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Preliminary Phytochemical and GC-MS Profiling of Ethanolic Extract of Leaves of *Calotropis gigantea Linn.* (Asclepiadaceae)

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ABSTRACT

Calotropis belong to the Asclepiadaceae family. It is also known as Akada, Aak, Mandar, Aakh etc. It has two species procera and gigantea. Here we study about *Calotropis gigantea*. The roots and leaves of *Calotropis gigantea* are used traditionally for the treatment of abdominal, tumors, skin diseases, wound, insect bites. The leaves of the plant contain primary and secondary metabolites. Quantitative analysis showed in the presence of alkaloids, glycosides, terpenoids, saponins, cardiac glycosides, flavonoids. *Calotropis gigantea* in a small dose is also useful in the treatment of cold, cough, asthma inflammatory diseases and loss of digestive and analgesic property. The medicinal properties of this plant represent it as a valuable source of a medicinal compound. This study summarizes that information concerning the phytochemical constituent present in the ethanolic extract of *Calotropis gigantea* in these constituents may be responsible for the pharmacological activity.

INTRODUCTION

Herbals plants are an effective source of traditional and modern medicines, used for primary health care. Plants are the richest source of bioactive organic chemicals on earth. The active metabolites like Phytochemicals from the medicinal plants were under exploration for the development of novel and biodegradable effective drugs as an alternative to the ineffective contemporary medicine. *Calotropis gigantea* has great medicinal importance to treat indigestion, rheumatism, diabetes, cancer cold, cough, cardiotonic, asthma, scabies etc[1]. The most important of these chemically active constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds. Many of these indigenous medicinal plants are also used for medicinal purposes [2, 3]. Natural products, which come out from medicinal plants are important for pharmaceutical research and for drug development as a source of therapeutic agents. At presents, the demand for herbal or medicinal plant products has increased significantly. In the recent past, there has been a growing interest in exploiting the biological activities of different Ayurvedic medicinal herbs, owing to their natural origin, cost-effectiveness and lesser side effects [4]. Medicinal plants are an 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 the existing antibiotics has lead researchers to scrutinize the antimicrobial compounds [5].

Nowadays synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars. In contradiction to this many medicines of the plant, origin had been used for a long time without any adverse effects. Exploring the healing power of plants is an ancient concept. For centuries, people have been trying to alleviate and treat disease with different plant extracts and formulations [6, 7]. Because of the present situation, there is a need for essential efforts that should be made to introduce new medicinal plants to develop cheaper drugs. Plants still represent a largely untapped source of structurally novel compounds that might serve as the lead for the development of novel drugs [8]. Gas chromatography-mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. GC-MS can also be used in airport security to detect substances in luggage or on human beings. However, fewer reports are available with respect to the pharmacological properties of the plant [9].

Keeping this in view, the present study has been undertaken to investigate the phytochemical constituents present in the ethanol extract of *Calotropis gigantea* leaf.

MATERIALS AND METHODS

Collection of medicinal plant

The Indian medicinal plant *Calotropis gigantea was* collected from the medicinal garden of DPS CPAS Puthuppally Kottayam., Kerala, India.

The plant was authenticated by Rogimon P.Thomas, Assistant professor Department of Botany, CMS College Kottayam (Kerala).

Preparation of Plant extract

The ethanolic extract of leaves of *Calotropis gigantea* was used in the study. The leaves were separated, freed from adhering moisture, dried in sunshade and powdered. The powdered material (32gm)[10] was packed in soxhlet apparatus and extraction was done using 450 ml of ethanol (60-70^{o}C) 56 hours. The extracts were filtered using filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using a rotary flask evaporator, and dried in a vacuum. The ethanolic extract yielded a dark greenish solid residue. The extract was then kept in the sterile bottle, under refrigerated conditions, until further use. The dry weight of the plant extract was obtained by the solvent evaporation and used to determine the concentration in mg/ml. The extract was preserved at 2 to 4° and sent an aliquot quantity for GC-MS analysis.

PHYTOCHEMICAL ANALYSIS OF THE EXTRACT:

A small portion of the dry extracts was subjected to the phytochemical test using Harbourne's (1983) methods to test for alkaloids, tannins, terpenoids, steroids, flavonoids, and glycosides.

