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Phytochemical Evaluation, GC-MS Analysis of Phytoactive Compounds and Antibacterial Activity Studies from *Calotropis gigantea*

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Abstract

Calotropis gigantea of Asclepiadacea family commonly referred as a perennial herb with a long history of use in traditional medicines. *Calotropis gigantea* [Asclepiadaceae] is a hoary, laticiferous shrubs tree, also known as "the milkweed". *Calotropis gigantea* contain chemical constituents such as cardenolides, flavonoids, terpenes, pregnanes and a non-protein amino acid and much more therapeutic uses and paves an essential way for drug development. The present investigation deals with the process of determining the phytocomponents, anti-microbial activity, GC-MS analysis with different extracts of *Calotropis gigantean* flower. The crude extracts of the sample showed good impact on its phytoconstituents such as steroids, terpenoids, tannins, saponins etc. The antimicrobial activity was performed against certain bacterial and fungal species for determing its inhibitory effect. The GC-MS analysis framed a complex of wide range of compounds present in the sample with different therapeutic uses. Thus the present study results provide a promising way for developing wide range of therapeutic agents.

Keywords: Phytochemical, Anti-microbial, Traditional plant, GC-MS.

INTRODUCTION

Herbal medicines have been used from the ancient times to the present day seems to be the effective source of traditional & modern medicines which are useful for primary health care[1]. Today, traditional medical practices have been recognized by the World Health Organization [WHO] as a building block of primary health care[2]. Herbs and plants have been in use as a source of therapeutic compounds in traditional medicinal system since ancient time[3]. There is a continuous need of the development of new effective antimicrobial drugs because of the emergence of new infectious diseases and drug resistance.

Medicinal plants are of great importance to the health of individual and communities. The medicinal value of these plants lies in some chemically active substances that produce a definite physiological action on human body [4]. Nowadays synthetic drugs are very expensive to develop and the cost of development ranges from 0.5 to 5 million dollars. On contradiction to this many medicines of plant origin had been used since long time without any adverse effects[5]. One such plant is *Calotropis gigantean which* is a common waste land weed and also known as giant milk weed [6].

Thus this traditional plant was considered to be drought resistant, salt tolerant to a relatively high degree, grows wild up to 900 meters throughout the country and prefers disturbed sandy soils with mean annual rainfall 300-400 mm [7]. It is a native of India, China and Malaysia and distributed in the following countries: Afghanistan, Algeria, Iran, Iraq, Israel, Kenya, Kuwait, Lebanon, Libyan, Arab, Mali, Mauritania, morocco, Mozambique, Myanmar, Nepal, Niger, Nigeria, Oman, Pakistan, Saudi Arabia[8].

As a part of its morphological identification, its stems are erect, up to 20 cm in diameter [9]. The leaves are broadly elliptical to oblong- obovate in shape, with the size of 9-20 cm x 6-12.5 cm. The cymes are 5-12.5 cm in diameter [10]. The inflorescence stalk is between 5-12 cm long, the stalk of an individual flower is 2.5-4 cm long[11]. Sepal lobes are broadly egg shaped with a size of 4-6 mm x 2-3 mm. Petal is 2.5-4 cm in diameter [12].

Calotropis plant parts have adequate pharmacological activity such as analgesic activity, cytotoxic activity, anti-diarrheal activity, anti-candida activity, anti-bacterial activity, antioxidant activity, anti-pyretic activity, cytotoxic activity, wound healing activity etc., these are mainly due to the active components present in the plant[13].

Moreover every part of this plant have been extensively used for various ailments and their diverse medicinal value are mainly because of the active constituents present in them such as alkaloids, steroids, terpenoids, flavanoids, saponins, proteins and aminoacids etc. They were identified by means of phytochemical analysis which paves an essential way for identifying these components[14]. Anti-microbial activity was performed to evaluate the effectiveness of *Calotropis gigantea* and their pharmaceutical values on some selected pathogens through their inhibitory effect against them. Gas chromatography Mass spectroscopy analysis was performed to identify the effective compounds present in *Calotropis gigantea*, a high defined technical system which is the most commonly used technique for the identification of certain phytocompounds and some unknown organic compounds from the pool of compounds available through their interpretation with respective to the spectra[15]. Thus the present study results provide a promising way for developing wide range of therapeutic agents.

