1a and b. Components were identified using the combination of retention index value and mass spectral matching against library standards.

CONCLUSIONS

In this study, a total of 15 phytoconstituents have been identified from *C. serratum* L. by GC/MS analysis. All the compounds have been reported for the first time from the plant. The presence of bioactive entities stakes the utilization of *C. serratum*. for various ailments in

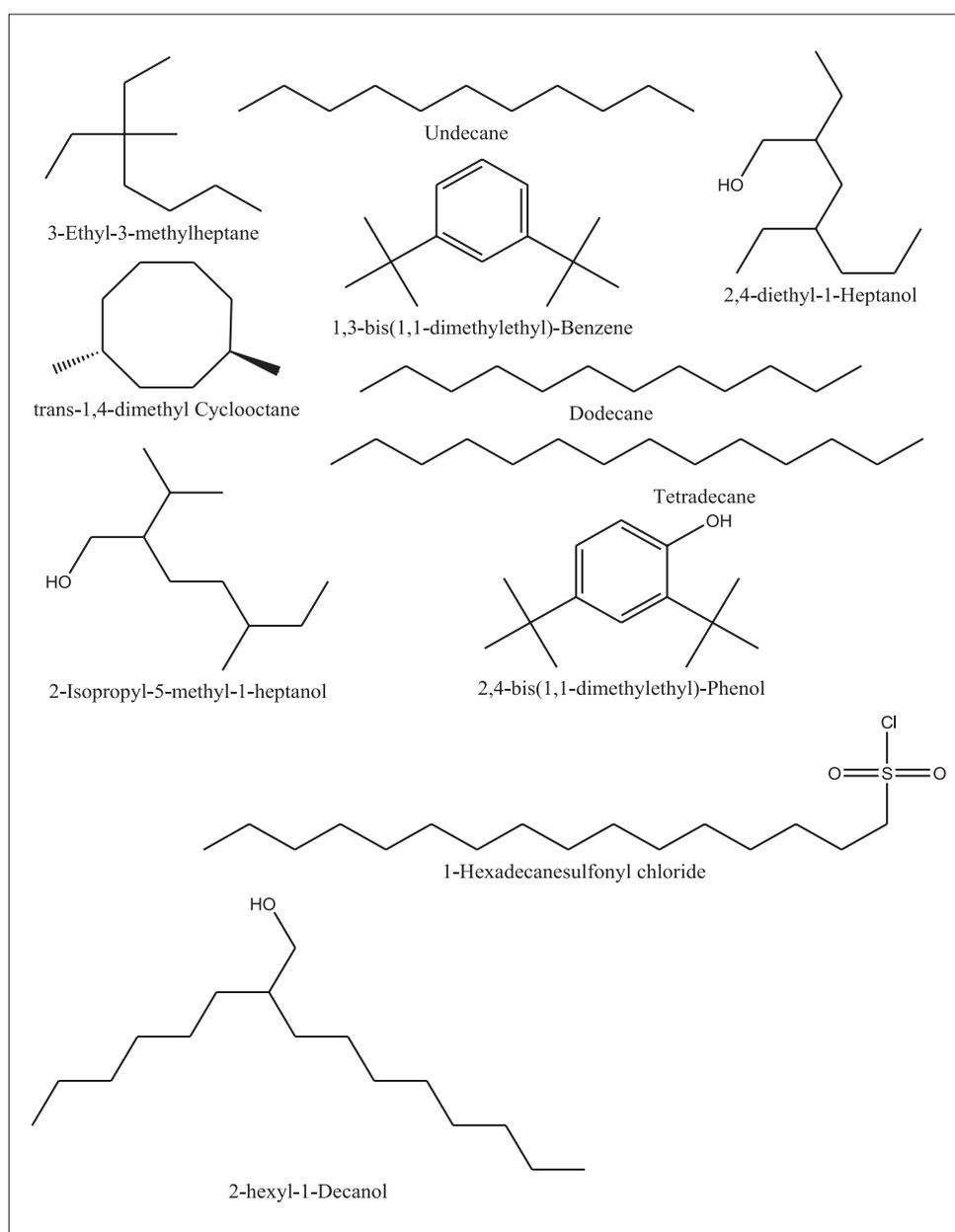


Fig. 1a: Identified phytoconstituents from roots of *Clerodendrum serratum* L. by gas chromatography-mass spectrometry technique

Table 1: Identified volatile compounds from the root of *C. serratum* L.

