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Review/Research

Gas Chromatography-Mass Spectrometry Analysis of Hydroalcoholic Extract of *Calotropis gigantea* Linn Leaves

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	Abstract
Published on: 14 July 2025	<i>Calotropis gigantea</i> Linn belongs to Apocynaceae, a large erect lactiferous shrub. Native to continental Asia and south East Asia and has been introduced in the pacific islands, Australia, Central and northern south America, south Africa, India, Srilanka, China, Thailand, Philippines, Malaysia, Indonesia and Cambodia. Tribal communities used this plant to treat bronchial asthma, cholera, convulsions, pneumonia, toothache, ringworm and small pox infections, fever, rheumatism, leprosy, constipation, wounds. The phytochemical reported the presence of cardenolides, triterpenoids, anthocyanins and hydrocarbons in it. The plant is reported for antimicrobial, antioxidant, antiasthmatic, anticonvulsant and it also contains hepatoprotective, hypoglycemic, procoagulant, abortifacient, cytotoxic, insecticidal and ovicidal effects. The collected leaves were washed with water; shade dried, powdered and extracted with hydroalcohol. The extract was concentrated and stored in air tight container for further use. The aim of this study was to characterize the phytoconstituents in hydroalcoholic <i>C.gigantea</i> leaves extract using Gas chromatography-Mass spectrometry (GC-MS). GC-MS chromatogram showed the presence of twenty phytoconstituents which may attribute to pharmacological properties.
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	Keywords: Gas chromatography-Mass spectrometry (GC-MS), <i>Calotropis gigantea</i> .

INTRODUCTION

Calotropis gigantea Linn belongs to Apocynaceae, a large erect lactiferous shrub. Native to continental Asia and south East Asia and has been introduced in the pacific islands, Australia, Central and northern south America, south Africa, India, Srilanka, China, Thailand, Philippines, Malaysia, Indonesia and

Cambodia[1,2]. *Calotropis* genus contains two different species *gigantea* (Purple flowered plant) and *procera* (White flowered plant)[3]. Santals tribe of Jharkhand and West Bengal used this plant as remedy for bronchial asthma, cholera, convulsions, pneumonia, toothache, ringworm and small pox infections. People of Kolayat tehsil in Rajasthan the plant to treat fever. People of Chitheri Hills of Dharmapuri and people of Raipur in Chhatisgarh plains used in rheumatism, People of North Kanara in Karnataka used to treat leprosy. People of Kamrup in assam used to treat constipation, Tribals of eastern Rajasthan used to get rid of wounds[4]. The phytochemical reported the presence of cardenolides from the latex and leaves, triterpenoids, anthocyanins from flowers and hydrocarbons in it[5]. It exhibited antimicrobial[6] antioxidant[7], antiasthmatic[8], anticonvulsant[9] and it also contains Hepatoprotective[10], Hypoglycemic[11], Procoagulant[12], abortifacient[13], cytotoxic[14], insecticidal[15] and ovicidal effects[16,17]. This research aims to analyze the presence of phytoconstituents present in the hydroalcoholic extract of *C. gigantea* leaves using Gas Chromatography–Mass Spectrometry (GC-MS) analysis.

MATERIALS AND METHODS

Collection of leaves and Authentication

Leaves were collected from Mangalakudi village, Madurai district, Tamil Nadu in the month of February 2025. The leaves was identified and authenticated by Dr.Stephen, Professor, Department of Botany, American College, Madurai-625 002. The herbarium of this specimen was kept in the department for further reference.

Preparation of hydroalcoholic extract of *Calotropis gigantea* (HAECG_L)

The collected leaves were washed with water; shade dried and powdered, five grams of powder was extracted with hydroalcohol (70%) by maceration technique until the complete extraction of material and filtered. The filtrate was concentrated to dryness and the percentage was found to be 14.34%w/w and stored in air tight container for further use.

Gas chromatography- mass spectroscopy analysis

Gas Chromatography–Mass Spectrometry (GC-MS) (Shimadzu QP 2020) is a powerful analytical technique that integrates gas chromatography and mass spectrometry to detect and characterize various compounds within a sample. As a hyphenated system, it offers excellent compatibility and is widely employed for identifying and quantifying biochemical constituents in medicinal plants.

Column

The column consists of fused silica, packed with SH-Rxi-5 Sil MS (30 m × 0.25 mm ID × 250 µm df). The separation of components was carried out using helium as the carrier gas, maintained at a constant flow rate of 1 ml/min. The injector temperature was set to 280°C.