MATERIALS AND METHODS

Calotropis gigantea flower were collected from the local area, Trichy market, Tamilnadu. The sample was completely cleansed with tap water and allowed to shade dry at room temperature. Then they were coarsely powdered by means of mechanical grinder.

Preparation of plant extract

5g of air dried powdered plant material was mixed in a conical flask with 100ml of warm water and different solvents like acetone, petroleum ether and chloroform then

shaken at 120rpm for 30 minutes and kept for 24h. After 24h, the extracts were filtered rapidly through four layers of gauge and then by a more delicate Whatman no.1 filter paper [125mm]. The resulting filtrate was then concentrated in a rotary evaporator for 24 hours. Concentrated extract was preserved in labeled sterile, airtight bottles at 4°C in refrigerator. These extract were used for experimental analysis.

Phytochemical screening

The flower extracts of *Calotropis gigantea* soaked in different solvents was analyzed for the presence of alkaloids, Tannins, Saponins, Phenolic compound, Steroids, Carbohydrates, Amino acid and Protein by the following procedure.

Test for alkaloids

To 2-3ml of test solution a few drops of wagner's reagent is added. Reddish brown color precipitates indicates the presence of alkaloids.

Test for steroids

To 2ml of extract, 2ml of acetic acid anhydride and 2ml of concentrated sulfuric acid were added. Brown ring coloration of the solution indicates the presence of steroids.

Test for terpenoids

To test solution, 2ml of chloroform and 1ml of concentrated sulfuric acid were added carefully to form a layer. A Reddish brown coloration of the interface indicates the presence of terpenoids.

Test for flavonoid

To 1ml of extract, a few ml of ammonia solution and concentrated sulfuric acid were added. Formation of intense yellow color in the flower extract indicates the presence of flavonoids.

Test for saponins

To 1 ml of test solution add few ml of sodium bicarbonate and shake well. Honey comb like froth indicates the presence of saponins.

Test for tannins

To one ml of extract, few ml of ferric chloride solution were added. Black/Blue and brownish green coloration of the solution indicates the presence of tannins.

Test for phenolic compound

To one ml test solution, 2ml of water and 10% aqueous ferric chloride solution were added. Blue or green coloration of the solution indicates the presence of phenolic compound.

Test for aminoacid

Dissolved a small quality of the extract in few ml of test solution and add 5% of ninhydrin reagent. Purple and bluish color indicates the amino acid.

Test for protein

To one ml of extract, one ml of concentrated nitric acid was added which results in the formation of white precipitate. Then it was boiled with 20% of sodium hydroxide and ammonia. Appearance of orange color shows the presence of proteins.

Test for carbohydrates

1 ml of extract is boiled with Barfoad's reagent. Appearance of reddish brown precipitate shows the presence of carbohydrates.

Anti-microbial activity

The crude extracts obtained from the plant sample were studied for its inhibitory action against *E.coli, Klebsiella. sp, Streptococcus. sp* and *Pseudomonas. sp*. Agar well diffusion method was performed and the zone of inhibition was measured. Sterilized Muller – Hinton agar medium 20 ml was poured into a sterile petriplate. Then the plate was covered and allowed to solidify. The sterile cotton swab was dipped into the culture suspension of bacteria. The agar surface of each plate was inoculated by swabbing. A sterile disc with different concentration of extracts [25µl, 50µl, 75µl, 100µl] was placed near the edge of the agar surface of the inoculated plates. All plates were incubated at 37°C for 24 hours. The diameter of the zones of inhibition appearing around the disc were measured to the nearest millimeter [mm] and recorded. The same procedure was followed for anti-fungal activity also.

