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Determination of Biologically active compounds in *Clerodendrum phlomidis* (L.) leaf extract using GC/MS

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Abstract
The bioactive components of *Clerodendrum phlomidis* leaf have been evaluated using GC/MS. The chemical compositions of the methanol extract of *Clerodendrum* phlomidis leaf was investigated using Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extracts were matched with the National Institute of Standards and Technology (NIST) library. GC/MS analysis of methanolic extract of *Clerodendrum phlomidis* revealed the existence of sixty nine compounds in leaf. The prevailing compounds of *Clerodendrum phlomidis* leaves were α – terpinene (RT 3.692), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (RT 33.142), Ethyl iso-allocholate (RT 35.367), Phytol isomer (RT 36.846). The results of this study offer a platform of using of *Clerodendrum phlomidis* leaf as herbal alternative for various diseases.

Keywords: *Clerodendrum phlomidis*, GC/MS, Bioactive components.

1. Introduction
Phytochemical simply means plant chemicals. "Phyto" is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. In addition, these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures [1]. Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants [2]. The valuable medicinal properties of different plants are due to presence of several constituents i.e. saponins, tannins, alkaloids, alkenyl phenols, glycol-alkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters [3]. Among them some are act as synergistic and enhance the bioactivity of other compounds.
In traditional systems of medicine, many formulations are used for the treatment of joint pain and RA. In Indian traditional system of medicine, the decoction of the roots of *Clerodendrum phlomidis* L.f. (Verbenaceae) is recommended to treat inflammatory diseases, swellings [4] and rheumatism [5]. It is considered to possess analgesic and antiasthmatic properties [6]. The decoction of leaves were used to treat bodyache [7], inflammations and bronchitis [8]. Crushed leaves were applied on joints to alleviate pain and stiffness of joints. The recent ethnobotanical survey carried out from our Institute, in Theni district have also revealed a high fidelity level for the use of *Clerodendrum phlomidis* for rheumatic pain [9]. *Clerodendrum phlomidis* is a common shrub distributed in India, Sri Lanka and South East Asia. The leaves are aromatic and deltoid-ovate. The inflorescence is a panicle with cream coloured flowers [10]. The antiinflammatory activity of Clerodendrum phlomidis was first reported by Surendrakumar [11]. The aim of this study to determine the organic compounds present in the *Clerodendrum phlomidis* (Family: Verbenaceae) leaves extract with the aid of GC-MS Technique, which may provide an insight in its use in tradition medicine.

2. Materials and Methods
2.1 Plant materials
The fully mature *Clerodendrum phlomidis* leaves were collected in April 2013 from Poyyundar kottai, Thanjavur District, Tamil Nadu, India from a single herb.

The leaves were identified and authenticated by Botanist, Dr. S John Britto, Department of Botany, St. Josephs College, Tiruchirappalli, Tamil Nadu, India. A Voucher specimen (VL 001) has been deposited at the Rapinat Herbarium, St. Joseph,s College, Tiruchirappalli, Tamil Nadu, India.

2.2 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 0.25 mm ID x 1 µMdf, composed of 100% Dimethyl polydiloxane), 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 µI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200 ºC, then 5 ºC/min to 280 ºC, ending with a 9 min isothermal at 280 ºC. 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 36 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 [12].

2.3 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.

3. Results and Discussion

Plants synthesize a dazzling array of additional components, called secondary metabolites, whose function has been debated. Many secondary metabolites are ”antibiotic” in a broad sense, protecting the plants against fungi, bacteria, animals, and even other plants.Every plant species contains chemicals that can affect some animals or micro-organisms negatively, strongly supporting the interpretation that secondary metabolites play a vital role in combating diseases and herbivores. ”Plants have been a rich source of medicines because they produce a host of bioactive molecules, most of which probably evolved as chemical defences against predation or infection” [3].

3.1 Gc-Ms Analysis

Twenty five compounds in *Clerodendrum phlomidis* leaf whereas twenty compounds in *Clerodendrum phlomidis* leaf was identified by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Fig 1 and Table 1). The prevailing compounds *Clerodendrum phlomidis* leaves were α – terpinene (RT 3.692), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (RT 33.142), Ethyl iso-allocholate (RT 35.367), Phytol isomer (RT 36.846). Table 2 represents the biological activity of phytocompounds.

The results obtained in this study thus suggest that the identified phytochemical compounds are bioactive constituents. Therefore, the data generated from these experiments provide the chemical basis for the wide use of this plant as therapeutic agent for treating various ailments. This study offers a platform for using *Clerodendrum phlomidis* leaf as herbal alternative for various diseases including diabetes, cancer, microbial infections, inflammations etc.

