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GC-MS ANALYSIS OF CHLOROFORM EXTRACT OF *CLEOME BURMANNI* W. AND A. (CLEOMACEAE)

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ABSTRACT: The present study was carried out to determine the presence of phytocomponents in the aerial parts of *Cleome burmanni* using Gas Chromatography- Mass Spectrometry (GC-MS) analysis. The mass spectrum of the crude chloroform extract was compared with the available library sources (NIST08 LIB, WILEY8 LIB). Sixteen components were identified. Sterols, terpenoids and hydrocarbons were the most prominent. The major components detected were 24(s)- Ethyl-3- alpha, 5-alpha-cyclocholest-22 (E)-en-6-one (19.29%), Delta-4-Sitosterol-3-one (14.75%), Cholest-4-en-3-one (12.35%), Stigmasta-5, 23-Dien-3-ol, (3-beta) (12.17%), Neophytadiene (6.83%), Hexatriacontane (6.22%), 1-alanine, N-(3-fluorobenzoyl) – undec-10-enyl ester (5.96%), phytol (5.67%), tetracontane (4.30%), 1,2-benzenedicarboxylic acid (3.25%). The other components were present in minute quantities.

INTRODUCTION: Medicinal plants are expensive gift from nature to human. The approval of traditional medicine as an alternative form of health care and the improvement of microbial resistance to existing antibiotics has led researchers to scrutinize the antimicrobial compounds¹.

Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway. The medicinal actions of plants unique to particular plant species or groups are consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct².

Screening active compounds from plants has led to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases including cancer and alzheimer's disease³.

The genus *Cleome* (Cleomaceae) is represented by 12 species in India⁴ and seven species in South India⁵. Many species of *Cleome* such as *C. viscosa*, *C.gynandra*, *C.chelidonii*, found growing as roadside weeds are reportedly used in traditional systems of medicines^{6,7}.

Cleome burmanni, a relatively common species of *Cleome*, is an erect herb with small purple flowers and with siliquaceous fruits. Reports on the phytochemical analysis and medicinal value of the plant is scarce or almost absent. However, a preliminary phytochemical screening has shown that many phytochemicals are present in the various extracts (methanol, chloroform and aqueous) of the plant.

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Phytochemicals such as alkaloids, tannins, saponins, steroids, terpenoids, cardiac glycosides, coumarins and volatile oils were present in the chloroform extract of *C. burmanni* and exhibited bioactivity^{8,9}.

The aim of the present study therefore, is to conduct the GC-MS analysis of the chloroform extract of *C. burmanni*.

MATERIALS AND METHODS:

Plant material: The plant sample, *C. burmanni* was collected from Kariavattom, Thiruvananthapuram and authenticated by the Curator, Department of Botany, University of Kerala. Voucher specimens are deposited in the herbarium of the Department (KUBH 5807).

Preparation of plant extract: Fresh plants of *C. burmanni* were collected, washed and air dried at room temperature. The dried material was homogenized to obtain a coarse powder and stored in air-tight bottles. About 20 gm of the powdered material was subjected to soxhlet extraction using 300 ml chloroform. The extract was concentrated under reduced pressure and preserved in the refrigerator until further use. Two microlitres of the extract was employed in GC-MS analysis.

GC-MS Analysis: The analysis of the extract was performed using GC-MS (Model: GC-MS-QP 2010,

Shimadzu, Tokyo, Japan) equipped with a VF 5ms fused silica capillary column of 30m length, 0.25mm diameter and 0.25 μ m film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. The carrier gas was Helium (99.99%) used at a constant flow rate of 1.51 ml/min. injector and mass transfer line temperature were set at 200°C and 240°C respectively. The oven temperature was set from 70 to 220°C at 10°C/min, held isothermal for three minutes and finally raised to 300°C at 10°C/min. Two microlitres of the sample was injected in a split mode with a scan range of 40 – 1000 m/z. The total running time of GC-MS was 30 min. The relative percentage of the extract was expressed as percentage with peak area normalization.

Identification of components: The identity of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with the NIST08 LIB¹⁰, WILEY8 LIB¹¹ library sources for matching the identified components from the plant material.

