



Original Article

Phytochemical screening of *Calotropis gigantea* by LC- HRMS

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Abstract:

Calotropis gigantea commonly known as 'Shweta Arka' is a highly medicinal drought resistant. Plant contains milky latex in the stem which is used as an antidote for snake poison in dried form. Whereas, dried leaves of the *Calotropis* plant are used as an expectorant and anti-inflammatory for the cure of paralysis and rheumatic pains. It contains pesticidal, insecticidal, fungicidal, anti-bacterial properties. The plant has been reported to be effective in more than hundred human diseases. Various bioactive compounds have been extracted from *C. gigantea* fruit follicle. Present investigation deals with the determination of bioactive constituents from the leaf of *C. gigantea* using LCMS and their comparative analysis. The LCMS analysis revealed the presence of total 22 bioactive compounds with valuable activity. Most of the compounds were found to be similar leaf and latex, but small variation was also observed in their chemical profile. The Compounds identified from the fruit follicle of *C. gigantea* were Cycloalliin, 1-(4-Hydroxy-2-methylpyrimid-5-ylmethyl)-3-(beta-hydroxyethyl) --2-methylpyridinium, 2- Dodecylbenzenesulfonic acid, Rimocidine, Sarcostin, Mycinamicin IV, Lycoperoside D etc. whereas the rest of the compounds were similar in both leaf and latex.

Keywords: *Calotropis gigantea*, phytochemical, fruit follicle, extraction, precipitate.

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Introduction:

Higher plants' potential as a source of novel pharmaceuticals is yet mostly untapped. Only a small portion of the estimated 250,000–500,000 plant species have been studied phytochemically, and an even smaller portion have been subjected to biological or pharmacological screening. Consequently, any phytochemical analysis of only a very small spectrum of a plant's constituents will be visible. In the search for new biologically active compounds, random screening has been particularly successful in the antibiotic field. (Pramila Kori, Prerana Alwa, 2014).

In current work the various extracts of a valuable medicinal plant *Calotropis gigantea* is systematically investigated by phytochemical tests and LCMS which may result into the presence of various bioactive compounds.

***Calotropis gigantea*:**

The various parts of the *C. gigantea* have been used as anticancer, antifertility, and an antidote for snakebites in the ancient system of medicine, among other conditions (Elakkiya, P et.al., 2012). Researchers claim that the plant *C. gigantea* has cytotoxic, antipyretic, and wound-healing properties. This plant is used to treat several conditions such as rheumatism, leprosy, nausea, vomiting, cough, and asthma. If used correctly, this plant's therapeutic benefits can be attributed to a variety of chemical metabolites found in its leaves, roots, flowers, fruits, sap, and other components. The plant might have pharmaceutical benefits. (Gauravkumar et. al., 2010)

It was established that *Calotropis procera* latex is responsible for the larvicidal properties by demonstrating the larvicidal efficacy of the latex against all three significant vector species, including *Ae. aegypti*, *An. stephesi*, and *Cx. quinquefasciatus*, vectors of dengue, malaria, and Lymphatic filariasis, respectively. (Manju singhi, et.al., 2004)

The methanol extract of *Calotropis gigantea* leaves was tested for its in-vitro antibacterial activity against *Salmonella typhi*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Escherichia coli*. This research provided strong evidence that *Calotropis gigantea* had a beneficial impact on *Pseudomonas fluorescens*. (*Calotropis gigantea*.<http://feedpedia.org/node/588>.)

By using the dip method in extract, the anti-feedant efficacy of *Calotropis procera* leaves was observed against third instar *Spodoptera litura* larvae. the highest activity found in the extract of chloroform (Ga. Bakavathiappan, 2012)

Material and Method:

Plant Materials:

1) Collection and identification of plant:

Calotropis gigantea plant fruit follicles was collected manually from the Karad tahasil from the roadside. The plant was authenticated by Department of Botany, Shivaji University, Kolhapur. The voucher specimens (STL 001) were deposited in the department for future reference.



