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PHYTOCHEMICAL SCREENING AND COMPARATIVE GC–MS ANALYSIS OF BIOACTIVE COMPOUNDS PRESENT IN METHANOLIC LEAF AND LATEX EXTRACT *Calotropis gigantea* (L)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Medicinal plants are considered as important source of promising bioactive compounds. *Calotropis gigantea* is a traditional medicinal plant which is known to have biochemical constituents with potential medicinal properties. Qualitative analysis showed the presence of alkaloids, terpenoids, saponins, tannins and cardiac glycosides and absence of flavonoids in ethanolic extract of *C. gigantea*, while the chloroform leaf extract showed absence of flavonoids and cardiac glycosides. Bioactive compounds since leaf and latex of *C. gigantea* utilized GC-MS and activity investigation. The GC-MS investigation uncovered the presence of complete 46 bioactive mixes (24 from leaves and 22 from latex) with significant movement. The majority of the mixes were discovered to be comparable in both leaf and latex, however little variety was likewise seen in their synthetic profile. The concoction mixes saw in just latex were 1-[(T-butyl) dimethyl silyl thin] butane, 1-Hexadecyne, Hexadecane, L-Glutamic corrosive, Phenol-3-isoproxy-5-methyl, Trocosane and Z-1,6-Tridecadiene. Mixes distinguished uniquely from the leaves were Azulene, Benalaxyl, Cis-vaccenic corrosive, Levomenol, Profenofos, β-Tocopherol and β-Sitosterol, while the remainder of the mixes were comparable in both leaf and latex. Major of bioactive compounds presents Gas Chromatography-Mass Spectroscopy (GC-MS) analysis. *C. gigantea* is a perennial plant abundantly found in all parts of the country (India) and wild in nature. The leaves of the plant were found to contain various primary and secondary metabolites.

Keywords: B-Tocopherol; *Calotropis gigantean;* GC-MS; phytochemical; phlobatannins.

1. INTRODUCTION

Medicinal plant extracts have been implicated for the treatment of diabetes mellitus since ages. The present study deals with extraction and evaluation of various phytochemical constituents in leaf, root and stem of *Calotropis procera* and *Calotropis gigantea* comparison of their anti-oxidant properties with antidiabetic drugs [1]. Major source of drug plants have been a rich source of medicines because having

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potential bioactive molecules, most of which probably participated as a chemical defence against predation or infection [2]. The world health organization has estimated that 80% of world population in developing countries depends on herbal medicine for their basic health care needs. Cancer is a serious health issue in different parts of the world. Cancer is defined as abnormal cell growth, which tends to invade other parts of the body. Cancer is of 2 types- Benign and Malignant. Benign tumours do not spread whereas malignant tumours tend to spread, divide, and lead to severe conditions. The risk factors are mostly associated with tobacco, obesity, excessive alcohol, certain infections, poor diet and lack of physical activity. *Escherechia coli* and have proved to be very good antioxidant potential and antibacterial activity. *Calotropis gigantea* has shown enormous protective properties and provides wide treatment opportunities hence it was analyzed for anti-proliferative activity on colorectal cancer [3]. The major challenges in herbal medicines are determining the overall quality, stability and efficiency of the herbal product. It is well known that the majority of herbal remedies are sensitive to the light and it has been reported that extracts of herbal plant may undergo photo-degradation reactions on exposure to UV light. The aim of this study was therefore to evaluate the phytochemical and antimicrobial activity of *calotropis gigantea* leaf extract in addition to these studies we also check the effect of light irradiation on the antimicrobial activity of ethanolic leaf extract of *Calotropis gigantean* [4]. The α-Amylase inhibition is considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes, obesity, dental caries and periodontal diseases [5]. Taking this into consideration, α-Amylase inhibition, antioxidant activity and phytochemical analysis of Nepalese originated *C. gigantea* (L.) Dry and were evaluated. TLC of fractions showed a compound at RF value at 0.45 in toluene: chloroform: methanol with mobile phase ratio 7:2:1 respectively [6]. Cancer is mostly managed by surgical removal, chemotherapy, and radiotherapy. However, there are side effects associated with these methods. Alternatively, herbal medicines are becoming popular for cancer treatment. *Calotropis gigantea* is a widely used plant in the traditional medical system. However, there are no reports on its potential in cancer management. Therefore, we aimed to examine the phytochemical composition and cytotoxic activity of *C. gigantea* methanolic leaf extract against three different cancer cell lines: HeLa (cervical), MCF7 (breast), and A549 (lung) [7]. The ethyl acetate fraction was analyzed by high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) for its flavonoid contents. flavonoids were isolated from this fraction and identified by mass spectrometry, infrared, HPLC and HPTLC [8]. The parricidal efficiency of the latex of *Calotropis procera* against all three important vector species such as *Ae, aegypti, An stephesi* and Cx. quinqe faciatus, vectors of dengue, malaria and Lymphatic filarial respectively in which it was proved that *Calotropis procera* latex is responsible for the parricidal properties [9].

