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DETERMINATION OF PHYTO-CONSTITUENTS IN *Clerodendrum inerme* (L) LEAF EXTRACT USING GC-MS

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ABSTRACT

GC-MS has become a highly recommended tool for monitoring and tracking organic compounds in the environment. GC-MS is exclusively used for the analysis of esters, fatty acids, alcohols, aldehydes, terpenes etc. The aim of this study was to carry out for identification of phytocompounds from the ethanolic extract of *Clerodendrum inerme* (L) leaf by Gas chromatography and Mass spectroscopy (GC-MS). GC-MS analysis of ethanolic 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 like tetradecane, Pentadecane, Tetradecanoic acid, Hexanoic acid, Tridecanoic acid, Kaempferol, Tannic acid, Dodecanoic acid, Ethyl ester, and Gallic acid in the ethanolic leaf extract of *Clerodendrum inerme*. Hence, the *Clerodendrum inerme* may use anticancer, anti-microbial activity, antioxidant and anti-inflammatory activity due to the presence of secondary metabolites in the ethanolic leaf extract. These findings support the traditional use of *Clerodendrum inerme* in various disorders.

Keywords: Gas chromatography and Mass spectroscopy, *Clerodendrum inerme*, Phytochemistry.

INTRODUCTION

Medicinal plants have been used in virtually all cultures as a source of medicine. Assurance of the safety, quality, and efficacy of medicinal plants and herbal products has now become a key issue in industrialized and in developing countries. The widespread use of herbal remedies and healthcare preparations is described in the Vedas and the Bible. Medicinal Plants have been used for thousands of years to flavour and conserve food, to treat health disorders and to prevent diseases including epidemics. The knowledge of their healing properties has been transmitted over the centuries within and among human communities. Active compounds produced during secondary metabolism are usually responsible for the biological properties of plant species used throughout the globe for various purposes. Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant

metabolism (Krishnaiah, et al., 2011). Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from amino acids), terpenes (a group of lipids) and phenolics (derived from carbohydrates) (Liu., 2010) Plant produces these chemicals to protect itself but recent *Clerodendrum inerme* (L). (Common name: Gloriybower; Tamil: Pinari) is a species of flowering plant in the family of Verbinaceae. This plant has simple leaves, white flowers. It is a tropical plant; it is mainly found in Indiaresearch 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 (Hamburger and Hostettmann, 1991) Within a decade, there were a number of dramatic advances in analytical techniques were powerful tools for separation, identification and structural determination of phytochemicals (Roberts and Xia, 1995).

. The aim of this paper is to determine the organic compounds present in the *Clerodendrum inerme* leaf extract with the aid of GC-MS Technique, which may provide an insight in its use in tradition medicine. This plant was used as a traditional medicine for curing many diseases like skin diseases, venereal infections, elephantiasis, asthma, tumors, and blood related diseases (Rao and Raju, 1984; Saleem et al., 1999; Saxena and Choubey, 1997).

MATERIALS AND METHODS

Plant materials

The flowers of *Clerodendrum inerme* (L) were collected from Kallanai, Thanjavur District, Tamil Nadu, India from a single shrub.

Preparation of extracts

The *Clerodendrum inerme* leaf were first washed well and dust was removed from the leaf. Then the leaf was dried at room temperature and coarsely powdered. The powder was extracted with methanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until 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 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 250ºC; ion-source temperature 280ºC. The oven temperature was programmed from 110ºC (isothermal for 2 min), with an increase of 10ºC/min, to 200ºC, then 5ºC/min to 280ºC, ending with a 9min isothermal at 280ºC. 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 Turbo Mass 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 defence mechanisms against, insects and herbivores. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-cancer, antioxidant, anti- fungal, anti-hepatotoxic and anti-ulcer actions (De-Fatima et al., 2006).