2) Extraction of plant material using solvents:

The fruits of *Calotropis gigantea* are follicular. Fruit formed of single carpel only that contains several seeds, dehisces by suture for the dispersal of seeds by wind. *Calotropis gigantea* fruit follicles were washed properly with running water and kept for drying in shade for a week and then only the follicular covering of fruits were grounded into fine powder in mixer grinder and used for the purpose of extraction. 10 grams of dried powder of follicular covering of the fruits was subjected to Soxhlet extraction with 200 ml of solvents starting from Alcohol, methanol and chloroform.

Soxhlet process was allowed to carry out till the complete exhaustion of sample material use for extraction with the maintenance of temperature below the boiling points of the solvents used. The extract so obtained were kept in rotary evaporator, so the extracts get in the semi solid form.

A small quantity of dry extracts were subjected for the phytochemical tests using Harborne (1983) methods to test for alkaloids, tannins, phenols, saponins, flavanoids and terpenoids.

Test for alkaloids:

About 0.2 g extract warmed with 2% H₂SO₄ for two minutes, filtered and few drops of Dragendorff'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.

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 use 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.

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.

Test for saponins:

About 0.2g of the extracts shaken with 5ml of distilled water and then heated to boil frothing (appearance of creamy mix of small bubbles) shows the presence of saponins.

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.

Test for Phenols:

About 0.5g of extract treated with 3-4 drops of Ferric chloride solution. A bluish black colour appeared, this shows presence of phenols.

Test for Terpenoids:

About 5 ml of extract added with 3 ml of con. H₂SO₄. It gives reddish brown ppt. This shows presence of Terpenoids.



Test for Steroids:

About 2 ml of extract dissolved in 2ml chloroform. Add side by side conc. H₂SO₄ carefully. It gives red ppt. This shows presence of steroids.

Test for Carbohydrates:

2ml of Fehling A and 2ml of Fehling B added into 2 ml of extract . Then mixture is boiled. This gives brick red ppt at bottom of test tube. It shows presence of carbohydrates.

LC-MS:

LC/MS analysis of *C.gigantea* leaves' hexane extract was performed using an Agilent Q-ToF G6540B connected to Agilent 1260 Infinity II HPLC. An alcoholic, methanolic and chloroformic extract (1 mL) was then injected into the LC/MS apparatus. Next, the data were displayed on a column, Agilent Eclipse XDB-C18, 3X150 mm, 3.5 micron (CAM-venture Laboratory, Pune).. LC oven temperature started at 40 °C and was held for 0- 2 min at 5% and then for 25 min with program rate 95 %.. The injector and detector temperatures were set at 300 °C to 350°C respectively. The mass range was scanned from 100 to 1700 amu. The identification of components was based on the comparison of their mass spectra with those of Meltin database.

The best instrument for locating and resolving chemical components in a combination is LC-MS. The active components are separated from the sample by LC. The analytes undergo MS following LC. The molecular weight, amount, structure, and identification of various

sample components are all revealed by the LC/MS results.

System components:

Interfacing LC and MS: *Calotropis gigantea* requires vacuum and a large gas load in order to separate the analytes from the liquid phase, MS just needs a little gas load and can handle roughly 1 mL/min of gas.

API stands for Atmospheric Pressure Ionisation and is a soft ionisation technique that can be used to analyse polar, non-polar, and labile substances. It is useful to analyse samples that contain proteins, polypeptides, and oligonucleotides using API-electrospray (API-ES).

Process:

API-ES is a process of ionization occurs in three basic steps:

- Nebulization and charging
- Desolvation
- Ion evaporation

1) Nebulization:

The nebulizing needle is used to deliver the HPLC effluent. A semi-cylindrical electrode with a high voltage receives the spray. Due to the potential difference between the needle and the electrode, a strong electrical field was produced. This field charges the liquid's surface, creating a spray of charged droplets. Gas flows in a circular pattern, which helps this approach.

2) Desolvation:

The charged sample droplets are drawn to the capillary sampling opening. The droplets and uncharged particles travel away when the nitrogen gas flow reverses.