Gas chromatography – mass spectrophotometer

GC-MS analysis of $CHCl_3$ and EtOAc fractions have been performed on a Hewlett Packard HP 6890 Gas Chromatography with Hewlett Packard 5973 mass spectrometer system equipped with a DB -5 capillary column [30 m x 0.25 mm id, film thickness 0.25 µm]. The oven temperature has programmed from 70- 240°C at the rate of 5°C/min. The ion source has set at 240 °C and electron ionization at 70 eV. Helium is used as the carrier gas at a flow rate of 1 mL/min. Scanning range was 35 to 425 amu. Interpretation of the mass spectrum of the unknown part has been 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 have been found out.

RESULTS

Preliminary phytochemical screening

Different extract of *Calotropis gigantea* showed the presence of various phytochemical components. The components such as alkaloids, steroids, terponoids, flavonoids, aminoacids, saponins, proteins and carbohydrates were considered to be present .Alkaloids and steroids were present in almost all the extracts. Tannins and phenolic compounds were absent in all samples refer toTable-1.

Anti-bacterial activity

The maximum level of antagonist was found in chloroform and acetone extracts of *Calotropis gigantea* and followed by aqueous and no results were found in petroleum ether against certain bacterial species such as *E.coli, Klebsiella. sp, Streptococcus. sp, Pseudomonas.sp.* refer to Table - 2.

Antifungal activity

Chloroform, petroleum ether, aqueous and acetone extract of the flowers were also tested for its antifungal activity against fungal species like *Aspergillus sp* and *yeasts sp*. In this case gradual decrease in growth was observed with increase in concentration of the extracts refer to Table-3.

Identification of phytoactive compounds by GC-MS

The results obtained from the GC-MS analysis paved an essential way for the identification of more than 60 different compounds refer to Table - 4. The components

present in them were detected by means of their retention time refer to Figure-1. A wide range of components that possess various pharmacological activities were also determined and gently tabulated refer to Table - 5.

Table 1: Phytochemical screening of flowers of *Calotropis gigantea*

Compounds	Acetone	Aqueous	Chloroform	Petroleum Ether
Alkaloids	+++	+++	+++	+++
Tannins	-	-	-	-
Saponins	+	+	+	+
Phenolic compounds	-	-	-	-
Steroids	++	+	+	+
Flavonoids	+	-	-	-
Terponoids	++	-	++	++
Amino acid	+	+	+	+
Carbohydrates	+	+	+	+
Protein	+	+	+	+

Table 2: Antibacterial Activity of *Calotropis gigantea*

Plant extract	Concentration	*E.coli*	*Kelbsiella*	*Streptococcus Sp*	*Pseudomonas Sp*
Acetone	25µl	3	8	4	3
	50µl	6	5	2	5
	75µl	2	3	6	8
	100µl	4	4	3	5
Chloroform	25µl	5	5	5	4
	50µl	4	7	3	6
	75µl	3	4	3	8
	100µl	5	4	7	5
Petroleum ether	25µl	-	-	-	-
	50µl	-	-	-	-
	75µl	-	-	-	-
	100µl	-	-	-	-
Aqueous	25µl	4	-	3	-
	50µl	5	-	3	-
	75µl	3	-	6	-
	100µl	2	-	4	-

Table 3: Anti-fungal activity of *Calotropis gigantea*

Plant extract	Concentration	*Aspergillus sp*	*Yeast*
Acetone	25µL	-	6
	50µL	-	3
	75µL	-	5
	100µL	-	4
Chlorofrom	25µL	2	3
	50µL	2	4
	75µL	3	2
	100µL	6	5
Petroleum ether	25µL	3	6
	50µL	10	2
	75µL	4	3
	100µL	6	2
Aqueous	25µL	3	-
	50µL	2	-
	75µL	4	-
	100µL	3	-