Identification of components

Based on the GC-MS analysis of the acid hydrolyzed solvent fraction for the different biochemical compounds, the methyl esterifies sample totally showed 40 peaks at various retention times (Fig. 1). The mass spectral analyses of the major peaks were identified as n-Hexadecanoic acid; 2-Methyl-Z,Z-3,13-octadecadienol and Androstan-17-one, 3-ethyl-3- hydroxy-, (5à)- and minor peaks were identified as Furfural; Benzene, 1-methoxy-4-(1-propenyl)-;Tricyclo[2.2.1.0(2,6)]heptane-3-methanol, 2,3-dimethyl-; Bicyclo [3.2.0]hept-2-ene, 2-methyl-; 4,5-Heptadien-2-ol, 3,3,6-trimethyl-; 2-Hexadecene, 3,7,11,15-tetramethyl- ,[R-[R*,R*-(E)]]-; 2-Pentadecanone, 6,10,14-trimethyl-; 2-Methyl-4-(1-methylethyl)-2- cyclohexenone; 1H-2-Benzopyran-1-one, 3,4-dihydro-3,8-dihydroxy-

3-methyl-, (-)- and 2H- 1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl-, Kaempferol, gallic acid and tannic acid. They were identified based on the results of NIST database. Furthermore, totally 40 compounds were identified and all were tabulated in the (Table-8), further, bioactive nature of all the biochemical compounds were shown as per the source of Dr. Dukes Phytochemical and Ethano botanical database (Table- 1). This clearly showed that the complexity and diversified biochemical components at the leaf extract of C. inerme.

Among the 40 compounds 8 compounds such as Hydrouracil, 1-methyl-1 (Organo silicon), 4H-Pyran-4 One 2,3 dihydro-3.5 dihydroxy-6-methyl-(Cycloalkene), 2(1H)- Naphthalenone, hexahy dro-1,1,4a-trimethyl-(Terpene alcohol) , Benzene, 1-methoxy-4-(1-Propeny1)-, (SNPL oil), Phenol,2,3,4,6-tetramethyl- (Organo silicon), Hexadecanoic acid, ethyl ester (Fatty acids), n-Hexadecanoic acid (Steriod compound), Kaempferol(Flavonoid compound), Possesses the anti-inflammatory activity according to Dr. Duke's data bases (Table- 2).

Among the 40 compounds 14 compounds such as 4H-Pyran-4 One 2,3 dihydro-3.5 dihydroxy-6-methyl- (Cycloalkene), Hydrouracil, 1-methyl-1(Organo silicon), Dodecanoic acid (Plastilizer compound), Cyclooctanol, acetate (Palmitic acid ester), 2,3a-Dimethylehexahydrobe nzofran-7a-o1 (Steriod compound), Tetradecanoic acid (Steriod compound), 3,7,11,15-Tetramethyl-2-hexadecen-1-o1(Amide), n-Hexadecanoic acid (Steriod compound), Phytol (Diterpene), 9-Octadecenoic acid, ethyl ester (Steriod compound), Androstan-17-one,3-ethyl-3-hydroxy-[5a]- (Aromatic compond), Squalene (Steriod compound),), Kaempferol (Flavonoid compounds),Tannic acid (Phenolic compound), possesses the antioxidant activity according to Dr. Duke's data bases (Table- 2).

Among the 40 compounds 14 compounds such as 4H-Pyran-4 One 2,3 dihydro-3.5 dihydroxy-6-methyl- (Cycloalkene), Hydrouracil, 1-methyl-1 (Organo silicon), Dodecanoic acid (Plastilizer compound), Hydrouracil, 1-methyl-1(Palmitic acid ester), 2,3a-Dimethylehexahydrobe nzofran-7a-o1(Steriod compound), Tetradecanoic acid (Steriod compound), 3,7,11,15-Tetramethyl-2-hexadecen-1-o1(Amide), n-Hexadecanoic acid (Steriod compound), Phytol (Diterpene), 9-Octadecenoic acid,ethyl ester(Steriod compound), Androstan-17-one,3-ethyl-3-hydroxy-[5a]- (Aromatic compound), Squalene (Steriod compound), Kaempferol (Flavonoid compound), Gallic acid, Tannic acid (Phenolic compound), possesses the anticancer activity according to Dr. Duke's data bases (Table- 2).