3) Ion evaporation:

Droplets reach a point where the columbic forces outweigh the cohesive forces as they contract. Until the analytes are desorbed into the gas phase, this process continues. These gas-phase ions enter the low-pressure area and the analyzer through the capillary sampling.

Result and Discussion:

Phytochemical analysis of *Calotropis gigantea* revealed the presence of sterols, flavonoids, alkaloids, saponins, glycosides, tannins, phenols and terpenoids, in the Chloroformic extract of fruit follicle. It is crucially significant as it has the largest number of phytochemicals and secondary metabolites. (Rubi Verma et al.,2019).

Table 01: Qualitative phytochemical analysis of *Calotropis gigantea* fruit follicle extract in chloroform

Sr. No	Test	Alcohol	Methanol	Chloroform
1	Alkaloids	+ve	+ve	+ve
2	Flavanoids	-ve	-ve	+ve
3	Tannins	-ve	-ve	-ve
4	Terpenoids	-ve	+ve	-ve
5	Saponins	-ve	+ve	+ve
6	Steroids	+ve	+ve	-ve
7	Glycosides	-ve	+ve	+ve
9	Phenols	+ve	+ve	+ve

Note - (+) present; (-) Absent

The best instrument for locating and resolving chemical components in a combination is LC-MS. The active components are separated from the sample by LC. The analytes undergo MS following

LC. The molecular weight, amount, structure, and identification of various sample components are all revealed by the LC/MS results.

Table 2: Phytochemicals screening of extracts of Fruit follicle of *Calotropis gigantea* by LC-MS

Sr. No.	Name of compound	Chemical formula	Rt. time	Score	Mass	Importance
1.	Goyaglycoside g	C43 H70 O14	810.4758	95.10	810.4758	Antioxidant Property
2.	2-Dodecylbenzenesulfonic acid	2-C18 H30 O3 S	12.556	93.58	326.1914	Microractors, Industrial production
3.	Mycinamicin IV	C37H61N O11	15.750	93.29	695.4251	A substance that kills or slows the growth of microorganisms , including bacteria, viruses, fungi and protozoans (via



						macrolide antibiotic)
4.	Melatonin	C13H16N2 O2	16.415	95.29	232.1222	Regulating plant growth and development
6.	Lycoperside D	C39H65N O12	16.008	93.53	739.4508	Anti inflammatory property
7.	Cycloalliin	C6H11N O3 S	3.277	90.89	177.0468	Anti-microbial activity
8	Myricanol 5-(6-galloylglucoside)	C37H61N O11	15.750	90.78	695.4251	Insecticidal properties
9.	Furaneol 4 glucoside	C12 H18 O8	18.183	97.83	290.1003	<i>antioxidant and anti-inflammatory properties</i>
10.	Hydroxytyrosol 1-O-glucoside	C14 H20 O8	18.795	97.36	316.1161	beneficial effects such as cancer chemoprevention by inducing apoptosis, cardioprotection and anti-atherogenic activity, skin photoprotection and anti-inflammatory activity.
11.	Erinacine P	C27 H40 O8	19.204	98.92	492.2720	nerve health-related activities
12.	Ethambutol	C10H24N2 O2	10.457	93.18	10.457	Treatment of tuberculosis
13.	N-Desmethylaminopyrine	C12 H15 N3 O	13.107	96.58	217.1220	Botanical pesticide
14	Coriandrone D	C18 H24 O7	14.328	99.00	352.1523	Insecticidal activity
15	Tetrahydrocortisone	C21 H32 O5	15.098	99.2	364.2248	Insecticidal activity
16.	(E)-1- Cinnamoylpyrrolidine	C13H15N0	24.193	95.38	201.1059	Pesticidal activity
17.	Nigakilactone C	C24 H34 O7	18.496	91.82	434.2289	Insecticidal activity
18.	Ergotamine	C33 H35 N5 O5	18.831	91.96	581.2643	Treatment against Migrains