Table 4: GC-MS analysis of *Calotropis gigantea*

Peak	Start time	End time	Component
1	4.025	4.12	2-FURANMETHANOL
2	4.35	4.44	1-BUTANOL, 3-METHYL-, ACETATE
3	4.625	4.79	BICYCLO[4.2.0]OCTA-1,3,5-TRIENE
4	4.79	4.855	1-PROPENE, 1-CHLORO-3-[[2-METHYL-2-PROPENYL]OXY
5	4.855	4.91	Methoxyacetaldehyde diethyl acetal
6	5.27	5.365	2-Cyclopenten-1-one
7	6.345	6.39	SILICIC ACID [H4SIO4], TETRAETHYL ESTER
8	6.39	6.47	2,4-Dihydroxy-2,5-dimethyl-3[2H]-furan-3-one
9	6.62	6.725	2-PROPENYL FORMATE
10	6.725	6.785	BENZENE, 1,3,5-TRIMETHYL
11	6.785	6.835	HEXANOIC ACID, ETHYL ESTER
12	7.815	7.895	2-[3-Carbethoxypropionamide]-3,4-dicarbethoxy-1- benzyloxymethyl pyrrole
13	8.655	8.77	Thymine
14	10.17	10.25	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl
15	11.305	11.39	1-Heptanol, 3-methyl
16	12.475	12.615	3-[DIDEUTEROMETHOXYMETHOXY]-1-OCTENE
17	14.07	14.185	2-METHOXY-4-VINYLPHENOL
18	15.71	15.795	trans-Linalool oxide [furanoid]
19	15.795	15.96	1-UNDECANOL
20	15.995	16.15	BENZENE, 1-CHLORO-4-METHOXY
21	16.93	17.13	D-Fructose, 1,3,6-trideoxy-3,6
22	19.34	19,44	N,N-BIS[2-HYDROXYETHYL]DODECANAMIDE
23	20	20.1	1-TETRADECANOL
24	20.495	20.835	1,3,4,5-TETRAHYDROXYCYCLOHEXANECARBOXYLIC ACID #
25	21.205	21.275	MOME INOSITOL
26	21.3	21.58	MOME INOSITOL
27	22.375	22.495	BENZENE, ETHYLPHENOXY
28	23.09	23.1	TETRADECANOIC ACID
29	23.715	23.815	1-Tetradecanol
30	23.925	24.02	Phenol, 2-[1-phenylethyl]-
31	26.52	26.75	n-Hexadecanoic acid
32	27.08	27.18	HEXADECANOIC ACID, ETHYL ESTER
33	28.895	28.98	Sulfurous acid, nonyl pentyl ester
34	29.205	29.315	9,12-Octadecadienoic acid [Z,Z]
35	29.315	29.49	9-Octadecenoic acid, 1,2,3-propanetriyl ester, [E,E,E]
36	29.49	29.565	Cyclohexane, 1,1'-[oxydi-2,1-ethanediyl]bis-
37	29.64	29.755	OCTADECANOIC ACID
38	29.755	29.865	Ethyl Oleate
39	30.365	30.47	Heptadecyl acetate
40	31.105	31.295	Ethanone, 1-[3-[[5-[3,3-dimethyloxiranyl]-3-methyl-2- pentenyl]oxy]phenyl]
41	31.705	31.8	Dodecane, 2-methyl
42	32.085	32.18	Pentadecanal
43	32.085	33.195	Phenol, 2,4-bis[1-phenylethyl]
44	33.325	33.344	Phenol, 2,4-bis[1-phenylethyl]
45	33.44	33.575	14-Heptadecenal
46	34.095	34.22	Phenol, 2,4-bis[1-phenylethyl]-
47	34.395	34.52	2-Methylhexacosane
48	34.52	34.59	Hexadecanoic acid, 2-hydroxy-1-[hydroxymethyl]ethyl ester
49	34.59	34.75	2,5-ETHANO-2H-AZOCINO[4,3-B]INDOLE, 4-ETHYLIDENE-1,3,4,5,6,7-HEXAH
50	34.75	34.94	Henicosanal
51	35.335	35.43	2-AMINOETHANETHIOL HYDROGEN SULFATE [ESTER]
52	35.69	35.78	TRITRIACONTANE
53	35.78	35.94	2H-1-BENZOPYRAN-6-OL,3,4-DIHYDRO-2,5,7,8-TETRAMETHYL-2-[4,8,12-TRIMETHYLTRIDECYL]-, [2R-[2R*[4R*,8R*]-
54	36.74	36.815	8-Tetradecyn-1-ol acetate
55	36.815	36.925	9-Octadecenoic acid, 1,2,3-propanetriyl ester, [E,E,E]
56	36.925	37.03	Eicosane
57	37.03	37.1	Propane-1,3-diamine, N,N'-bis[3-ethoxy-1-methyl-3-oxo-1- propenyl]
58	37.115	37.25	Octadecanoic acid, 2,3-dihydroxypropyl ester
59	38.09	38.195	2-Methylhexacosane
60	38.195	38.435	Squalene
61	38.485	38.565	Cyclohexanamine, 3,6-diethenyl-2,2-dimethyl-N-[2- methylpropylidene]
62	38.88	39.075	Stigmasterol
63	39.295	39.455	TETRAPENTACONTANE
64	39.565	39.67	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23- hexamethyl-, [all-E]
65	39.99	40.095	IBOGAMINE, 12-METHOXY