GCMS of ethanolic as major compounds present study provides the insight that *Calotropis procera* ethanolic extract can serve as bio fungicide for controlling disease caused by *Alternaria alternata*. Thus there will be natural origin of the fungicide which will have no side effects [10]. Bioactive compounds in medicinal plants are compounds produced by plants that having pharmacological or therapeutic properties such as, anti-malarial, anti-diabetic, antioxidant, antimicrobial, anti-carcinogenic, and, anti-cholinergic activities. According to the previous studies, antioxidants are reported to relief the function of immune cells against free radicals [11]. Therapeutic potential of this plant as it is likely to have more therapeutic properties than are currently known. The leaf of *Calotropis* contains ascorbic acid, o-pyrocatechic acid and also contains β-amyrin, taxasterol, tarasterol and β-sitosterol. Therefore, the present study was undertaken to evaluate the total phenolic, flavonoid and DPPH antioxidant activity of *C. procera* leaves and fruits extract (Furthermore, GC-MS analysis has carried out to identify the bioactive constituent present in this plant [12].

Many studies have identified that the process of co-paralysis can be used to enhance the quantity and quality of bio-oil. Polystyrene, also known as thermocol is an aromatic polymer with high molecular weight and it is used in the manufacture of several goods and as packaging material. Therefore in order to understand the role of various phytochemicals present in *C. gigantea*, we carried out this study to identify the possible phytochemical compounds along with its functional groups present in the methanolic extract of *Calotropis gigantea* leaves. Our study is formulated to identify the possible phytochemical compounds along with its functional groups present in the methanolic extract of *Calotropis gigantea* leaves [13]. The data presented in this study indicated that *C. gigantea* ability for iron binding and could reduce the generation of hydroxyl radicals. In the metal chelating activity, Ferrozine can quantitatively chelate with Fe^{2}+ and form a complex with a red colour. The adverse effect of *calotropis* consumption is reported to cause lesion, eruption and blisters were taken by patients for treatment of joint pain [14]. Based on the medicinal treatments and pharmaco-therapeutic uses of *C. procera*, more phytochemical studies in

different methods are needed to investigate the chemical components of this plant, therefore, this study was aimed to extract the leaves of *C. procera* growing in Iraq, using two different polarities of solvent extractor, and to identify the chemical components using GC-MS Technique. Also to study the antibacterial activity of leaves extract. This is the first study on the phytochemical and antimicrobial activity of the leaves of *C. procera* in Ira [15].

The yield of bio-oil from CPS pyrolysis was very low and it was found to contain mainly phenolic compounds. The co-pyrolysis of CPS and WPS improved the yield and the quality of bio-oil. The co-pyrolysis of CPS with WPS reduced the phenolic compounds and increased the ester content [16]. The GC-MS analysis showed that CPS bio oil consisted of mainly phenolic compounds and few oxygenated, aliphatic and cyclic compounds. The CPS-WPS bio-oil was composed of mono-aromatics, esters and nitrogenised compounds. The CPS-WPS bio-oil also possessed improved calorific value and viscosity [17]. The solution was then boiled at 60-70ºC for 18 hours on water bath, filtered, evaporated to dryness, & final residue was then subjected to GC-MS analysis. The extract was then subjected to slandered phytochemical analytical tests i.e. Wagner's test for alkaloids, Molish test for carbohydrates, Borntrager's test for glycosides, Lead Acetate test for phenolic compounds, Alkaline test for flavanoids, Xantho-protein test for protein and amino acid, Foam test for saponins, Salkowski test for steroids and terpenoids for isolation and characterization of primary products [18]. Plant growth hormones, auxins: indole-3-butyric acid and indole-3- propionic acid, were then analyzed through GC–MS in the using their respective isotopic internal standards [19]. The use of medicinal plants has an ancient origin in different cultures around the world and their preparation is basically due to producing a spectrum of secondary metabolites. To promote the use of medicinal plants as potential sources of antioxidant, it is important to thoroughly find out their composition and activity and thus confirm their use.