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 Ethanobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

GC-MS ANALYSIS

Gas chromatography has a very wide field of applications. But, its first and main area of use is in the separation and analysis of multi component mixtures such as essential oils, hydrocarbons and solvents (Kadhim et al., 2016). Intrinsically, with the use of the flame ionization detector and the electron capture detector (which have very high sensitivities) gas chromatography can quantitatively determine materials present at very low concentrations. It follows, that the second most important application area is in pollution studies, forensic work and general trace analysis. Because of its simplicity, sensitivity, and effectiveness in separating components of mixtures, gas chromatography is one of the most important tools in chemistry (Altameme et al., 2015). It is widely used for quantitative and qualitative analysis of mixtures, for the purification of compounds, and for the determination of such thermo chemical constants as heats of solution and vaporization, vapor pressure, and activity coefficients (Andrew Marston, 2007; Kalavathi and Sagayagiri, 2014). A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies. Higher plants as sources of

bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on green plants represent a reservoir of effective chemotherapeutants, these are non-phytotoxic, more systemic and easily biodegradable (Bliesner, 2006).

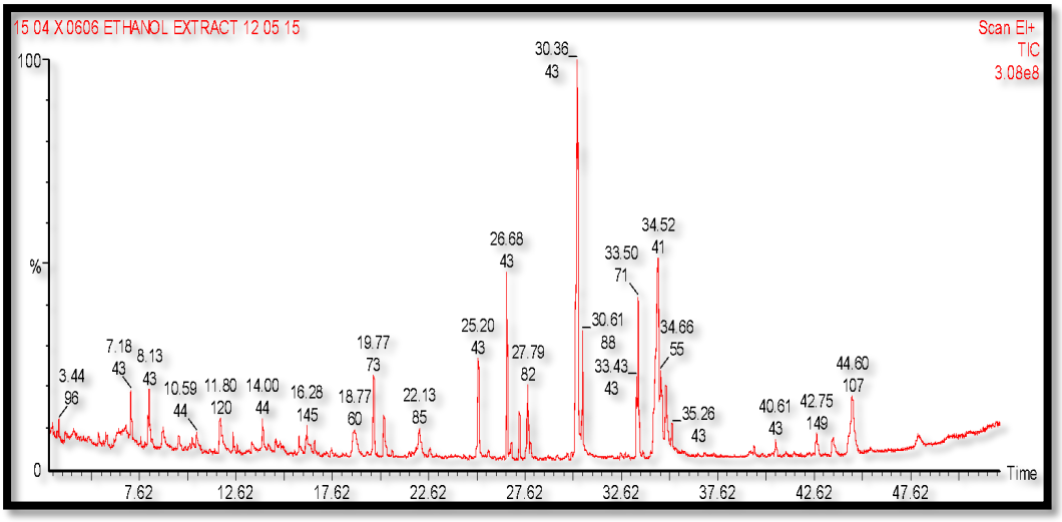

Figure 1: Chromatogram obtained from GC-MS with the extract of *Clerodendrum inerme*(L).

Table -1: Phyto- Components identified in ethanolic extract of *Clerodendrum inerme* (L). leaves (GC- MS study).