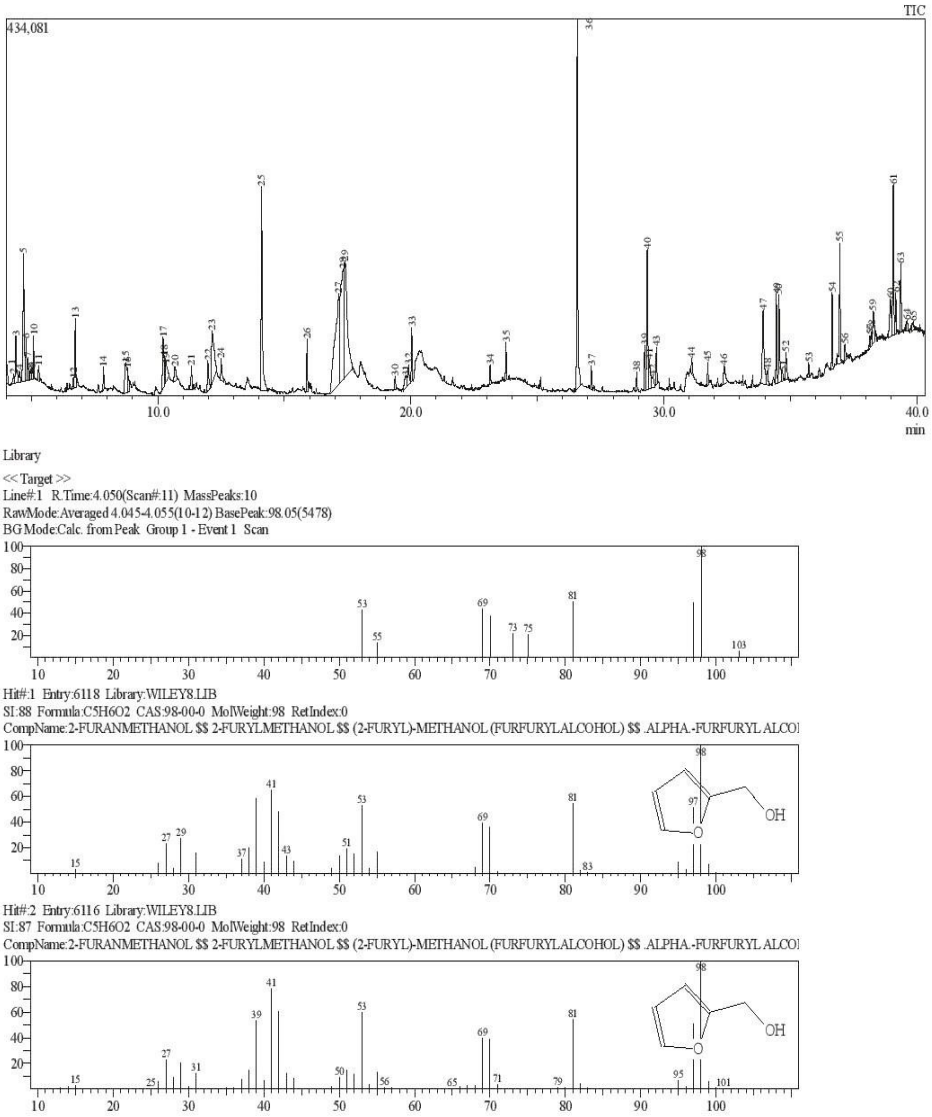

Fig. 1: Graphical representation of gas chromatogram and mass spectrometry (GC-MS)