RESULTS AND DISCUSSION: The phyto components present in the chloroform extract of *Cleome burmanni* were identified by GC-MS analysis, GC-MS running time being 30 min. The GC-MS chromatogram of chloroform extract of *C. burmanni* is presented in **Fig. 1**.

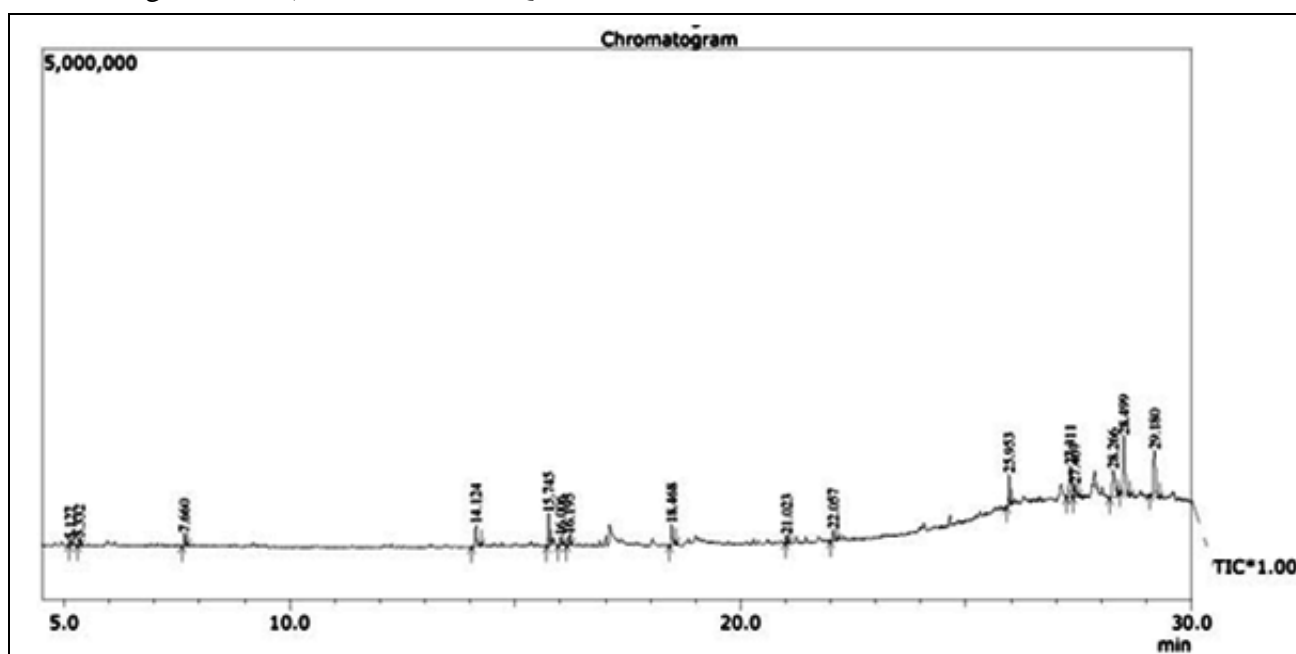


FIGURE 1: GC-MS CHROMATOGRAM OF *CLEOME BURMANNI* W. & A.

The active compounds in the chloroform extract of the plant, their retention time (RT), molecular formula, weight and concentration is provided below (**Table 1**).

TABLE 1: PHYTOCOMPONENTS IN THE CHLOROFORM EXTRACT OF *CLEOME BURMANNI*.