19.	Dukunolide A	C26 H26 O9	19.120	92.63	482.1575	Insecticidal activity
20.	Aloin	C21 H22 O9	20.395	95.72	418.1259	Pesticidal activity
21.	Oleamide	C18 H35 N O	22.639	95.46	281.2720	Neuropathy target
22.	Celerin	C15 H16 O4	23.564	96.44	260.1050	Natural pesticide
23	Stearamide	C18 H37 N O	23.917	99.47	283.2878	Insecticidal activity
24	Palmitoyl-EA	C18 H37 N O2	24.249	94.74	299.2824	Anti-Inflammatory, Antioxidant and Crystallographic

With regard to the anti-oxidant activity, this extract displayed the strongest LC-MS signals for ascorbic acid, margarolic acid, brevifolincarboxylic acid, quercetin 3O-glycoside, kuguacin H, cucurbitacin E, 3malonylmomordicin I, and goyaglycoside G. The presence of brevifolincarboxylic acid in this fruit and the antioxidant activity of 3malonylmomordicin I and goyaglycoside G are both reported in this study for the first time. Additionally, the loading plots identified probable isolates of unidentified chemicals with antioxidant properties. (Vikneswari Perumal, et. al., 2021)

Lycoperoside H can improve epidermal dehydration and suppress inflammation by increasing SC ceramide and steroidal anti-inflammatory activity. (Shogo Takeda, 2021). Goyaglycoside g and 2-Dodecylbenzenesulfonic acid are having antioxidant property with microreactors and industrial production. Mycinamicin IV is a substance that kills or slows the growth of micro organisms , including bacteria, viruses, fungi and protozoans (via macrolide antibiotic). Melatonin regulates the plant

growth and development. Lycoperoside D and Cycloalliin are having anti inflammatory and anti microbial property. Myricanol 5-(6-galloylglucoside) is having a insecticidal activity. Furaneol 4 glucoside shows the anti inflammatory and anti oxidant property. Hydroxytyrosol 1-O-glucoside shows beneficial effects such as cancer chemoprevention by inducing apoptosis, cardioprotection and anti-atherogenic activity, skin photoprotection and anti-inflammatory activity. Erinacine P having properties with nerve health-related activities. Ethambutol used in the treatment of Tuberculosis. N-Desmethylaminopyrine is a Botanical pesticide. Coriandrone D and Tetrahydrocortison are having a insecticidal property. (E)-1- Cinnamoylpyrrolidine and Nigakilactone C shows the insecticidal and pesticidal properties. Ergotamine shows the property in the treatment of Migrains. Dukunolide A and Aloin are having insecticidal and pesticidal properties. Oleamide having a property of Neuropathy target. Celerin and Stearamide shows property of natural pesticide and insecticidal

activity. Palmitoyl-EA having a properties of Crystallographic.
Anti-Inflammatory, Antioxidant and