4. Acknowledgement

The authors are grateful to Dr. S. Velavan, Director, Harman Institute of Science Education and Research (www.harmanresearchcentre.com), Thanjavur, Tamil Nadu for providing necessary facilities.

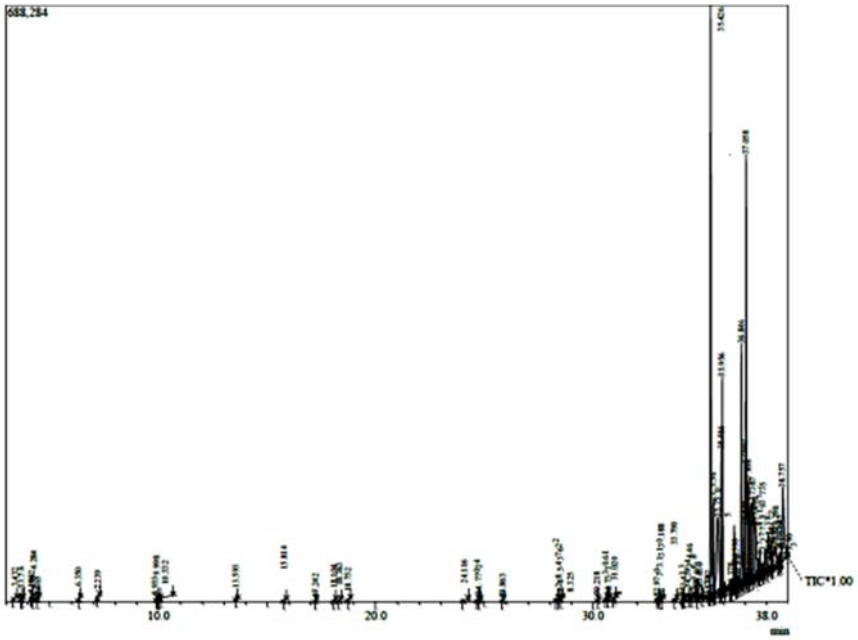
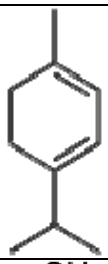
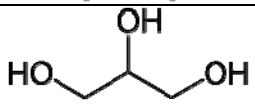
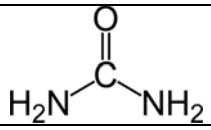
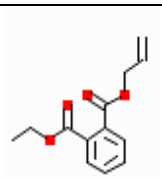
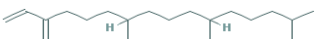
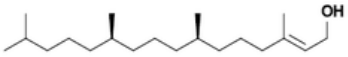
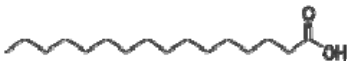
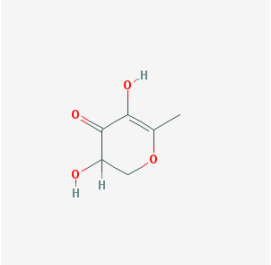
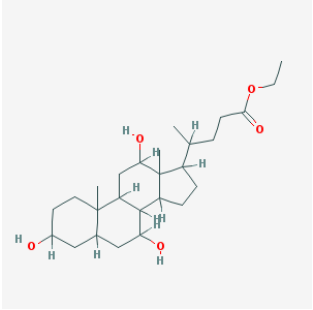
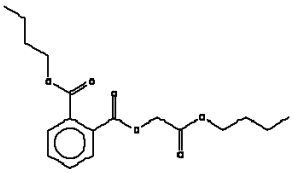
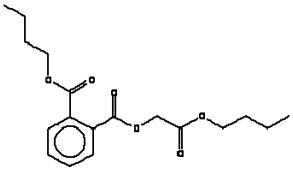
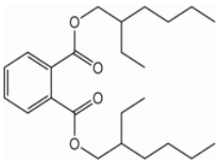
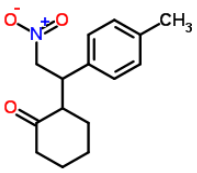
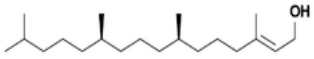
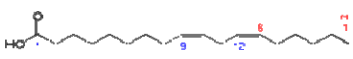
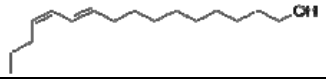
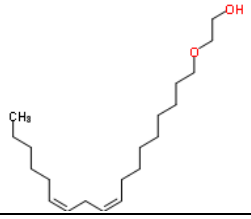


