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Preliminary Phytochemical Screening and GC- MS Profiling of Ethanolic Flower Extract of *Calotropis gigantea* Linn. (Apocynaceae)

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ABSTRACT

Calotropis gigantea Linn is popularly known as the swallow-wort or milkweed and is used as one of the most important drug in Traditional System of Medicine to treat various ailments. The aim of this study is to screen the phytochemicals present in the ethanolic flower extract of *Calotropis gigantea* and further analysis of the components present in it by GC-MS analysis. Ten grams of flower power was sequentially extracted by ethanol. The results showed the presence of phytochemical compounds of alkaloids, tannins, phenol, flavonoids, sterols, anthraquinones, proteins and quinones in the flower extract. The GC-MS analysis of the ethanolic extract revealed the presence of 14 major compounds. This study forms a basis for the biological characterization and importance of the compounds identified and creates a platform to screen many bioactive components to treat many diseases.

Keywords: *Calotropis gigantea*, Phytochemical Screening, GC-MS Analysis.

1. Introduction

The Indian subcontinent is rich in medicinal plants and is one of the richest countries in terms of genetic diversity of medicinal plants. It exhibits a wide range in topography and climate. Moreover the agro climatic conditions are conducive for introducing and domesticating new exotic plant varieties [1]. Several plants have been used in folklore medicine. The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare. Medicinal plants are of great importance to the health of individual and communities. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on human body.

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 sources of therapeutic agents. At presents the demand for herbal or medicinal plant products has increased significantly.

In the recent past, there has been 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 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]. Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway. Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health facilities and services are inadequate [6].

Calotropis gigantea Linn (Apocynaceae) is a glabrous or hoary, laticiferous shrubs or small trees, about 3-4 m tall commonly known as the swallow-wort or milkweed. Its stems are erect, up to 20 cm in diameter. The leaves are broadly elliptical to oblong-obovate in shape, with the size of 9-20 cm x 6-12.5 cm but subsessile. The cymes are 5-12.5 cm in diameter. The inflorescence stalk is between 5-12 cm long, the stalk of an individual flower is 2.5-4 cm long. 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. It has clusters of waxy flowers that are either white or lavender in colour. Each flower consists of five pointed petals and a small, elegant "crown" rising from the center, which holds the stamens. The plant has oval, light green leaves and milky stem [7]. The flower of the plant contains the cardiac glycosides, calotopin, uscharin, calotoxin, calactin, uscharidin and gigantin. The flower also contains the protease calotropin DI and DII and calotropin FI and FII [8].

Nowadays synthetic drugs are very expensive to develop and whose 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. 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 [9]. As a result 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 large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs [10].

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

2. Materials and Methods

2.1 Collections of test Materials

Fully developed flowers of the plant namely *Calotropis gigantea* were collected from the natural habitat of Coimbatore locale of $11^0$1'N $76^0$56'S Longitude.

2.2 Preparation of Flower Powder and Extract

Fresh flowers were collected washed in water and dried at room temperature. After drying for 2 to 3 weeks, the flowers were ground in an electric pulverizer to get the powder. 10 g of the flower powder was weighed using an electronic balance (Denver XS-210) and made into packets using Zerohedge filter paper (A Grade, SD's). The powder was subjected to extraction with 500 ml of ethanol for 8 h using a Soxhlet apparatus [11,12]. The flower extracts thus obtained were concentrated by distillation and dried by evaporation in a water bath at 40 ^{0}C. The residue thus obtained was stored in tightly closed glass vials in the refrigerator for further use.

2.3 Phytochemical screening

2.3.1 Test for Alkaloids

2.3.1.1 Mayer's test

A fraction of extract was treated with Mayer's test reagent (1.36 gm of mercuric chloride and 5 gm of potassium iodide in 100 ml of water) and observed for the formation of cream coloured precipitate.

2.3.1.2 Wagner's test

A fraction of extract was treated with Wagner's reagent (1.27 gm of iodine and 2 gm of potassium iodide in 100 ml water) and observed for the formation of reddish brown colour precipitate.

2.3.1.3 Hager's test

A few ml of extract was treated with Hager's reagent (saturated aqueous solution of picric acid) and observed for the formation of prominent yellow precipitate.

2.3.2 Test for Tannins

2.3.2.1 Acetic Acid Test

The extract was treated with acetic acid solutions and observed for the formation of red colour solution.