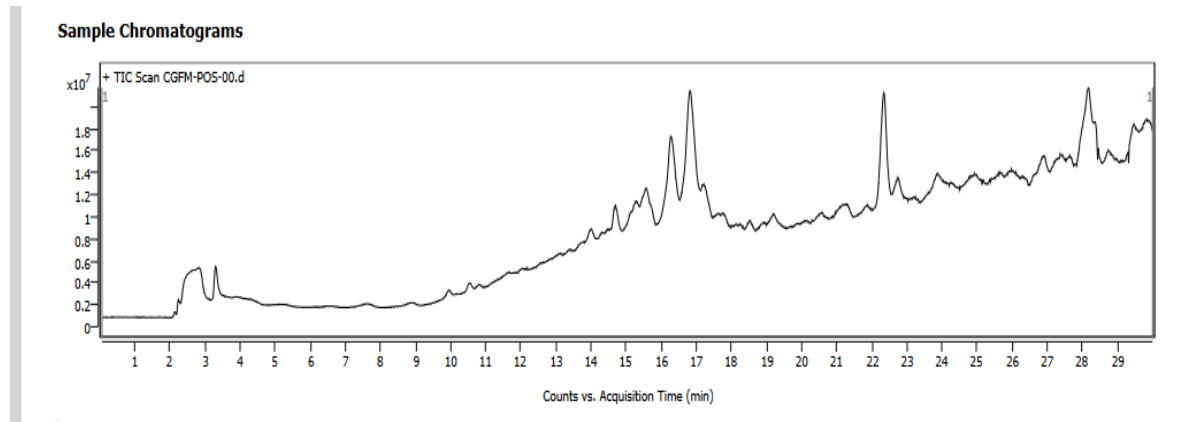


Fig. 1: Liquid chromatogram of the Methanolic extract of fruit follicle of *Calotropis gigantea*

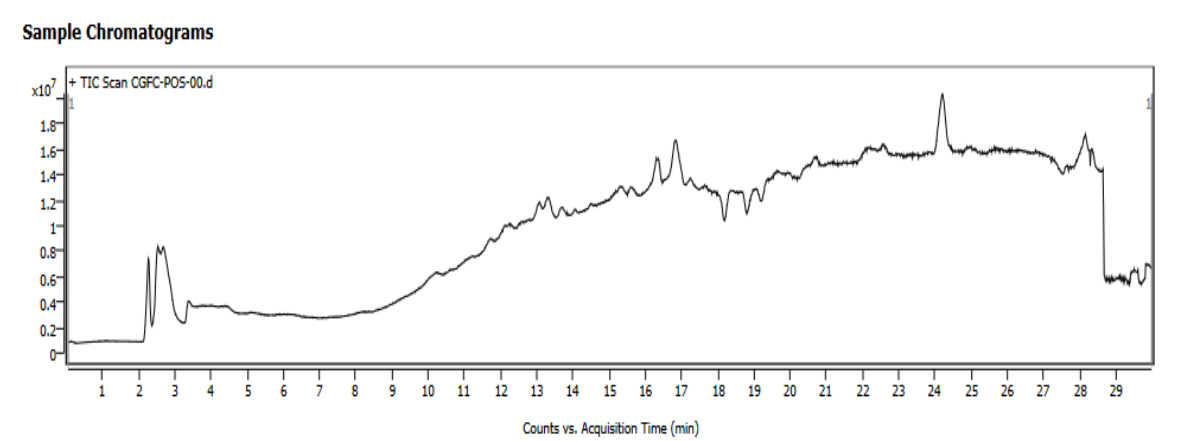


Fig. 2: Liquid chromatogram of the Chloroformic extract of fruit follicle of *Calotropis gigantea*

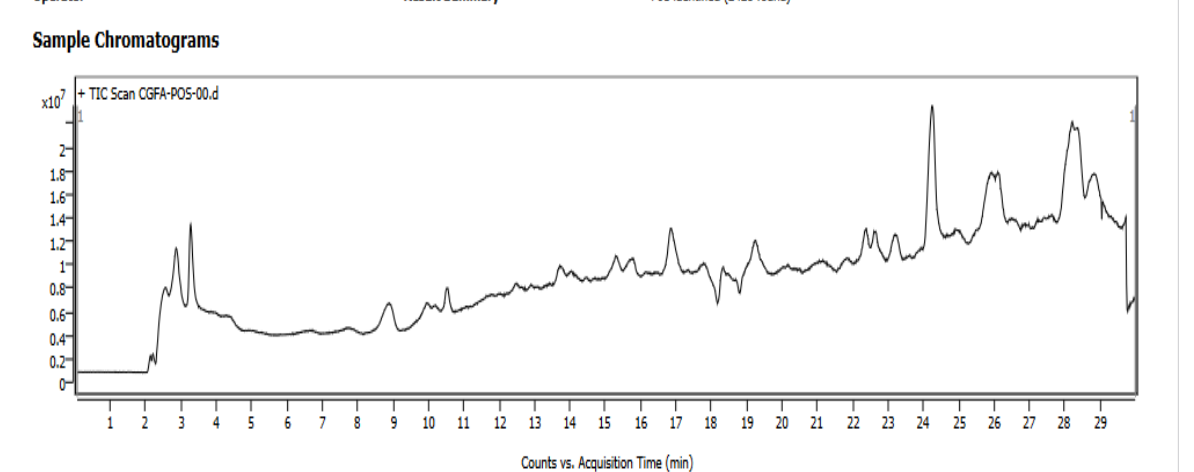
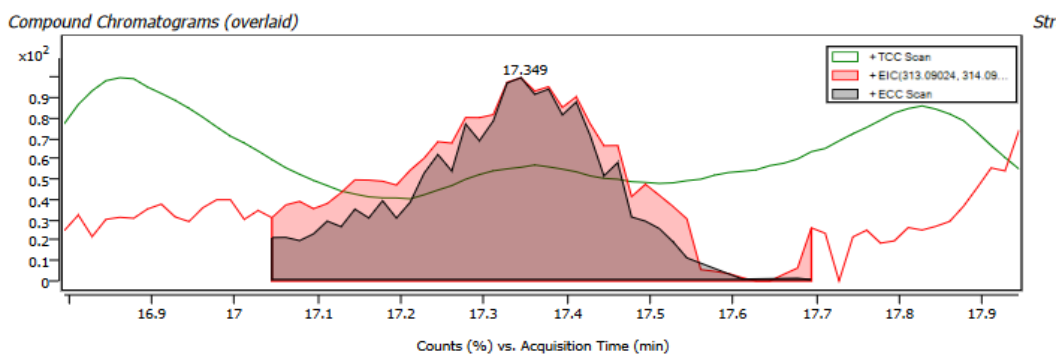