2. MATERIALS METHODS

2.1 Plant Collection

The fresh leaves of *Calotropis gigantea* were collected from Saliyamangalam, Thanjavur District, Tamil Nadu, India.

2.2 Plant Material

The *Calotropis gigantea* leaf and latex was dried under shade, mechanically reduced to a moderately coarse powder, and stored in amber-colored airtight containers. The coarse type of the medication was utilized for the assurance of physicochemical boundaries like dampness content, debris esteems, expanding file, frothing record, unfamiliar natural issue, extractive qualities, and fluorescence analysis.

2.3 Phytochemical Studies

Secondary metabolites in the present studies were carried out on the plant sample revealed the presence of medicinally active constituents. Beneficial drugs and to improve the patient health.

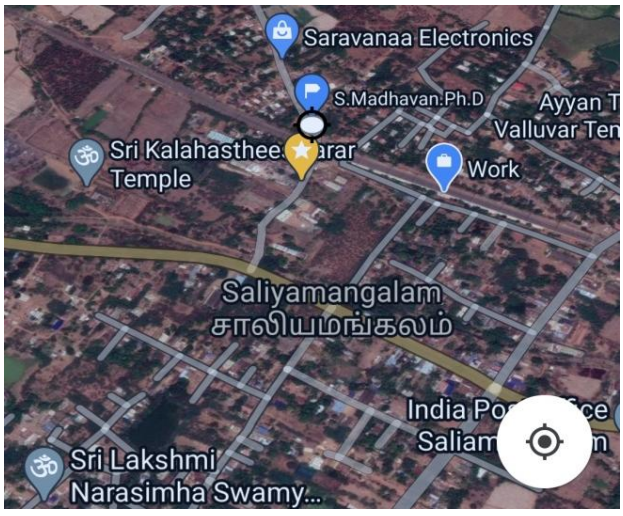


Photo 1. Study Area

2.4 Preparation of Extracts

The powdered plant samples of leaf and latex (100 g) were used for successive solvent extraction (500 ml) with increasing order of polarities like ethanol, methanol, water, chloroform, ethyl acetate and petroleum ether. At that point it is kept in an orbital shaker at 190-220 rpm for 48 hours. The supernatant was collected, filtered through Whatman No.1 filter paper and the extract were concentrated by a Rotary flask evaporator at a specific temperature was used based on the solvent system. Each time before extracting with the next solvent the residue was dried thoroughly to remove the solvent used. The obtained dried extract was then accurately weighed, stored in small vials at -20°C and used for the following studies.

2.5 Phytochemical Screening

The preliminary phytochemical evaluation was carried out by using standard procedure [20-22].

2.6 Test for Tannins

About 0.5 g of the dried powder leaf and latex samples were boiled in 20 ml of water in a test tube and then filtered. A few drops of zero. 1% ferric chloride was added and observed for brownish green or a blue-black coloration.

2.7 Test for Phlobatannins

The deposition of a red precipitate once associate binary compound extract of every rootstalk sample was cooked with 1 Chronicles binary compound acid was taken as proof for the presence of phlobatinins.

2.8 Test for Saponin

a) About 1 ml of alcoholic extract was diluted separately with 20 ml of distilled water and shaken in a graduated cylinder for 15 minutes. A one cm layer of froth indicates the presence of saponins.
b) To 1 ml of the extract, 1 ml of alcoholic vanillin solution was added which was followed by the addition of few drops of concentrated sulphuric acid. A deep violet colour confirms the presence of saponins.