S. No	Compound Name	RT	Peak Area %	Molecular Formula	Molecular Weight	Compound Nature
1	Furfural	3.44	0.42	$C_5H_4O_2$	96	Alkaloid
2	2-Furancarboxaldehyde, 5-methyl	5.49	0.51	$C_6H_6O_2$	110	Organo Silicon
3	1-amino-2,6,dimethylpiperdine	7.18	1.66	$C_7N_{16}N_2$	128	Monoterpene
4	Hydrouracil, 1-methyl-1	8.13	0.82	$C_5H_8N_2O_2$	128	Organo silicon
5	4H-Pyran-4 One 2,3 dihydro-3.5 dihydroxy-6-methyl-	3.67	0.85	$C_6H_8O_4$	144	Cycloalkene
6	Benzaldehyde, 4 methyl	11.80	3.72	C_8H_8O	144	Organo silican
7	2(1H)- Naphthalenone, hexahy dro-1,1,4a-trimethyl-	12.48	0.79	$C_{13}H_{20}O$	192	Terpene alcohol
8	Benzene, 1-methoxy-4-(1- Propeny1)-	12.69	0.22	$C_{10}H_{12}O$	148	SNPL oil
9	Phenol,2,3,4,6-tetramethyl-	13.44	0.85	$C_{10}H_{14}O$	148	Organo silicon
10	2-Cyclopentene-1-acetaldehyde,2-formyl-a,3-dimethyl-	14.00	1.31	$C_{10}H_{14}O$	166	Oilly hydrocarbon s
11	n-Decanoic acid	14.67	0.73	$C_{10}H_{20}O_2$	172	Phenolic compound
12	Tricyclo[2,2,1,0[2,6]heptanes-3-methanol,2,2-dimethyl-	14.90	0.22	$C_{10}H_{16}O$	152	Hydro carbons
13	Bicyclo[3,2,0]hept-2-ene,2-methyl-	15.89	0.45	C_8H_{12}	108	Hydro carbons
14	7-Azaindole-3-carboxaldehyde	16.28	0.62	$C_8H_6N_{20}$	146	Fatty acid
15	D-Allose	18.77	4.00	$C_6H_{12}O_6$	180	Triterpene

16	Dodecanoic acid	19.77	3.86	$C_{12}H_{24}O_{20}$	200	Plastilizer compound
17	Cyclooctanol, acetate	20.30	2.69	$C_{10}H_{18}O_2$	170	Palmitic acid ester
18	2,3a-Dimethylehexahydrobe nzofran-7a-o1	22.68	0.54	$C_{10}H_{18}O_2$	170	Steriod compound
19	Tetradecanoic acid	25.20	5.27	$C_{14}H_{28}O_2$	228	Steriod compound
20	4,5-Heptadien-2-01,3,3,6-trimethyl-	25.73	0.43	$C_{10}H_{18}O$	154	Terpene alcohol
21	3,7,11,15-Tetramethyl-2-hexadecen-1-o1	26.68	5.81	$C_{20}H_{40}O$	296	Amide
22	2-Hezdecene,3,7, 11,15-tetramethyl-[R-R,R-(E)]-	26.83	0.03	$C_{20}H_{40}$	280	Alcohol compound
23	2-Pentadecanone,6,10,14-trimethyl-	26.91	0.24	$C_{18}H_{36}O$	152	Silicon based Polymer
24	2-Methyl-4-(1methyllethyl)-2-cyclohexenone	27.91	0.31	$C_{10}H_{16}O$	152	Steriod
25	1H-2-Benzopyran-1-one,3,4-dihydro-3,8-dihydroxy-3-methyl- [-]	29.30	0.28	$C_{10}H_{10}O_4$	0.28	Polysterols
26	2H-1-Benzopyran 6,7,-dimethoxy-2,2-dimethyl-	29.85	0.13	$C_{13}H_{16}O_3$	220	Amide
27	n-Hexadecanoic acid	30.36	25.06	$C_{16}H_{32}O_2$	256	Steriod compound
28	Hexadecanoic acid, ethyl ester	30.61	3.20	$C_{18}H_{36}O_2$	284	Fatty acid
29	Phytol	33.50	3.72	$C_{20}H_{14}O$	296	Diterpene
30	2-Methyl1-Z,Z-3,13-Octadecadienol	34.52	13.7	$C_{19}H_{36}O$	280	Organo Silicon Compound
31	9-Octadecenoic acid,ethyl ester	34.66	1.56	$C_{18}H_{36}O_2$	310	Steriod compound
32	Octadecenoic acid	34.94	2.53	$C_{20}H_{36}O_2$	284	Organo Silicon Compound
33	Octadecenoic acid,2-methyl- methyl ester	35.24	0.76	$C_{20}H_{40}O_2$	312	Silicon based polymer
34	Cinnolin-3[2H]-one,5,6,7,8-tetrahydro-5-ecetylhydrazono-7,7-dimethuyl-	40.61	0.76	$C_{12}H_{16}N_4O_2$	248	Aromatic compound
35	Andrograoholide	43.59	1.71	$C_{20}H_{16}N_4O_2$	350	Alkene compound
36	Androstan-17-one,3-ethyl-3-hydroxy-[5a]-	44.60	8.22	$C_{21}H_{34}O_2$	318	Aromatic compound
37	Squalene	48.01	1.81	$C_{30}H_5O$	410	Steriod compound
38	Kaempferol	2.700	5.55	$C_{15}H_{10}O_6$	302	Flavonoid
39	Gallic acid	3.550	66.6	$C_{76}H_{52}O_{46}$	500	Flavonoid
40	Tannic acid	4.833	5.27	$C_{76}H_{52}O_{46}$	500	Phenol