Condition

A 1 µL sample of the hydroalcoholic extract was injected into the instrument. The oven temperature was programmed as follows: initially set at 50°C for 3 minutes, then increased to 180°C at a rate of 15°C per minute.

Mass Detector

The mass detector was operated under the following conditions: transfer line temperature set at 290°C, ion source temperature at 230°C, and electron impact ionization mode at 70 eV. The scan time was 0.2 seconds with a scan interval of 0.1 seconds, and fragment ions were recorded in the range of 50 to 600 Da. The obtained spectra of the components were compared with the reference spectra available in the GC-MS NIST (2017) library.

RESULTS AND DISCUSSION

Gas chromatography – Mass spectrometry (GC-MS) identified the presence of twenty phytoconstituents are shown in the Table 1. and its biological activity are shown in Table 2 and the chromatogram was displayed in Fig 1.

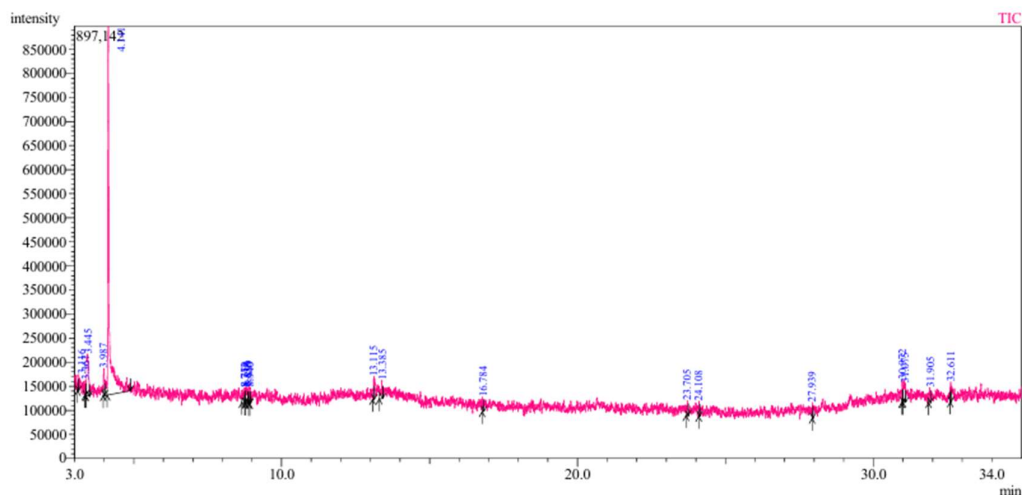


Fig 1: Gas Chromatography - Mass Spectrometry of HAECGL

Table 1: Identification of phytoconstituents in HAECGL by GC-MS

Peak	R.Time	Area	Area %	Height	Height %	Molecular Formula	Molecular Weight	Phytoconstituents
1	3.116	45726	1.10	33850	2.24	C ₁₃ H ₁₁ ClO	218	Benzene, 1-(chloromethyl)-3-phenoxy-
2	3.367	46241	1.12	31478	2.08	-	-	-
3	3.445	414162	10	86605	5.73	C ₆ H ₁₄ O ₂	118	2,2-dimethoxybutane
4	3.987	118706	2.87	56474	3.74	C ₇ H ₁₂ O ₂	128	2-Buten-1-ol, propanoate
5	4.141	233709	56.44	76489	50.60	C ₇ H ₁₂ O ₂	128	2-Buten-1-ol, propanoate
6	8.743	118432	2.86	30784	2.04	C ₁₁ H ₁₁ NO	173	Oxazole, 4-benzyl-5-methyl-
7	8.770	35903	0.87	32580	2.16	-	-	-
8	8.835	105627	2.55	33615	2.22	C ₁₆ H ₃₄ S	258	Octane, 1,1'-thiobis-
9	8.899	63748	1.54	36953	2.44	C ₅ H ₉ ClO ₂	136	Propanoic acid, 3-chloro-, ethyl ester
10	8.940	41431	1	33350	2.21	C ₈ H ₁₃ N ₃ OS ₂	231	Urea, 1-methyl-3-(3,5,5-trimethyl-2-thioxothiazolidin-4-ylideno)-
11	13.115	103731	2.50	47275	3.13	C ₁₉ H ₂₅ N ₃ OS	343	p-Anisaldehyde 4-[1-adamantyl]-3-thiosemicarbazone
12	13.385	116273	2.81	38583	2.55	C ₈ H ₁₆ S	144	3-n-Butylthiolane
13	16.784	68821	1.66	30591	2.02	-	-	-
14	23.705	67804	1.64	31348	2.07	-	-	-
15	24.108	35900	0.87	31381	2.08	-	-	-
16	27.939	30931	0.75	33117	2.19	C ₅ H ₄ BrNOS	205	5-Bromo-2-mercaptopyridine-1-oxide
17	30.972	109908	2.65	46915	3.10	C ₂₂ H ₃₄ O ₃ Si	374	Androsta-1,4-dien-3-one, 6-hydroxy,17-trimethylsilyloxy-, (6.beta.,17.beta.)-
18	31.075	177036	4.28	39170	2.59	-	-	-