2.9 Test for Flavonoids

a) To 5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each rhizome extract followed by the addition of concentrated H2SO4. A yellow coloration discovered in every extract indicated the presence of flavonoids. The yellow coloration disappeared on standing.
b) A few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow coloration was discovered indicating the presence of flavonoids.
c) A portion of the powdered rhizome sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and four millilitre of the filtrate was agitated with one millilitre of dilute ammonia resolution. A yellow coloration was discovered indicating a positive take a look at for flavonoids.

2.10 Test for Steroids

a) Libermann-burchards test

To 1.0 ml plant extract, 1.0 ml of concentrated sulphuric acid was added followed by the addition of 2.0 ml of acetic anhydride solution. A greenish colour developed and it turned blue to indicate the presence of steroids.

b) Salkowski reaction

To 2.0 ml sample extract, 1.0 ml of concentrated sulphuric acid was added carefully along the sides of the tube. A red colour was produced in the chloroform layers.

2.11 Test for Terpenoids

a) Horizon test

To one millilitre of extract, 2 ml of tri-chloroacetic acid was added. The formation of yellow to red precipitate shows the presence of terpenoids.

b) Liebermann test

To 1 ml of extract 3 ml of acetic acid and few drops of concentrated sulphuric acid were added. Color changed from red to blue indicating the presence of terpenoids.

2.12 Test for Triterpenoids

To 10 mg of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of Conc. H_2SO_4. The formation of reddish-violet colour indicates the presence of tri-terpenoids.

2.13 Test for Alkaloids

a) A pair of millilitre aliquot of the extract was treated with the Dragen-deroff's chemical agent. An orange-red precipitate is produced immediately indicating the presence of alkaloids.
b) 1 ml aliquot of the extract was treated with a few drops of Mayer's reagent. The formation of white or pale yellow precipitate showed the presence of alkaloids.

2.14 Test for Carbohydrates

a) Fehling's test

The extract was treated with 5 ml of Fehling's solution (A and B) and kept at a boiling water bath for 5 min. The formation of a yellow or red colour precipitate indicates the presence of reducing sugar.

b) Benedict's test

To 1 ml of the extract, added 5 ml of Benedict's solution and kept at boiling water bath for 5 min. Red, yellow or green precipitate indicates the presence of reducing sugars.

2.15 Test for Proteins

About 1 ml of rhizome sample was taken and added 1 ml of 40% Sodium hydroxide and added slowly in the sides of test tubes of few drops of copper sulfate. The appearance of violet or pink color indicates the presence of proteins.

2.16 Test for Anthraquinones

To 5 ml of the extract, the solution was hydrolyzed with diluted Conc. H_2SO_4 extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink hue recommended a positive reaction for anthraquinones.

2.17 Test for Polyphones

Ethanol (10.0 ml) was added to each extract and the resulting solution (3.0 ml) was transferred in test tubes and warmed in a water bath (15 minutes). Three drops of freshly ready ferrous cyanide resolution were added to the extract resolution. The formation of a blue-green colour indicated the presence of poly-phenols.

2.18 Test for Cardiac Glycosides

Above 5 ml was treated with 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was underplayed with 1 ml of focused sulfuric acid. A brown ring of the interface indicates deoxy sugar characteristics cardiac glycosides. A violet ring may appear below the brown ring while in the acidic layer, a greenish ring may form just gradually through thin layers.

2.19 Gas Chromatography- Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis of the sample was performed using a Shimadzu GCMS-QP2010 gas chromatograph-mass spectrometer interfaced with a Turbo Mass quadrupole mass spectrometer, fitted with an Rtx-5 fused silica capillary segment (30 X0.25 mm, with 1 Cm film thickness). The oven temperature was programmed from 100°C to 320°C at 100°C/min and hold for 10 min. Helium was used as carrier gas at a flow of 1.0 mL/min. The injector temperature was 250°C, injection size 1 µL neat, with a split ratio of 1:10. The interface and MS ion source were maintained at 320°C and 200°C respectively and the mass spectra were taken at 70eV with a mass scan range of 40-700 AMU (atomic mass unit). Data handling was done using GCMS solution software.

2.20 Identification of Compounds

Interpretation of the mass spectrum of GC-MS was conducted using the mass spectral database of the National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The range of the obscure segment was contrasted and the range of the realized segments put away in the NIST library. The name, sub-atomic weight, and structure of the parts of the test materials was determined.