2.3.2.2 Dilute HNO_3 Test

The extract was treated with dil. HNO_3. The extract turns from reddish to yellow colour which indicates the presence of tannins.

2.3.3 Test for Phenols

2.3.3.1 Ferric chloride test

The fraction of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black colour

2.3.3.2 Liebermann's test

The extract was heated with sodium nitrite, added H_2SO_4 solution diluted with water and excess of dilute NaOH was added and observed for the formation of deep red or green or blue colour.

2.3.4 Test for Flavonoids

2.3.4.1 NaOH test

A small amount of extract was treated with aqueous NaOH and HCl, observed for the formation of yellow orange colour.

2.3.4.2 H_2SO_4 test

A fraction of the extract was treated with concentrated H_2SO_4 and observed for the formation of orange colour.

2.3.5 Test for Sterols

2.3.5.1 Liebermann-Burchard test

Extract (1 ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark pink or red colour.

2.3.6 Test for Terpenoids

2.3.6.1 Liebermann-Burchard test

Extract (1 ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark green colour.

2.3.7 Test for Saponins

2.3.7.1 Foam Test

The extract or dry powder was vigourosly shaken with water and observed for the formation of persistent foam.

2.3.8 Test for Anthraquinones

2.3.8.1 Borntrager's test

About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia and observed for the formation of pink or deep red colouration of aqueous layer.

2.3.9 Test for Proteins

2.3.9.1 Ninhydrin test (Aqueous)

The extract was treated with aqueous ninhydrin and observed for the presence of blue colour, indicating the presence of amino acid or purple colour indicating the presence of protein.

2.3.9.2 Ninhydrin (acetone)

Ninhydrin was dissolved in acetone; the extract was treated with ninhydrin and observed for the formation of purple colour.

2.3.9.3 Biuret test

The extract was heated in distilled water and filtered. The filtrate was treated with 2% copper sulphate solution, 95% ethanol and potassium hydroxide and observed for the formation of pink ethanolic layer.

2.3.10 Test for Quinones

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

2.4 GC– MS analysis

Mass experiments were performed on GC (T8000 Top CE) combined with Mass Spectrometer (Md 800 FIS ONS). Sample was dissolved in methanol and introduced into the column TR-5-MS capillary standard non-polar by splitless injection system. Ultra high purity helium was introduced as the buffered collision gas with flow rate of 1.0 ml/min. The source temperature for ionization was set at 250 ^{0}C. All the experiments were performed on the positive ion mode.

3. Results and Discussion

The present study carried out on the ethanol flower extracts of *C. gigantea* revealed the presence of medicinally active constituents. The phytochemical constituents of the flower investigated are summarized in Table 1. Alkaloids, tannins, phenol, flavonoids, sterols, anthroquinones, proteins and quinones were present in the flower extract. Terpenoids and saponins were found to be absent. Similar observations were reported by Edeoga *et al.*,[3] in which both *S. acuta* and *T. procumbens* possessed very high levels of alkaloids and flavonoids, and are employed in medicinal uses. In another study Bartholomew *et al.,* [13] revealed the presence of steroids, alkaloids, saponins, tannins, cardiac glycosides, flavonoids, phlobatannins, anthraquinones, and terpenes, while cyanogenic glycosides were absent. The quantitative analysis yielded high levels flavonoids, alkaloids, polyphenols, and moderate levels of tannins and saponins.

Table 1: Phytochemical constituents present in ethanol flower extract of *C. gigantea*

Sl. No	Constituents	Ethanol flower extract of *C. gigantea*
1	Alkaloid	+
2	Tannin	+
3	Phenol	+
4	Flavanoids	+
5	Sterol	+
6	Terpenoids	−
7	Saponin	−
8	Anthraquinones	+
9	Protein	+
10	Quinones	+

The presence of tannins suggests the ability of this plant to play a major role as antidiarrhoeal and antihemorrhagic agent [14], while alkaloids has been implicated for its detoxifying and antihypertensive properties [15,16]. Furthermore, the presence of phenol observed in the ethanolic extract is an indication that the plant might play an important role as dietary antioxidants. Phenolic compounds prevent oxidative damage in living systems [17,18].