Fig. 3: Liquid chromatogram of the alcoholic extract of the fruit follicle of the *Calotropis gigantea*



Compound Spectra (overlaid)

Fig 4: Liquid chromatogram of the methanol extract of fruit follicle of *Calotropis gigantea*, Furaneol 4 glucoside.

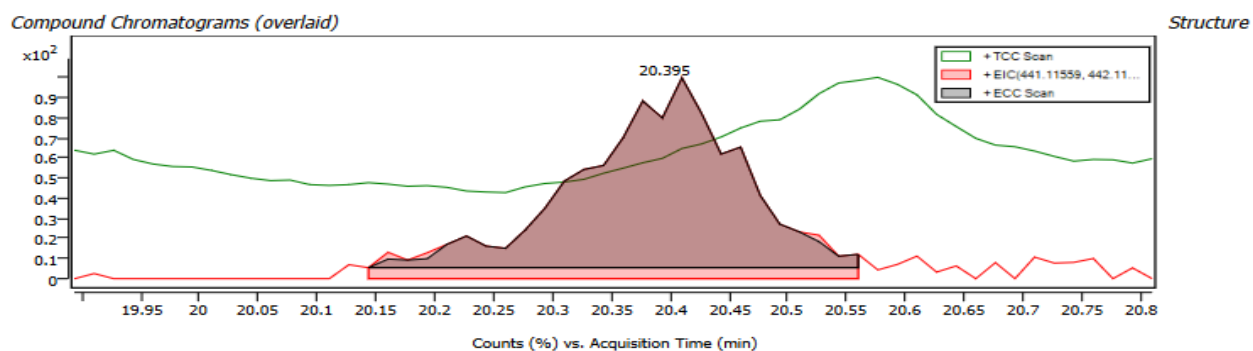


Fig 5: Liquid chromatogram of the methanolic extract of fruit follicle of *Calotropis gigantea*, Aloin

Conclusion:

The present phytochemical study of *C. gigantea* fruit follicle extract was studied using various extracts with increasing polarities. The information about the phytochemicals of *C. gigantea* has been published. The fruit follicles have antibacterial, anti-inflammatory, pesticidal, insecticidal, anti-oxidant, anti-microbial, anti-cancerous, botanical pesticidal properties. It is utilized in Ayurvedic medicines to cure migraines, constipations, neuropathy, in tuberculosis. In agriculture fields, *Calotropis gigantea* can be used as biopesticides to prevent from harmful effects of chemical pesticides.

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