3. RESULTS AND DISCUSSION

Every constituent plays an important role and deficiency of anyone constituent may lead to abnormal developments in the body [23]. India is probably the biggest maker of therapeutic spices on the planet. The Indian customary medicinal services framework, Ayurveda gives a moderately sorted out database and more thorough depiction of natural materials, a considerable lot of which have been utilized as layouts for novel medication advancement. Nature has provided a complete storehouse of remedies to cure all ailments of mankind by providing our drugs in the form of herbs, plants and algae to cure the incurable diseases without any toxic effects. Nowadays allopathic system usage was decreased due to side effects, adverse reactions, so now a day's herbal drugs usage was increased due to fewer side effects and patience acceptance in these way herbal drugs usage was increased. In the present study, the

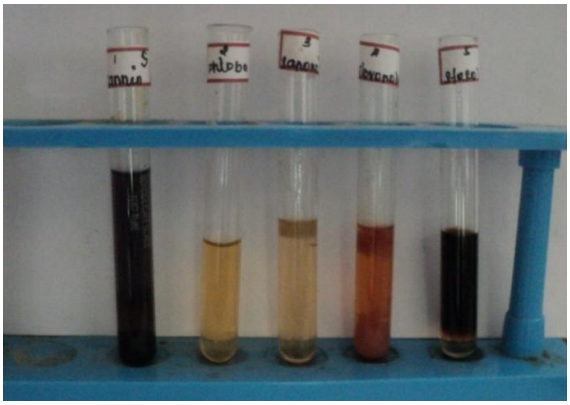
attempt is made to the ethyl acetate extract showed mild to moderate activity and better anthelmintic activity when compared to petroleum ether extract [24]. These are compounds present in plant derived-foods that induce biological activities and various salubrious functions in the body. For instance, in this way phyto-nutrients advance the capacity of the invulnerable framework, acts directly against microscopic organisms and infections, diminishes aggravation and are additionally connected with the treatment and anticipation of cancer, cardiovascular disease and many other maladies affecting the health or well being of an individual. Plant biochemistry is epitomize the considerable diversity of organic substances that are intricate and accumulated through plants, the chemical composition of these substances, their biosynthesis turn over and metabolism in plants, their Innate circulation and their biological medicinal plants are supportive for therapeutic as well as for remedial of human diseases, since of the occurrence of phytochemical constituents [25].

Potential natural source of antioxidants and could have greater importance as therapeutic agent in preventing or slowing diabetic activity that mean extract of *C. gigantea* flowers can be used as anti-diabetic agents. The present ethanol botanical study on *Calotropis gigantea* leaves confirm the existence of various biological active molecules with its possible functional groups. Moreover, these leaves are already in use for a wide range of treatments. Traditionally such as fever, indigestion, cough, cold, asthma, nausea, vomiting, diarrhea etc. The present study may be an initiative for further phytochemical and pharmacological investigations required to separate the novel active compounds from the leaves to formulate new drug in order to treat incurable diseases.

Table 1. Qualitative analysis of *Calotropis gigantea* leaf extract

S. no	Analysed phytochemicals factor	Methanol	Ethanol	Water	Chloroform	Ethyl acetate	Petroleum ether
1.	Tannin	++	+	+	+	+	+
2.	Phlobatannins	+	+	++	-	-	+
3.	Saponin	-	+	++	+	-	-
4.	Flavonoids	+++	++	-	+	++	++
5.	Steroids	++	-	+	-	+	+
6.	Terpenoids	+	+	+	-	-	+
7.	Triterpenoids	+	+	-	+	+	+
8.	Alkaloids	+++	++	+	+	+	+
9.	Carbohydrate	+	+	+	-	-	-
10.	Protein	++	-	++	-	+	-
11.	Anthraquinone	-	-	+	-	+	+
12.	Polyphenol	++	+	++	+	-	-
13.	Glycoside	+	-	-	+	+	+

Indications: "+" means positive activity, "-" means negative activity
Tannin, Phlobatannins, Saponin, Flavonoids, Steroids, Terpenoids, Triterpenoids, Alkaloids, Carbohydrate, Protein, Anthraquinone, Polyphenol and Glycoside