1. Test for alkaloids:

About 0.2 g extract warmed with 2% H_2SO_4 for two minutes, filtered and few drops of Dragendroff's reagent added orange-red precipitate indicates the presence of alkaloids. And or filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

2. Test for glycosides: The extracts hydrolyzed with HCl solutions and neutralized with NaOH solutions. A few drops of Fehling solution A and B were added. Red precipitate indicates the presence of glycoside. Another test used was Benedict's test, in which the filtrates were treated with Benedict's reagent and heated gently. Orange-red precipitate indicates the presence of reducing sugars.

3. Test for tannins: Small quantity of extracts mixed with water, heated, filtered and ferric chloride added. A dark green solution indicates the presence of tannins.

4. Test for steroids: 2ml of the extract was taken in a test tube and 2 ml of chloroform and 2 ml of concentrated H_2SO_4 was added, the formation of the reddish-brown ring at the junction of two liquids, shows the presence of steroids

5. Test for flavonoids: Extract of about 0.2 g of the extracts shaken with 5ml of distilled water and then a few drops of 10% lead acetate solution is added. A yellow or dirty white precipitate shows the presence of flavonoids. [11]

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the ethanolic extract showed the presence of alkaloids, glycosides, tannins, steroids, flavonoids.

GC-MS Profiling

Working procedure:

Central instrument unit Thrissur Kerala carried out GC-MS

One-microliter sample was subjected for the study. The Instrument used, Varian, CP-3800 Saturn 2200 GC/MS/MS with factor four VF-5MFcolumn.Oven temperature maintained at 1000C for 1.5 minutes, the temperature gradually increased to2700 c at 50 c per minute, and 1µlitre sample was injected for analysis. Helium gas 99.9 % was used as the carrier gas, the flow rate of carrier gas was1ml per minute sample injected temperature was maintained at 250^0 C and the split ratio is 20 throughout the experiment period. The ionization mass was done with 70 eV. The mass spectra were recorded for the mass range 40-600 m/z for 60 minutes. Identification of compound was based on a comparison of their mass spectra. As the compound separated, on elution through column were detected in electronic signals. The m/z

obtained was calibrated through graph obtained, which was called the mass spectrum graph, which is the fingerprint of the molecule. The identification of the compound was based on the comparisons of their mass spectra with Nist library.

Applications of GC -MS

1) Very minute amounts of a substance can be measured.

2) Various temperature programs can be used to make the readings more meaningful; for example to differentiate between substances that behave similarly during the GC process.

3) Gas Chromatography is used in the separation and analysis of multi-component mixtures such as essential oils, hydrocarbons, and solvents.

4) 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.

5) The most important application area is in pollution studies, forensic work and general trace analysis. [12]

GC-MS chromatogram (*Calotropis gigantea*)