Sl. No.	Retention Time	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
1.	5.127	2,5,5- Trimethyl heptane	C ₁₀ H ₂₂	142	0.86
2.	5.332	Decahydronaphthalene	C ₁₀ H ₁₈	138	1.20
3.	7.660	Dodecane	C ₁₂ H ₂₆	170	2.26
4.	14.124	1-alanine,N-(3-fluorobenzoyl)undec-10-enyl ester	C ₂₁ H ₃₀ FNO ₃	363	5.96
5.	15.745	Neophytadiene	C ₂₀ H ₃₈	278	6.83
6.	16.006	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	268	1.87
7.	16.195	2,6,10-Trimethyl,14-ethylene-14-pentadecne	C ₂₀ H ₃₈	296	1.99
8.	18.468	Phytol	C ₂₀ H ₄₀ O	296	5.67
9.	21.023	Methyl abietate	C ₂₁ H ₃₂ O ₂	316	1.03
10.	22.057	1,2-benzenedicarboxylic acid	C ₂₄ H ₃₈ O ₄	390	3.25
11.	25.953	Hexatriacontane	C ₃₆ H ₇₄	506	6.22
12.	27.311	Stigmasta-5,23-dien-3-beta.-ol	C ₂₉ H ₄₈ O	412	12.17
13.	27.407	Tetracontane	C ₄₀ H ₈₂	562	4.30
14.	28.266	Cholest-4-en-3-one	C ₂₇ H ₄₄ O	398	12.35
15.	28.499	24(S)-ethyl-3-alpha,5-alpha-cyclocholest-22(E)-en-6-one	C ₂₉ H ₄₆ O	410	19.29
16.	29.180	Delta-4-sitosterol-3-one	C ₂₉ H ₄₈ O	412	14.75

The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peaks indicate the relative concentrations of the components present in the plant. The mass spectrometer analyses the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to various peaks at different m/z ratio. These mass spectra are fingerprint of that compound which can be identified from the data library.

The present study on GC-MS revealed the presence of 16 active compounds. The phytoconstituents identified were mainly sterols, terpenoids and alkanes. Of the major components detected, four were sterols such as 24(s)- Ethyl-3- alpha, 5-alpha-cyclocholest-22 (E)-en-6-one (19.29%), Delta-4-Sitosterol-3-one (14.75%), Cholest-4-en-3-one (12.35%), Stigmasta-5,23-Dien-3-ol, (3-beta) (12.17%); four were terpenoids such as Neophytadiene (6.83%), 1-alanine, N-(3-fluorobenzoyl) – undec-10-enyl ester (5.96%), phytol (5.67%), 1,2- benzenedicarboxylic acid (3.25%) and two were alkanes such as Hexatriacontane (6.22%) and tetracontane (4.30%).

Six other components were also detected in minute quantities in the plant extract. As mentioned above, the compound, 24 (S)-Ethyl-3-alpha,5.alpha-cyclocholest-22 (E)-en-6-one is a steroidal sapogenin possessing wide range of biological activities. It also helps in the synthesis of sex and adrenocortical hormones. The second major compound was delta-4-sitosterol which is reported to possess antimicrobial, antioxidant, anti-inflammatory, antiasthma and diuretic properties ¹². The compound, Cholest-4-en-3-one is a novel drug for Amyotrophic lateral sclerosis ¹³.

Stigmast-5,23-dien-3β-ol is a sterol compound possessing anti-inflammatory and antioxidant property ¹⁴. Phytosterols are known to play a significant role in cholesterol metabolism in animals and many previous studies have focused on this aspect ¹⁵. The phytosterols could lower the cholesterol (LDL) levels in a safe and effective way and therefore they are considered suitable ingredients of functional foods.

Neophytadiene, is a fatty acid-related compound which plays an important part in competitive inhibition of cyclooxygenase or lipoxygenase in an inflammation reduction, resulting in decreased production of prostaglandins and leukotrienes ¹⁶.

Hexatriacontane and tetracontane are hydrocarbons; tetracontane are reported to possess anti-inflammatory and analgesic activity¹⁷. Phytol, an acyclic diterpene alcohol, is a constituent of chlorophyll in plants and precursor for the manufacture of synthetic forms of vitamin-E¹⁸.

The study thus shows that the weed, *C. burmanni* is a rich reservoir of medicinally useful phytoconstituents which can be utilized beneficially by isolating these compounds using appropriate methods.

CONCLUSION: The present study is the first report on the GC-MS analysis in *Cleome burmanni*. Sixteen chemical constituents were identified from the chloroform extract of the plant. Presence of medicinally useful phytocomponents in the extract implies the phytopharmaceutical importance of the plant. Further studies are needed to ascertain the pharmacological activity of the concerned compounds.

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