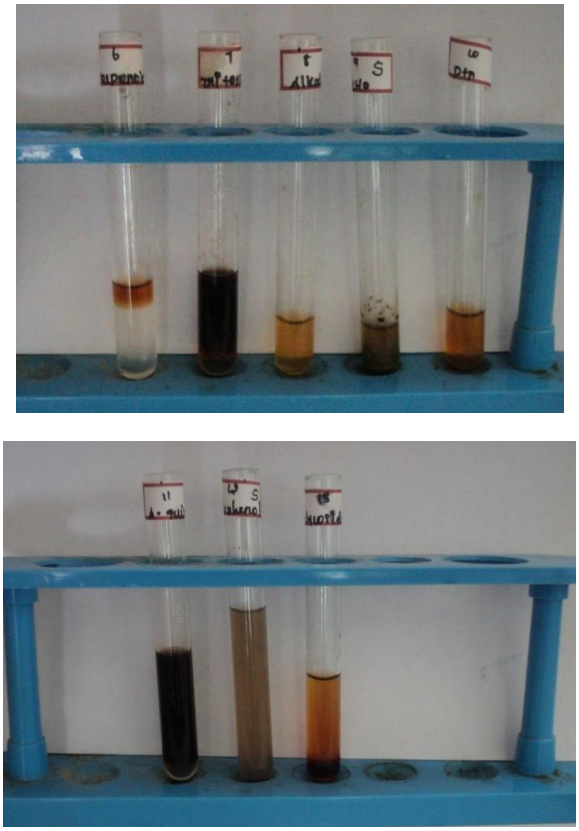


Fig. 1. Qualitative analysis of phytochemicals analysis *Calotropis gigantea leaf* methanolic extract

Indications: "+" means positive activity, "-" means negative activity
Tannin, Phlobatannins, Saponin, Flavonoids, Steroids, Terpenoids, Triterpenoids, Alkaloids, Carbohydrate, Protein, Anthraquinone, Polyphenol and Glycoside

Table 2. Qualitative analysis of *Calotropis gigantea* latex extract

S. no	Analysed phytochemicals factor	Methanol	Ethanol	Water	Chloroform	Ethyl acetate	Petroleum ether
1.	Tannin	++	+	+	+	+	+
2.	Phlobatannins	+	+	++	-	-	+
3.	Saponin	-	+	++	+	-	-
4.	Flavonoids	+++	++	+	-	-	++
5.	Steroids	++	-	+	-	+	-
6.	Terpenoids	-	+	-	-	-	+
7.	Triterpenoids	+	+	-	+	+	+
8.	Alkaloids	-	-	+	+	+	-
9.	Carbohydrate	+	+	+	+	+	-
10.	Protein	++	-	+	-	+	-
11.	Anthraquinone	+	-	+	-	+	+
12.	Polyphenol	++	+	+	+	-	-
13.	Glycoside	+	-	-	+	+	+

Indications: "+" means positive activity, "-" means negative activity
Tannin, Phlobatannins, Saponin, Flavonoids, Steroids, Terpenoids, Triterpenoids, Alkaloids, Carbohydrate, Protein, Anthraquinone, Polyphenol and Glycoside