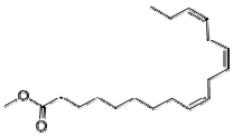
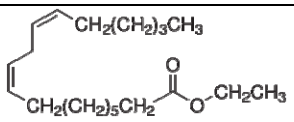
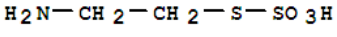
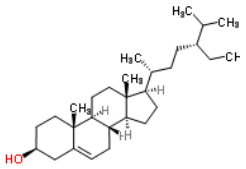
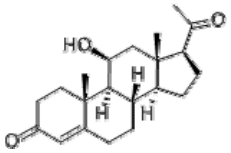
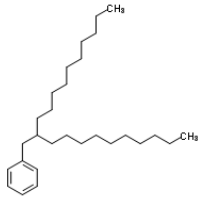
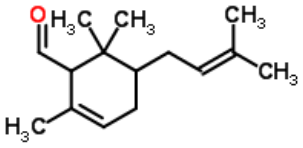
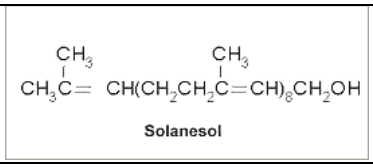
Table 1: Phyto-components identified for *Clerodendrum phlomidis* Sample (GCMS)

Compound Name	RT	Peak Area %	Molecular Formula	Molecular Weight	Compound nature
α - terpinene	c	0.03	$C_{10}H_{16}$	136	Terpenoid
1,2,3-Propanetriol (CAS) Glycerol	10.332	2.29	$C_3\ H_8\ O_3$	92	Polyol
Urea-N15	10.332	2.29	$CH_4\ N^{15}N\ O$	60	Organic compound
1,2-Benzenedicarboxylic acid, diethyl ester (CAS) Ethyl phthalate	28.325	1.36	$C_{12}\ H_{14}\ O_4$	222	phthalate ester,
Phthalic acid, allyl ethyl ester (CAS) Ethylallylphthalate	28.325	1.36	$C_{13}\ H_{14}\ O_4$	234	phthalate ester,
2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS) Phytol	33.790	1.40	$C_{20}\ H_{40}\ O$	296	Acyclic diterpene alcohol
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	33.142	0.59	$C_6H_8O_4$	144	Flavonoid fraction
Ethyl iso-allocholate	35.367	0.06	$C_{26}H_{44}O_5$	436	Steroid
Hexadecanoic acid (CAS) Palmitic acid	35.424	14.84	$C_{16}\ H_{32}\ O2$	256	Fatty acid
Eicosanoic acid (CAS) Arachidic acid	35.424	14.84	$C_{20}\ H_{40}\ O_2$	312	Saturated fatty acid
1,2-Benzenedicarboxylic acid, dibutyl ester (CAS) Butyl phthalate	35.565	2.45	$C_{16}\ H_{22}\ O_4$	278	Phthalate, an ester of phthalic acid
Phthalic acid, butyl ester, ester with butyl glycolate (CAS) 1,2-Benzenedicarboxylic acid, 2-butoxy-2-oxoethyl butyl ester (CAS) Butyl (Butoxycarbonyl)Methyl Phthalate	35.565	2.45	$C_{18}\ H_{24}\ O_6$	336	Phthalate, an ester of phthalic acid
1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) Bis(2-ethylhexyl) phthalate	35.750	5.83	$C_{24}\ H_{38}\ O_4$	390	Diester of phthalic acid
Rac-2,6-Dimethyl-4-nitro-3-phenylcyclohexan-1-one	35.916	3.19	$C_{14}\ H_{17}\ N\ O_3$	247	Unknown
2-(2-nitro-1-p-tolyl-ethyl)-cyclohexanone	35.956	4.46	$C_{15}\ H_{19}\ N\ O_3$	261	
Benzol, 1,2-bis(1-buten-3-yl)-	36.514	1.42	$C_{14}\ H_{18}$	186	Organic compound
2-Hydroxy-4-methylbenzaldehyde	36.846	5.82	$C_6\ H_5\ BO_3$	136	
Phytol isomer	36.846	5.82	$C_{20}\ H_{40}\ O$	296	Diterpene
9,12-Octadecadienoic acid (Z,Z)- (CAS) Linoleic acid	37.002	2.73	$C_{18}\ H_{32}\ O_2$	280	Unsaturated omega-6 fatty acid
Bombykol	37.002	2.73	$C_{16}\ H_{30}\ O$	238	Pheromone
Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)- (CAS) 2-Cis,Cis-9,12-Octadecadienyloxy Ethanol	37.002	2.73	$C_{20}\ H_{38}\ O_2$	310	-
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (CAS) Methyl linolenate	37.058	12.39	$C_{19}\ H_{32}\ O_2$	292	-
Ethyl linoleolate	37.058	12.39	$C_{20}\ H_{36}\ O_2$	308	
Thiosulfuric acid ($H_2S_2O_3$), S-(2-aminoethyl) ester	37.168	3.64	$C_{20}\ H_7\ N\ O_3\ S_2$	157	
Stigmast-5-en-3-ol, (3.beta.,24S)- (CAS) Clionasterol	37.298	4.00	$C_{29}\ H_{50}\ O$	414	Steroids
Pregn-4-ene-3,20-dione, 11-hydroxy-, (11.beta.)- (CAS) 11.beta.-Hydroxyprogesterone	37.381	5.51	$C_{21}\ H_{30}\ O_3$	330	-
Benzene, (2-decyldodecyl)- (CAS) 11-Benzylheneicosane	37.493	2.18	$C_{28}\ H_{50}$	386	--
8-Nitro-11-dodecanolide	38.390	1.49	$C_{12}\ H_{21}\ NO_4$	243	-
Geranyl Linalool isomer	38.757	4.68	$C_{20}\ H_{34}\ O$	290	Terpenoids