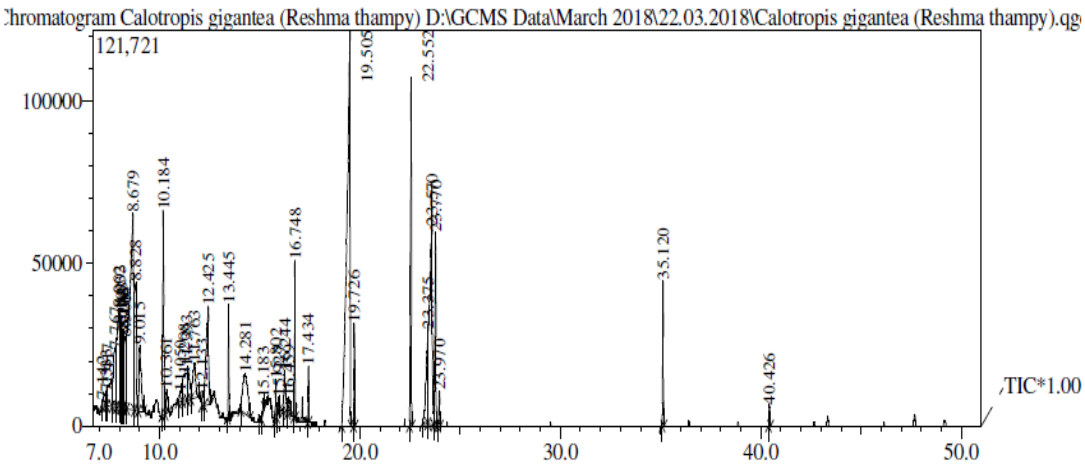

Table number: 1

Peak Report

Peak	R. time	Area	Area %	Height	Height %	Name	Base m/z
1	7.142	27160	0.40	1628	0.15	1-BUTANOL	56.00
2	7.333	16666	0.25	4131	0.38	1-PYRROLIN-N-OXIDE	85.05
3	7.417	108879	1.61	6149	0.56	PENTANE, 2,2-DIMETHYL	57.05
4	7.767	177458	2.62	17849	1.63	1,3-DIOXOLANE,2,4,5-TRIMETHYL-	101.00
5	8.002	273175	4.03	30439	2.78	N-(tert-butyl)UREA	58.00
6	8.033	138157	2.04	30526	2.79	CYCLOBUTANONE,2 METHYL-4-HYDROXY	58.00
7	8.108	48861	0.72	25623	2.34	PROPANENITRILE, 3-(METHYLTHIO)-	61.00
8	8.142	35190	0.52	24267	2.22	GLYCERALDEHYDE	61.00
9	8.175	46881	0.69	23750	2.17	PENTABORANE(11)	62.05
10	8.206	66295	0.98	24256	2.22	ETHYNE, DIFLUORO	62.00
11	8.300	154202	2.28	22008	2.01	D1-METHANAMINE, N-METHOXY-	62.05
12	8.679	890088	13.14	60500	5.53	2-OXOPENTANEDIOIC SCID	101.00
13	8.828	273761	4.04	39379	3.60	GLYCERIN	61.00
14	9.015	146089	2.16	20159	1.84	BENZOFURAN, 2,3-DIHYDRO	120.05
15	10.184	227746	3.36	63068	5.77	2-METHOXY-4-VINYLPHENOL	150.05
16	10.361	38099	0.56	7435	0.68	L-LYXONIC ACID, 5-DEOXY-, GAMMA.-LACTONE	70.05
17	11.050	32751	0.48	3069	0.28	BUTANE,2-NITRO	57.00
18	11.208	109592	1.62	8004	0.73	OXIRANE,(BUTOXYMETHYL)-	57.00
19	11.393	83414	1.23	10191	0.93	ACETALDEHYDE, SEMICARBAZONE	60.00
20	11.763	119017	1.76	10769	0.99	PROPANENITRILE,2,2-DIMETHYL	84.05
21	12.133	21667	0.32	4524	0.41	NITROUSACID, BUTYL ESTER	60.00
22	12.425	280763	4.14	30293	2.77	2-PIPERIDINEMETHANOL, ALPHA-ETHYL-	84.05
23	13.445	84311	1.24	35420	3.24	3’,5’-DIMETHYLACETOPHENONE	180.00
24	14.281	20989	3.10	12468	1.14	BETA-D-GLUCOPYRANOSE,1,6	60.00

						ANHYDRO	
25	15.183	12852	0.19	3867	0.35	2-PENTENE,3-METHYL-,(Z)-	69.05
26	15.802	32054	0.47	12307	1.13	NONANOIC ACID	73.00
27	15.927	13413	0.20	4827	0.44	TRIPROPYLBORANE	97.05
28	16.244	64886	0.96	12539	1.15	2,6,6-TRIMETHYL-BICYCLO(3,1,1)HEPT-3-YLAMINE	98.05
29	16.459	29042	0.43	5342	0.49	DIMETHYL(3-TERT-BUTYLACETOXY)-3-METHYL-2-OXOBUTYL)PHOSPHONATE(18)O	124.05
30	16.748	87956	1.30	48324	4.42	NEOPHYTADIENE	68.05
31	17.434	29558	0.44	17436	1.60	3-NONEN-1-OL,(Z)	55.00
32	19.505	133430	19.70	121721	11.14	HEXADECAOIC ACID	73.00
33	19.726	75720	1.12	31585	2.89	HEXADECANOIC ACID, ETHYL ESTER	88.05
34	22.552	342102	5.05	107105	9.80	PHYTOL	71.05
35	23.357	175223	2.59	29246	2.68	1,1’-BICYCLOPENTYL	67.05
36	23.570	621755	9.18	61313	5.61	9,12,15-OCTADECATRIENAL	79.05
37	23.770	190371	2.81	59451	5.44	9,12,15-OCTADECATRIENOIC ACID(Z,Z,Z)-	79.05
38	23.970	35177	0.52	10779	0.99	PENTACHLOROBROMOBENZENE	55.05
39	35.120	97074	1.43	44867	4.10	UNKNOWN TERPENE IN GRAPES	69.05
40	40.426	22345	0.33	6451	0.59	DI-ALPHA-TOCOPHEROL	165.05
		6773775	100.00	1093065	100.00		