To explore the importance of any medicinal plant the initial step is to screen for its phytochemicals, as it gives a broad idea regarding the nature of compounds present in it. In the present study, the flower of *C. gigantea* was preliminarily screened for the phytochemicals. The ethanolic extract was found to be rich in all the phytoconstituents. Earlier report on the phytochemical analysis of ethanolic extract of *Tylophora pauciflora* also revealed the presence of alkaloids, flavonoids, tannins, phenols, saponins, steroids, aminoacids and proteins, oils and fats, terpenoids and carbohydrates [19].

The phytoconstituent rich ethanolic extract of *C. gigantea* flower was subjected to Gas Chromatography-Mass spectrometry (GC-MS) analysis. The result revealed the presence of 14 major compounds (Fig 1 and 2). Similar observation was reported by Nishaa *et al.,* [20] in which the phytoconstituent rich ethanolic extract of *Maranta arundinacea*. L subjected to GC-MS analysis revealed the presence of 49 compounds. Similar work was reported for chemical composition analysis of essential oil of *Curcuma amada* by Vishnupriya *et al.,* [21]. The name, molecular formula and molecular weight of the compounds were given in Table 2.

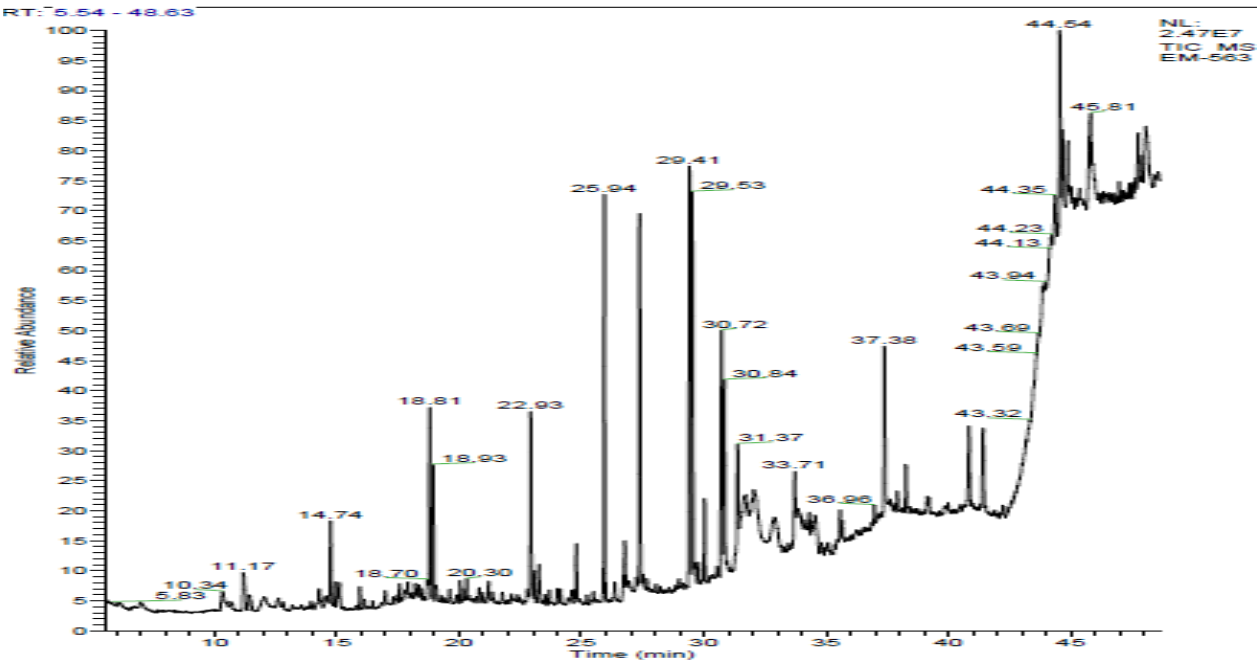

Fig 1: GC-MS Chromatogram of the ethanolic flower extract of *C. gigantea*

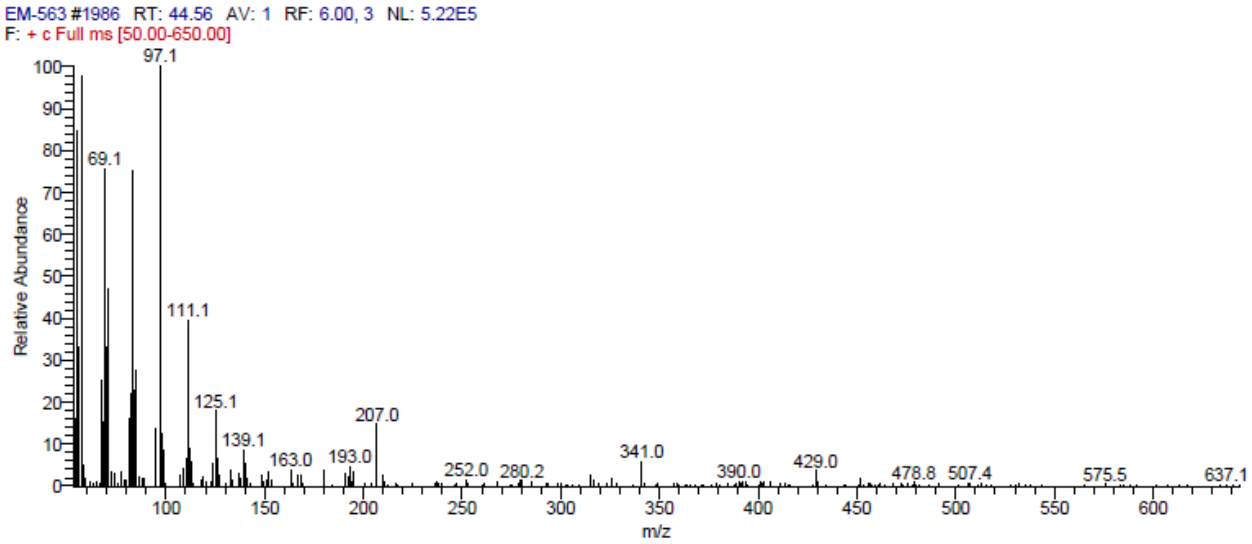

Fig 2: Mass spectrum at (RT: 44.56)

Table 2: Phytocomponents identified in the ethanolic extract of the flower of *Calotropis gigantea* by GC-MS analysis

Sl.No	RT	Compound name	Molecular formula	Molecular weight
1	14.74	1-Tetradecene	$C_{14}H_{28}$	196
2	18.81	3-ethyl-1-tetradecene	$C_{16}H_{32}$	224
3	22.93	3-Octadecene, (E)	$C_{18}H_{36}$	252
4	25.94	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270
5	29.41	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294
6	30.72	Ethyl linoleate	$C_{20}H_{36}O_2$	308