Peak RT (minutes)	Compound detected	% Peak area	Molecular formula	Molecular weight	CAS number	Activity reported
4.108	3-ethyl-3-methylheptane	9.52	C ₁₀ H ₂₂	776	17302-01-01	Biomarker for urinary cell carcinoma [19]
5.054	Undecane	8.82	C ₁₁ H ₂₄	804	1120-21-4	Antimicrobial agent [20]
5.118	1-heptanol, 2,4-diethyl	5.19	C ₁₁ H ₂₄ O	736	80192-55-8	Cosmetics [21]
5.354	Cyclooctane, 1,4-dimethyl-, trans-	3.35	C ₁₀ H ₂₀	726	13151-98-99	Vehicle hydrocarbon [22]
6.946	Dodecane	17.46	C ₁₂ H ₂₆	832	112-40-3	Oxygen vector [23]
7.899	Benzene, 1,3-bis (1,1-dimethylethyl)	74.9	C ₁₄ H ₂₂	901	1014-60-4	Lipid oxidation [24]
8.688	2-isopropyl-5-methyl-1-heptanol	3.71	C ₁₁ H ₂₄ O	761	91337-07-4	Antimicrobial [25]
10.171	Tetradecane	26.7	C ₁₄ H ₃₀	836	629-59-4	Biomedical use [26]
11.929	Phenol, 2,4-bis (1,1-dimethylethyl)-	35.1	C ₁₄ H ₂₂ O	880	96-76-4	Antioxidant [27]
12.232	1-hexadecanesulfonyl chloride	4.97	C ₁₆ H ₃₃ ClO ₂ S	722	38775-38-1	Antifungal and Antioxidant [28]
12.500	1-decanol, 2-hexyl-	4.68	C ₁₆ H ₃₄ O	734	2425-77-6	Commercially used [29]
15.614	Fumaric acid, dodecyl 2-methylallyl ester	9.19	C ₂₀ H ₃₄ O ₄	562		Not reported
15.791	7H-cyclohepta[a] naphthalen-7-one, 8,9,10,11-tetrahydro-9,9-dimethyl-	26.7	C ₁₇ H ₁₈ O	547	64184-19-6	Not reported
17.493	Hexadecanoic acid, methyl ester	76.5	C ₁₇ H ₃₄ O ₂	827	112-39-0	Antibacterial and antifungal [30]
19.537	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	13.8	C ₁₉ H ₃₄ O ₂	867	112-63-0	Anticancer activity [30]

CAS: Chemical abstracts service

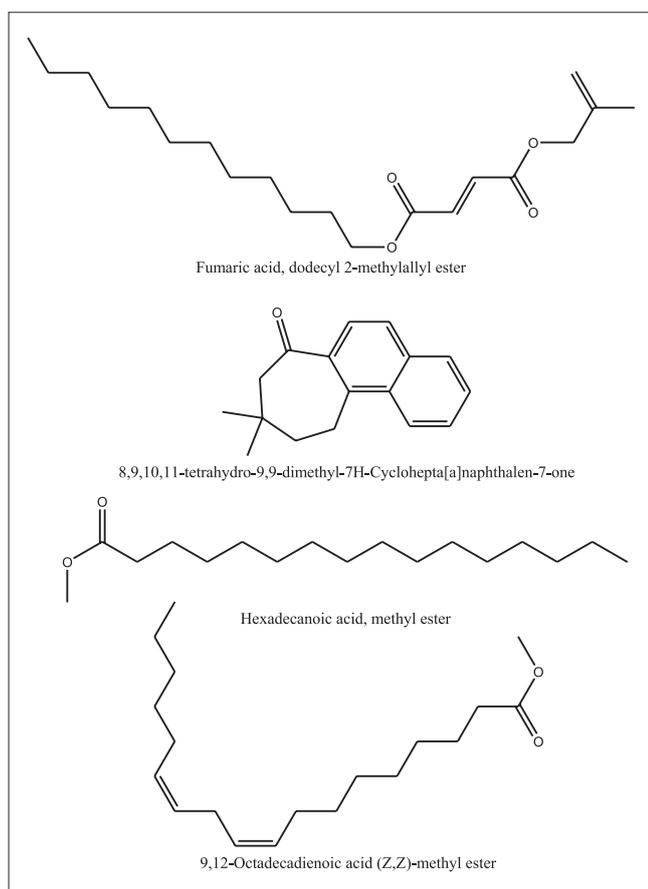


Fig. 1b: Identified phytoconstituents from roots of *Clerodendrum serratum* L. by gas chromatography-mass spectrometry technique

traditional system of medicine. The plant contains several bioactive phytoconstituents, and further study is needed to discover and establish bioactivity for various compounds present in the plant. This study provides a scientific basis for the traditional use of plant as well as documents volatile principles present in it. Volatile oil is found to have biological significance with antibacterial, antifungal, antiseptic property, etc. Bharangi has a wide range of medicinal value in regard

to the constituents identified. Compound hexadecanoic acid and methyl ester showed highest percentage of about 76.5 having medicinal value of antibacterial and antifungal property, whereas benzene, 1,3-bis(1,1-dimethylethyl) was found to be 74.9% having lipid oxidation activity. Other compounds identified were having antimicrobial, anticancer, cosmetic, and commercial value. Moreover, the method can also be used as quality control measure for Bharangi plant.

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