Source: Dr. Duke's phytochemical and ethno botanical databases.

Table- 2: Biological activity of phyto-components in *Clerodendrum inerme (L)*. leaves (GC-MS study).

S. No	Compound Name	Function and uses
1	Furfural	Antifungal, antimicrobial and **anticancer activity**.
2	2-Furancarboxaldehyde, 5-methyl	**Antitumor**, antimicrobial, nonlinear optical properties.
3	1-amino-2,6,dimethylpiperdine	Antimicrobial, antimagnetic, antimicrobial activities.
4	Hydrouracil, 1-methyl-1	**Antioxidant, anti-inflammatory, anticancer activities.**
5	4H-Pyran-4 One 2,3 dihydro-3.5 dihydroxy-6-methyl-	**Antioxidant, anti-inflammatory, anticancer activities.**
6	Benzaldehyde, 4 methyl	Antimicrobial, **anticancer**, antibacterial, antifungal activities.
7	2(1H)- Naphthalenone, hexahy dro-1,1,4a-trimethyl-	**Antitumor, anti-intlammatory**, antiproliperative activities.
8	Benzene, 1-methoxy-4-(1-Propeny1)-	Antibacterial, **anti-inflammatory**, antifungal, **antioxidant.**
9	Phenol,2,3,4,6-tetramethyl-	**Anticancer, anti-inflammatory** markers, cardiovascular prevention anti virulence properties.
10	2-Cyclopentene-1-acetaldehyde,2- formyl-a,3-dimethyl-	Synthetic intermediates
11	n-Decanoic acid	Antiproliferative effect, antimicrobial activity.
12	Tricyclo[2,2,1,0[2,6]heptanes-3-methanol,2,2-dimethyl-	Nucleoside antibiotics
13	Bicyclo[3,2,0]hept-2-ene,2- methyl-	Antimicrobial and antifungal activity.
14	7-Azaindole-3-carboxaldehyde	Antimicrobial, **antitumor**
15	D-Allose	Antiproliferative, emulsification activity.
16	Dodecanoic acid	Antimicrobial, **antioxidant**, cytotoxicity properties.
17	Cyclooctanol, acetate	Cytotoxicity **antioxidant**, antifungal, antibacterial, antimicrobial.
18	2,3a-Dimethylehexahydrobe nzofran-7a-o1	Cytotoxicity, **antioxidant**, antifungal, anti-bacterial, antimicrobial.
19	Tetradecanoic acid	Antimicrobial, **antioxidant**, antibacterial activity.
20	4,5-Heptadien-2-01,3,3,6- trimethyl-	Antibacterial and antifungal activities.
21	3,7,11,15-Tetramethyl-2-hexadecen-1-o1	Antibacterial, cytotoxicity, **antioxidant,** antimicrobial activity.
22	2-Hezdecene,3,7, 11,15- tetramethyl-[R-R,R-(E)]-	Antifungal, antimicrobial, cytotoxicity effect

