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Research Article

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GC-MS IDENTIFICATION OF PHYTOCOMPOUNDS IN THE METHANOLIC EXTRACT OF *Clerodendrom phlomides*

R. Elakkiya¹ and K. Murugaiah^{2*}

¹Research Scholar, Department of Biochemistry, Enathi Rajappa Arts and Science College, Enathi, Pattukkottai – 614615, Tamil Nadu, S. India

²Department of Biochemistry, Enathi Rajappa Arts and Science College, Enathi, Pattukkottai – 614 615, Tamil Nadu, S. India

ABSTRACT

The aim of this study was to carry out for identification of bioactive compounds from the whole plant methanolic extract of *Clerodendrom phlomides* by Gas chromatography and Mass spectroscopy (GC-MS). GCMS analysis of methanolic extract was done by standard protocol using the equipment Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. The GC-MS analysis revealed the presence of various compounds Octadecanoic acid, methyl ester, n-Hexadecanoic acid, Methyl 9-cis,11-trans-octadecadienoate, 2-Hexadecen-1-ol, 3,7,11,15-tetrame, Hexadecanoic acid, methyl ester and Squalene in the methanolic extract of *Clerodendrom phlomides*. These findings support the traditional use of *Clerodendrom phlomides* in various disorders.

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*Corresponding author

K. Murugaiah
Department of
Biochemistry, Enathi
Rajappa Arts and
Science College, Enathi,
Pattukkottai – 614 615,
Tamil Nadu, S. India

INTRODUCTION:

Plants have been an important source of medicine with qualities for thousands of years. Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines [1]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to elect crude plant extracts with potentially useful properties for

further chemical and pharmacological investigations [2].

Phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated with and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turn over and metabolism, their natural distribution and their biological function [3].

Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from aminoacids), terpenes (a group of lipids) and phenolics (derived from carbohydrates) ^[4]. Plant produces these chemicals to protect itself but recent research demonstrates that emphasizes the plant source of most of these protective, disease-preventing compounds. A true nutritional role for phytochemicals is becoming more probable every day as research uncovers more of their remarkable benefits ^[5]. Within a decade, there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation, identification and structural determination of phytochemicals ^[6].

The chosen medicinal plant namely as *Clerodendrom phlomides* leaves L belongs to the Verbanaceae family. *Clerodendrom phlomides* Gaertn.f. (Verbanaceae) is widely distributed in India, Nepal and Bhutan. In India, the species is distributed from Himachal Pradesh to Assam, Tripura, West Bengal, Bihar and Orissa, Eastern districts of Madhya Pradesh extending further to the Eastern Ghats of Andhra Pradesh ^[7]. The literature survey revealed that no biological activity and phytochemical works has been done so far with the oleoresin of this plant. The biological activity was screened against the micro organisms causing skin allergies, diarrhea and dysentery. A recent study with methanol extract of mature leaves reported anti-inflammatory and antinociceptive activity ^[8-13]. The aim of this study is to determine the organic compounds present in the *Clerodendrom phlomides* extract with the aid of GC-MS Technique, which may provide an insight in its use in tradition medicine.

MATERIAL AND METHODS

Plant materials:

The fully mature *Clerodendrom phlomides* leaves were collected in April 2013 from Tamil University, Thanjavur District, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Dr.S.John Britto, The Director, the Rapiant Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamil Nadu, India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of extracts:

The collected *Clerodendrom phlomides* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 70% ethanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used. The extract contained both polar and non-polar phytocomponents of the plant material used.

GC-MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polydioxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µl was employed (split ratio of 10:1) injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min, to 200°C, then 5°C/min to 280°C, ending with a 9min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver 5.2.0

RESULTS AND DISCUSSION

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. These substances serve as plant defense mechanisms against, insects and herbivores. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-fungal, anti-hepatotoxic and anti-ulcer actions ^[14].

Figure 1: Chromatogram obtained from the GC/MS with the extract of *Clerodendrom phlomidis*.

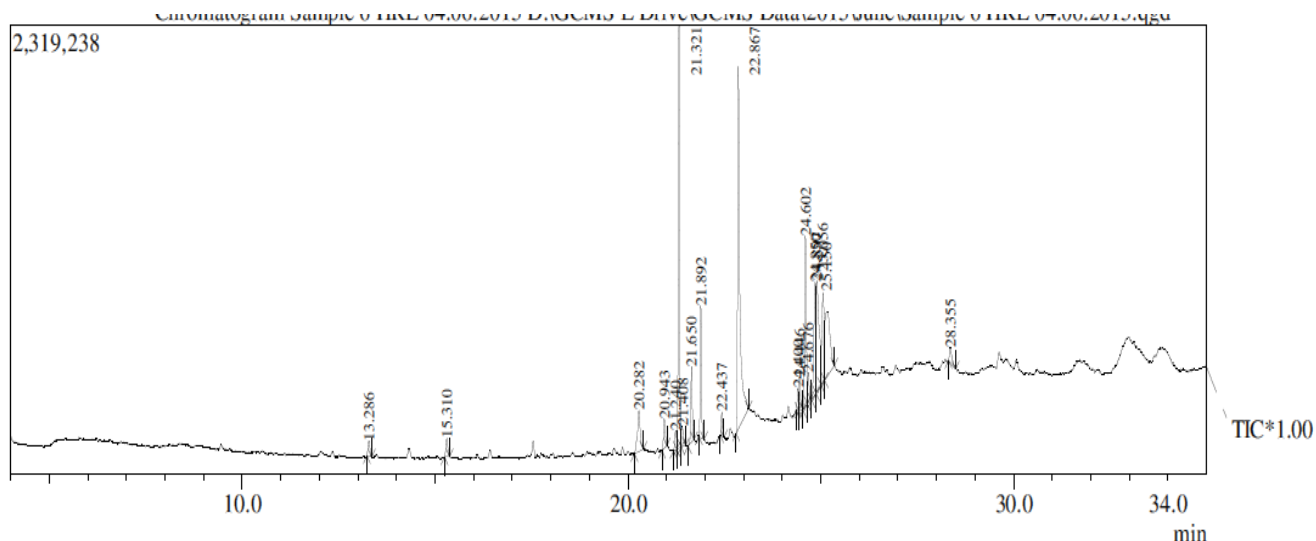


Table 1 Shows the components identified in methanolic extract of *Clerodendrom phlomidis* (GC MS study)