7	31.37	Methyl 17-methyl-octadecanoate	$C_{20}H_{40}O_2$	312
8	33.71	Docosane	$C_{22}H_{46}$	310
9	33.71	Nonacosane	$C_{29}H_{60}$	408
10	37.38	Tricosane	$C_{23}H_{48}$	324
11	44.35	4' Methyl-2 Phenylindole	$C_{15}H_{13}N$	207
12	44.54	Heptacosane	$C_{27}H_{56}$	380
13	44.54	Octadecane, 3-ethyl-5-(2-ethylbutyl)	$C_{26}H_{54}$	366
14	45.81	5,12-Naphthacenedione, 8-ethyl-7,8,9,10-tetrahydro-1,6,8,11-tetrahydroxy	$C_{20}H_{18}O_6$	354

The results of the GC-MS analysis provide 14 major peaks determining the presence of phytochemical compounds with different therapeutic activities (Fig 1). The mass spectrum showed the characteristic peaks at (M-14), (M-15), (M-19) (M-28), (M-44) which confirmed the presence of hydrocarbon, methyl, hydroxyl, carbonyl, carboxylic acid functional groups in the extract (Fig 1 and 2). The results lead to the identification of number of compounds from the GC fractions of the ethanolic extract of *C. gigantea*. In another work of Paranthaman *et al* [22] the GC-MS results indicated the presence of twelve phytochemical constituents from ethanolic extract of the leaves of *Amaranthus caudatus.* The presence of various bioactive compounds justifies the use of the flower of *C. gigantea* for various ailments by traditional practitioners.

Efforts in this regard have focused on plants because of their use from historical times and the fact that a good portion of the world's population rely on plants for the treatment of infections and non-infectious diseases. The isolation of individual phytochemical constituents in the plants and subjecting it to pharmacological activity will definitely give fruitful results. The current pioneering study suggests that ethanolic extract is a potent therapeutic agent. It paves the way for the development of several treatment regimens based on this extract. In addition, further research is necessary to purify the active compounds responsible for therapeutic activity.