Table 5: Biological activity of various compounds of *Calotropis gigantea*

S.no	Compounds	Activity
1.	2-FURANMETHANOL	Anti-bacterial activity
2.	1-BUTANOL, 3-METHYL-, ACETATE	Anti-microbial activity
3.	BICYCLO[4.2.0]OCTA-1,3,5-TRIENE	Anti-cancer activity
4.	Hexadecanoic acid, ethyl ester	Anti-oxidant activity and flavouring agent
5.	Stigmasterol	Anti-angiogenic and anti-cancer activity
6.	Squalene	Anti-oxidant and anti-tumor property
7.	SILICIC ACID [H4SIO4], TETRAETHYL ESTER	Bone mineralization
8.	2,4-Dihydroxy-2,5-dimethyl-3[2H]-furan-3-one	Flavour analysis and antimicrobial activity
9.	Eicosane	Anti-fungal activity
10.	Henicosanal	Anti- microbial activity
11.	Mome inositol	Anti-alopecic, anti-cirrhotic and anti-neuropathic activity

DISCUSSION

Pramila et al evaluated the phytocomponents present in the root and latex extracts of the plant sample, the root aqueous extracts showed good range of components such as alkaloids, glycosides, tannins, saponin and flavanoids whereas the latex acetone extract showed quiet higher range of alkaloids, glycosides, tannins, saponins, flavanoids, terpenoids[16]. The present study deals with the process of evaluating the flowers of *Calotropis gigantea* , acetone extract showed the presence of those components discussed above when compared with other.

Gaurav kumar *et al* performed the antibacterial activity for aqueous extract of C*alotropis gigantea* leaves against *Staphylococcus aureus* and *Klebsiella .sp*, maximum inhibitory effect was found in *Staphylococcus aureus* whereas as the least inhibitory effect was in *Klebsiella .sp* [17].

Shopna *et al* evaluated the antimicrobial activity of *Calotropis gigantea* that showed maximum inhibition in chloroform and ethyl acetate extracts than methanol extract and among the tested organisms *Staphylococcus aureus* showed higher resistant followed by *E.coli* [18], thus the present study reveals that maximum effect was found in chloroform and acetone followed by aqueous and no effect on petroleum ether. The antifungal activity reveals that the present sample had significant effect in chloroform and petroleum ether extracts followed by aqueous and acetone extract of the tested organisms, *Aspergillus.sp* shows good result when compared with yeast.

Shalini sharma *et al* evaluated the active components present in the leaves and latex of *Calotropis gigantea* using methanol solvents, totally 46 compounds were identified among that 22 were from latex and 24 from leaves of the sample[19]. The current study also enfolds the identification of wide range of bioactive compounds that may involve in various other biological activity and thus totally 64 compounds were discovered from the acetone extract of this sample.

CONCLUSION

The present study clearly indicates that the acetone, aqueous, chloroform, petroleum ether extracts of *Calotropis gigantea* potentially exhibited wide range of phytoconstituents. Generally this sort of plant constituents paves an essential way for treating widespread human ailments as part of a remedial source. Amidst the variety of secondary metabolites found in these sample alkaloids, glycosides, terpenoids, flavanoids played a unanimous role which possesses anti-bacterial, anti-inflammatory and analgesic property. Further, among the pool of biologically active compounds nearly 65 compounds that possess predominant medicinal properties were determined through GC-MS analysis. These all served as evidence which strongly states that *Calotropis gigantea* would play a tremendous role as folk medicine. Further, research is needed to analyse those biologically active phyto components and their mode of action that will pave an essential way for utilizing in the world of pharmacy with nil side effects.

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Conflict of Interests -Declared none

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