Table 2: Biological activity of Phyto-components in *Clerodendrum phlomidis* (L) leaf

Compound Name	Structure	Biological activity**
α - terpinene		Used in cosmetics and food industries, pharmaceutical Antifungal activity
1,2,3-Propanetriol (CAS) Glycerol		Food and Pharmaceuticals
Urea-N15		Fertilizer
1,2-Benzenedicarboxylic acid, diethyl ester (CAS) Ethyl phthalate		Plasticizers
Phthalic acid, allyl ethyl ester (CAS) Ethylallylphthalate		Plasticizers
Neophytadiene		Antihelmeththic Properties
2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS) Phytol		Precursor for the manufacture of synthetic forms of vitamin E and vitamin K1. used in the fragrance industry and used in cosmetics, shampoos, toilet soaps, household cleaners, and detergents
Hexadecanoic acid (CAS) Palmitic acid		Palmitic acid is mainly used to produce soaps, cosmetics, and release agents
Eicosanoic acid (CAS) Arachidic acid		Arachidic acid is used for the production of detergents, photographic materials and lubricants
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-		Antimicrobial, anti-inflammatory

Ethyl iso-allocholate		Antimicrobial Diuretic Anti-inflammatory Anti-asthma
1,2-Benzenedicarboxylic acid, dibutyl ester (CAS) Butyl phthalate		Used as a plasticizer for vinyl foams, which are often used as floor tiles. Other uses are in traffic cones, food conveyor belts, and artificial leather
Phthalic acid, butyl ester, ester with butyl glycolate (CAS) 1,2-Benzenedicarboxylic acid, 2-butoxy-2-oxoethyl butyl ester (CAS) Butyl(Butoxycarbonyl) Methyl Phthalate		Used as a plasticizer for vinyl foams, which are often used as floor tiles. Other uses are in traffic cones, food conveyor belts, and artificial leather
1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) Bis(2-ethylhexyl) phthalate		Plasticizer
2-(2-Nitro-1-P-Tolyl-Ethyl)-Cyclohexanone		Antimicrobial
Benzol, 1,2-Bis(1-Buten-3-Yl)-	-	-
2-Hydroxy-4-methylbenzaldehyde	-	-
Phytol isomer		Precursor for the manufacture of synthetic forms of vitamin E and vitamin K1
9,12-Octadecadienoic acid (Z,Z)- (CAS) Linoleic acid		Useful in oil paints and varnishes
Bombykol		-
Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)- (CAS) 2-Cis,Cis-9,12-Octadecadienyloxy Ethanol		Antimicrobial activity

9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (CAS) Methyl linolenate		Flavour, Fungicide, pesticide, perfumery Anti -inflammatory
Ethyl linoleolate		Antimicrobial activity
Thiosulfuric acid (H2S2O3), S-(2-aminoethyl) ester		Activity not reported
Stigmast-5-en-3-ol, (3.beta.,24S)- (CAS) Clionasterol		Antihepatotoxic, Antiviral, Antioxidant, Cancer¬ preventive, Hypocholesterolemic
Pregn-4-ene-3,20-dione, 11-hydroxy-, (11.beta.)- (CAS) 11.beta. -Hydroxyprogesterone		Antimicrobial activity
Benzene, (2-decyldodecyl)- (CAS) 11-Benzylheneicosane		Antimicrobial activity Flavor, Perfumery
1-formyl-2,2,6-trimthyl-3-cis- (3-methyl-2-butne-1-yl)5-cyclohexene,(.alpha.-cis-sesquicyclocitral)		Antimicrobial activity
Solanesol		Used in synthesis of high-value bio-chemicals such as vitamin-K
Geranyl linalool isomer		Antioxidant

**Source: Dr. Duke's phytochemical and ethnobotanical databases [13].

5. References

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