Table number: 2

Activity of bioactive compounds identified in ethanolic extract of *Calotropis gigantea*[13.14]

Serial number	Compound name	Activity
1	Hexadecanoic acid, Ethyl ester	Hemolytic, Antioxidant, Flavor, Alpha reductase inhibitor
2	Phytol	Anti-inflammatory, Anticholesteremic
3	9,12,15 – Octadecatrienoic acid, (z,z,z)	Anti-arthritic, Anti-inflammatory, Anti eczemic,Anti acne, Cancer preventive
4	di-alpha-Tocopherol	Vitamin E
5	Pentachlorobromobenzene	Inflammation, Wounds, Psoriasis
6	1,1-Bicyclopentyl	Eczema, Psoriasis, Seborrheoeic dermatitis
7	3-Nonen-1-ol,(z)-	Murine leukemia
8	Acetaldehyde semicarbazone	Antiviral, Anticancer
9	Neophytadiene	Antiseptic, Anti-inflammatory.
10	Beta-D-Glucopyranose, 1,6-anhydro	Anti-microbial
11	Nonionic acid	Anti-microbial
12	Oxirane, (butoxymethyl)-	Anti-viral
13	Benzofuran,2,3-dihydro	Anti-inflammatory, Anti-tumor, Anti-coagulant, Alzimers disease inhibitor
14	2-oxopentanedioic acid	Immunomodulator
15	2-Methoxy-4-vinylphenol	Anti-cancer, Anti- diabetic, Anti-oxidant
16	Unknown terpenes	Anti-inflammatory, Expectorant

GC -MS analysis has been found to be an ideal technique for the analysis of volatile and semi-volatile bioactive compounds. The total number of the compound identified in the ethanolic extract was 40. The GC-MS retention time and percentage peak of the individual compounds were demonstrated. It contains terpenoids, saponins, flavonoids, alkaloids, steroids. The major Phytoconstituents present in ethanolic extract was 9,12,15 –

octadecatrienoic acid(z,z,z)- retention time 23.570 peak area 9.18% , Hexadecanoic acid, Ethyl ester retention time 19.505 peak area 19.70% , Phytol retention time 22.552 peak area 5.05% , Unknown terpenes retention time 35.120 peak area 1.43% ,Oxirane (butoxymethyl)- retention time 11.208 peak area 1.62%, di-alpha-Tocopherol retention time 40.426 peak area 0.33% , Pentachlorobromobenzene retention time 23.970 peak area 0.52% , Neophytadiene retention time 16.748, peak area 1.30%.

CONCLUSION

Now a day the identification of bioactive compounds from the medicinal plant has increased. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. The ethanolic extract of *Calotropis gigantea* frequently used as a natural remedy for many diseases. The identification of this study is based on the peak area of the compound (which represents the percentage of that compound). In the present study, 40 compounds were identified from leaves extract of *Calotropis gigantea* using ethanol as a solvent. However, the isolation of individual phytochemical constituents may proceed to find a novel drug. Therefore, the GC-MS method is a direct and fast analytical approach for identification of terpenoids, glycosides, alkaloids, and steroids and only a few grams of plant material is required. The results reveal that the extract has a quite number of chemical constituents, which may be responsible for many pharmacological activities. Further studies are needed on this extracts in order to isolate, identify, characterize and elucidate the structure of these compound.

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