Peak	R.Time	Area %	Height %	Molecular Formula	Name of the compounds
1	13.286	0.82	0.85	C ₁₅ H ₂₄	Bicyclo[7.2.0]Undec-4-Ene, 4,11,11-Trimethyl-8-Methylene-, [1R-(1R*,4E,9S*)]- \$\$
2	15.310	0.98	1.00	C ₁₄ H ₂₂ O	3-Buten-2-One, 4-(2,5,6,6-Tetramethyl-2-Cyclohexen-1-Yl
3	20.282	3.24	2.27	C ₁₁ H ₁₆ O ₃	2(4H)-Benzofuranone, 5,6,7,7a-Tetrahydro-6-Hydroxy-4,4,7a-Trimethyl-, (6s-Cis)
4	20.943	1.47	1.63	C ₁₁ H ₁₈ O ₂	2,6,8-Trimethylbicyclo[4.2.0]Oct-2-Ene-1,8-Diol
5	21.240	0.91	1.07	C ₁₁ H ₁₈ O ₂	2,6,8-Trimethylbicyclo[4.2.0]Oct-2-Ene-1,8-Diol
6	21.321	16.55	23.38	C ₂₀ H ₄₀ O	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl-
7	21.408	1.05	1.10	C ₁₁ H ₂₂	1-Decene, 8-Methyl
8	21.650	3.03	4.20	C ₂₀ H ₄₀ O	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol
9	21.892	4.79	7.30	C ₂₀ H ₄₀ O	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol
10	22.437	0.87	1.31	C ₁₉ H ₃₈ O ₂	Octadecanoic Acid, Methyl Ester
11	22.867	23.35	20.08	C ₁₆ H ₃₂ O ₂	N-Hexadecanoic Acid \$\$ Hexadecanoic Acid

12	24.400	0.87	1.22	C ₁₉ H ₃₄ O ₂	Methyl 9-Cis,11-Trans-Octadecadienoate
13	24.446	1.34	1.78	C ₁₈ H ₃₄ O ₂	Cyclopropaneoctanoic Acid, 2-Hexyl-, Methyl Ester
14	24.602	7.59	9.42	C ₂₀ H ₄₀ O	2-Hexadecen-1-ol, 3,7,11,15-Tetramethyl
15	24.676	1.20	1.65	C ₁₇ H ₃₄ O ₂	Hexadecanoic Acid, Methyl Ester
16	24.850	6.77	6.18	C ₁₅ H ₂₈ O ₂	Cyclopentadecanone, 2-Hydroxy-
17	24.897	9.24	6.13	C ₁₈ H ₃₀ O ₂	9,12,15-Octadecatrienoic Acid, (Z,Z,Z)-
18	25.056	5.94	4.97	C ₁₈ H ₃₆ O ₂	Octadecanoic Acid
19	25.150	8.94	3.62	C ₃₀ H ₅₀	Squalene
20	28.355	1.07	0.83	C ₁₈ H ₃₆ O	Octadecanal
		100.00	100.00		

Table 2: Activity of phyto-components identified in the methanolic extracts of the *Clerodendrom phlomidis* by GC-MS.

S.NO.	R.Time	Name of the compound	Biological activity **
1	21.321	2-Hexadecen-1-ol, 3,7,11,15-tetrame	Antimicrobial, Anti-inflammatory
2	21.650	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Cancer-Preventive Antimicrobial, anti-inflammatory, anti-diuretic, Antioxidant
3	22.437	Octadecanoic acid, methyl ester	Anti-tumour
4	22.867	n-Hexadecanoic acid	Antioxidant, hypocholesterolemic nematocide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor
5	24.400	Methyl 9-cis,11-trans-octadecadienoate	Anti cancer
6	24.602	2-Hexadecen-1-ol, 3,7,11,15-tetrame	Cancer-Preventive Antimicrobial, anti-inflammatory, anti-diuretic, Antioxidant
7	24.676	Hexadecanoic acid, methyl ester	Antioxidant, hypocholesterolemic , Antiandrogenic , hemolytic, Alpha reductaseinhibitor.
8	25.150	Squalene	Antibacterial, Antioxidant, cancer-preventive, Antitumor, Immunostimulant, Perfumery, Pesticide, Sunscreen.

**Source: Dr.Duke's phytochemical and ethnobotanical databases [Online database].

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. The biological activities listed (Table 2) are based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

GC-MS ANALYSIS

Thirty compounds were identified in *Clerodendrom phlomides* by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1 and Fig 1). The prevailing compounds Octadecanoic acid, methyl ester, n-Hexadecanoic acid, Methyl 9-cis,11-trans-octadecadienoate, 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl, Hexadecanoic acid, methyl ester and Squalene were found in this plant.

The investigation concluded that the stronger extraction capacity of methanol could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases.

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