23	2-Pentadecanone,6,10,14- trimethyl-	Antibaterial, antifungal, antimicrobial activity.
24	2-Methyl-4-(1methyllethyl)-2- cyclohexenone	Herbicides, fungicides, antimicrobial and nematicidal substences.
25	1H-2-Benzopyran-1-one,3,4- dihydro-3,8-dihydroxy-3-methyl- [-]	In vitro cytotoxicity and **free**
26	2H-1-Benzopyran 6,7,-dimethoxy- 2,2-dimethyl-	Docking studies, antimicrobial,
27	n-Hexadecanoic acid	Antimicrobial, adioxident,
28	Hexadecanoic acid, ethyl ester	Antimicribial, **anti-inflammatory**, antibacterial, antidiarrhoeal, antiviral activities.
29	Phytol	Antinociceptive, **antioxidant**, antibacterial, **anti-inflammatory, anticancer** activity.
30	2-Methyl1-Z,Z-3,13-Octadecadienol	Antimicrobial, antifungal activity.
31	9-Octadecenoic acid,ethyl ester	Antimicrobial, antifungal, **antioxidan**t.
32	Octadecenoic acid	Antimicrobial, **treatment of cancer**
33	Octadecenoic acid,2-methyl- methyl ester	Antibacterial, antimicrobial activity,
34	Cinnolin-3[2H]-one,5,6,7,8- tetrahydro-5-ecetylhydrazono-7,7- dimethuyl-	Antimicrobial antifungal and antibacterial activity.
35	Andrograoholide	Antibacterial, **antitumor**, antivirus, antidiabetic activities.
36	Androstan-17-one,3-ethyl-3- hydroxy-[5a]-	**Antioxidant**, antiulcer, antibacterial activities.
37	Squalene	**Antioxidant** and **antitumor** activity.
38	Kaempferol	**Antioxidant, anti-inflammatory, anticancer,** antiallergic, and antidiabetic activities.
39	Gallic acid	Antibacterial, antifungal, and antimicrobial.
40	Tannic acid	**Antioxidant, antitumour**, and antibacterial activities.

Source: Dr. Duke's phytochemical and ethano botanical databases.

The principle of gas chromatography is adsorption and partition. Within the family of chromatography-based methods gas chromatography (GC) is one of the most widely used techniques. GC-MS has become a highly recommended tool for monitoring and tracking organic pollutants in the environment. GC-MS is exclusively used for the analysis of esters, fatty acids, alcohols, aldehydes, terpenes etc. (Abeer Fauzi Al-Rubaye et al., 2017; Kalavathi and Sagayagiri, 2016; Velavan,. 2015).

In the present study, forty compounds were identified in *Clerodendrum ineme* leaf by GC-MS analysis. The active compounds with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1 and Fig 1). The prevailing compounds were tetradecane, Tetradecane, Pentadecane, Tetradecanoic Acid, Hexanoic Acid and tridecanoic acid. Anandhi and Ushadevi, (2013) and Balaji and Kilimozhi, (2014) identified the hexadecane, dodecanoic acid, nonadecane, eicosane, tetradecanoic acid, oleic acid, heptacosane, 9,12- octadecenoic acid, ethyl ester; n-hexadecanoic acid; 1,2-benzenedicarboxylic acid and 9- octadecenoic

acid (Z)-ethyl ester were reported in *Clerodendrum inerme* and *C. phlomidis* leaves.

CONCLUSION

GC-MS is widely used in pharmaceutical industries for analytical research and development, quality control, quality assurance, production, pilot plants departments for active pharmaceutical ingredients (API), bulk drugs and formulations. The prevailing compounds were, Tetradecane, Pentadecane, Tetradecanoic acid, Hexanoic acid, Tridecanoic acid, Kaempferol, Tannic acid, Dodecanoic acid, were identified in *Clerodendrum inerme* leaf by GC-MS analysis. 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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