19	31.905	50639	1.22	31294	2.07	C ₁₁ H ₁₂ Si	172	Methyl(ethenyl)bis(but-3-en-1-ynyl)silane
20	32.611	52960	1.28	41502	2.75	C ₂₇ H ₂₄ N ₈ O ₂ S ₂	556	1,1'-(4-Methyl-1,3-phenylene)bis(3-[5-(p-tolyl)-1,3,4-thiadiazol-2-yl]urea)

Table 3: List of Biological activity of phytoconstituents identified in HAECGL by GC-MS

S.No.	Name of the Bioactive compound	Derivative	Biological activity
1	Benzene, 1-(chloromethyl)-3-phenoxy-	Benzene	-
2	2,2-dimethoxybutane	1,3-dioxolanes	Antibacterial and anticancer activity [18]. Antidermatophytic and antimicrobial activity[19].
3	2-Buten-1-ol, propanoate	Propanoate	Antimicrobial activity[20].
4	Oxazole, 4-benzyl-5-methyl-	oxazole	Analgesic, anti-inflammatory, antidepressant, anticancer, antimicrobial, antidiabetic, antiobesity, antioxidant, anti progesterone activity[21].
5	Octane, 1,1'-thiobis-	Sulfide	-
6	Propanoic acid, 3-chloro-, ethyl ester	Propanoic acid	Antioxidant, antitumour, analgesic, antimicrobial and antidiabetic activity[22].
7	Urea, 1-methyl-3-(3,5,5-trimethyl-2-thioxothiazolidin-4-ylideno)-	Urea	-
8	p-Anisaldehyde 4-[1-adamantyl]-3-thiosemicarbazone	Thiosemicarbazone	Antibacterial, antifungal and anti proliferative activity [23].
9	3-n-Butylthiolane	Thiolane	Its derivative investigated for potent uncompetitive inhibitors against alcohol[24]
10	5-Bromo-2-mercaptopyridine-1-oxide	Pyridine	-
11	Androsta-1,4-dien-3-one, 6-hydroxy,17-trimethylsilyloxy-, (6.beta.,17.beta.)-	-	-
12	Methyl(ethenyl)bis(but-3-en-1-ynyl)silane	Silane	
13	1,1'-(4-Methyl-1,3-phenylene)bis(3-[5-(p-tolyl)-1,3,4-thiadiazol-2-yl]urea)	Thiadiazole	Anticancer activity[25].

From the identified phytoconstituents 2,2-dimethoxybutane, Oxazole,4-benzyl-5-methyl-, 1,1'-(4-Methyl-1,3-phenylene)bis(3-[5-(p-tolyl)-1,3,4-thiadiazol-2-yl]urea) produce anticancer activity. 2,2-dimethoxybutane, p-Anisaldehyde 4-[1-adamantyl]-3-thiosemicarbazone exhibits antibacterial activity. 2,2-dimethoxybutane, 2-Buten-1-ol, propanoate, Oxazole,4-benzyl-5-methyl-, Propanoic acid, 3-chloro-, ethyl ester reported for antimicrobial activity. Oxazole,4-benzyl-5-methyl-, Propanoic acid, 3-chloro-, ethyl ester has analgesic, antioxidant and antidiabetic activity. 2,2-dimethoxybutane shows antidermatophytic activity. Oxazole,4-benzyl-5-methyl- reported for anti inflammatory, antidepressant, antiobesity and antiprogestosterone activity. Propanoic acid, 3-chloro-, ethyl ester exhibits antitumor activity. p-Anisaldehyde 4-[1-adamantyl]-3-thiosemicarbazone has antifungal and antiproliferative activity. 3-n-Butylthiolane derivative is investigated for potent uncompetitive inhibitors against alcohol.

CONCLUSION

The study determined that the hydroalcoholic extract of *Calotropis gigantea* leaves contains twenty phytoconstituents with promising pharmacological properties. These phytochemical compounds may serve as a valuable source for therapeutic drug development, contributing to activities such as analgesic, antibacterial,

anticancer activity, antidiabetic, antimicrobial activity. The findings highlight its pharmacological potential and lay the groundwork for future research.

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