4. Conclusion

The investigation concluded that the stronger extraction capacity of ethanol could have produced number of active constituents which are responsible for many biological activities. So that it might be utilized for the development of traditional medicines and further investigation is in need to elute novel active compounds from the medicinal plants which may create a new way to treat many incurable diseases. Gas chromatography and mass spectroscopy analysis showed the existence of various compounds with variable chemical structures. At end point it can be concluded that the *in vivo* studies on biological systems can open up new way for natural drugs that can also be employed for clinical trials which may generate successful results in future.

5. References:

1. Mitchell RN, Cotran RS. In: Robinsons Basic Pathology. 7th Ed, Harcourt Pvt. Ltd, New Delhi, India, 2000, 33-42.
2. Aiyelaagbe OO, Osamudiamen MP. Phytochemical screening for active compounds in *Mangifera indica* leaves from Ibadan, Oya State. Plant Sci Res 2009; 2(1):11-13.
3. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal Plants. African Journal of Biotechnology 2005; 4 (7):685-688.
4. Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohani DP, Biyani MK *et al*. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. Phytochemistry 2003; 63:97-104.
5. Sumathi, Parvathi. Antimicrobial activity of some traditional medicinal plants. J Med Plant Res 2010; 4:316-321.
6. Zaidan MRS, Rain NA, Badrul AR. *In vitro* screening of five local medicinal plants for antibacterial activity using disc diffusion method. Trop Biomed 2005; 22:165-170.
7. Carol JP, Jignesh HP, Mayuree AP, Anar JP. A comprehensive review on plant *Calotropis gigantea*. International Journal of Institutional Pharmacy and Life Sciences 2012; 2(2):463-470.
8. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd Ed, Vol. III, International Book Distributors, Dehradun, 1999, 191-192, 420-422, 993-994, 2045-2047.
9. Li RK, Ciblak MA, Nordoff N, Pasarell L, Warnock DW, Mcginnis MR. *In vitro* activities of voriconazole, itraconazole, and amphotericin B against *Blastomyces dermatitidis, Coccidioides immitis,* and *Histoplasma capsulatum*. Antimicrobial. Agents. Chemother 2000; 44 (6):1734-1736.
10. Cowan MM. Plants products antimicrobial agents. Clin Microbial Rev 1999; 14:564-584.
11. Harbourne JB. Phytochemical Methods. Chapman and Hall, Ltd, London, 1973, 49-188.
12. Vogel AI. Text book of practical organic chemistry. The English language book society and Longman, London, 1978, 1368.
13. Bartholomew OI, Maxwell E, Bitrus HJ. Phytochemical Constituents and *in vitro* Antioxidant Capacity of Methanolic Leaf Extract of *Oxytenanthera abyssinica* (A. Rich Murno). European Journal of Medicinal Plants 2013; 3(2):206-217.
14. Asquith TN, Butler LG. Interaction of condensed Tannins with selected proteins. Phytochem 1986; 25(7):591-1593.
15. Trease GE, Evans WC. Pharmacology 11th Ed, Bailliere Tindall Ltd, London, 1989, 60-75.
16. Zee-cheng RK. Anticancer research on Loranthaceae plants. Drugs Future 1997; 22(5):515-530.
17. Block G. The data support a role of antioxidants in reducing cancer risk. Nutr Rev 1992; 50:207-213.
18. Hertog MGL, Feskens EJM. Dietary antioxidant flavonoids and risk of coronary heart disease the Zutphen Elderly Study. Lancet 1993; 342:1007-1011.
19. Starlin T, Raj CA, Ragavendran P, Gopalakrishnan VK. International Research Journal of Pharmacy 2012; 3:180-183.
20. Nishaa, S, Vishnupriya, M, Sasikumar JM, Gopalakrishnan VK. Phytochemical Screening and GC-MS Analysis of Ethanolic Extract of Rhizomes of *Maranta arundinacea* L. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2013; 4(2):52-59.
21. Vishnupriya M, Nishaa S, Sasikumar JM, Darsini DTP, Hephzibah Christabel PH. International Research Journal of Pharmacy 2012; 3: 99-103.
22. Paranthaman R, Praveen KP, Kumaravel S. GC-MS Analysis of Phytochemicals and Simultaneous Determination of Flavonoids in *Amaranthus caudatus* (Sirukeerai) by RP-HPLC. J Anal Bioanal Techniques 2012; 3(5):1000147.