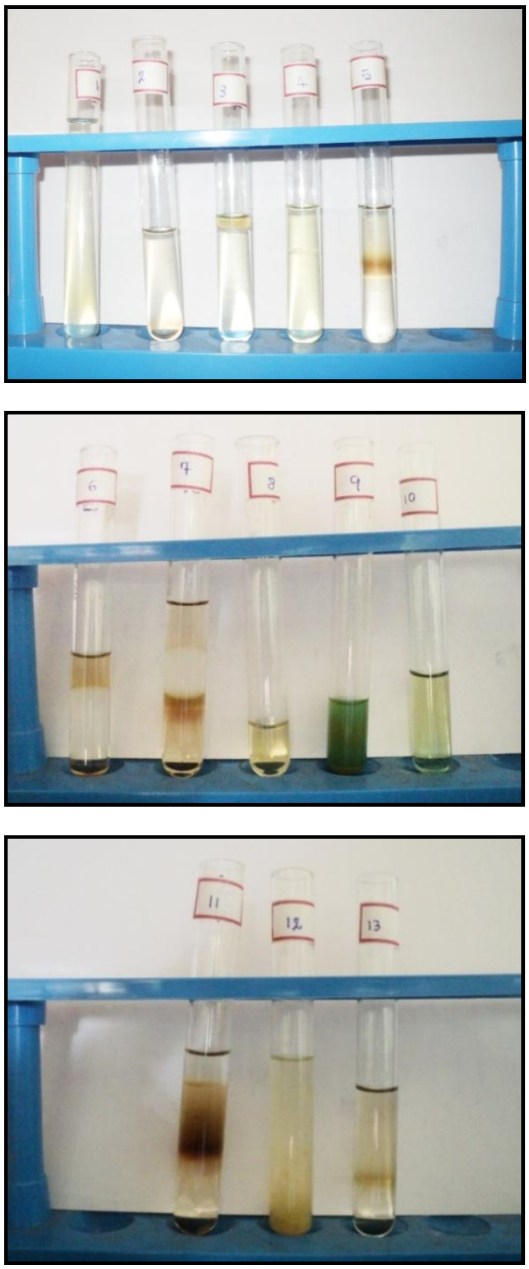


Fig. 2. Qualitative analysis of phytochemicals analysis *Calotropis gigantea* latex methanolic extract

Indications: "+" means positive activity, "-" means negative activity
Tannin, Phlobatannins, Saponin, Flavonoids, Steroids, Terpenoids, Triterpenoids, Alkaloids, Carbohydrate, Protein, Anthraquinone, Polyphenol and Glycoside

Now a day the identification of bioactive compounds from medicinal plants has increased. This study was screened for the phytochemical constituents of the methanolic *C. gigantea* leaf extract. The presence of active compounds that belong to flavonoids, alkaloids, and terpenoids indicates the medicinal importance of the plant [7]. The antioxidant properties of extracts were evaluated and compared with anti-diabetic drugs (insulin, metformin and pioglitazone) [1]. The presence or absence of functional groups in an organic molecule determines the manner in which that organic molecule will fragment. The presence or absence of various mass peaks in each spectrum was used to deduce the structure of the compounds. The compounds identified are phthalic acid, butane, methyl palmitate, n-hexadecanoic acid, phytol etc [26].

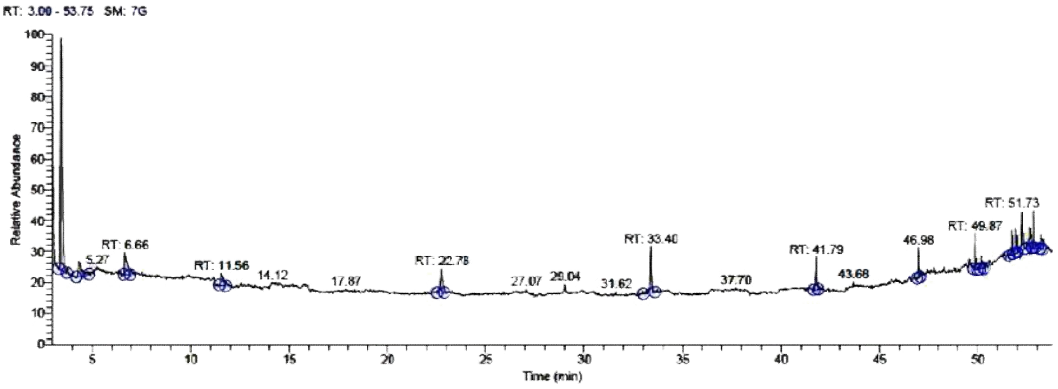


Fig. 3. GC-MS chromatogram of *Calotropis gigantea* leaves extract

Table 3. GCMS analysis - bioactive compounds *Calotropis gigantean* methanolic leaves extract

S. no.	Compound name	Retention time	Peak area (%)	Molecular formula	Molecular weight
1.	1-Octanol-3,7-dimethyl	22.78	5.78	C10H22O	158.28
2.	2- Methoxy 4-vinyl phenol ethanone	53.21	1.87	C9H10O2	149.0
3.	4-Methyl-2-phenylindole	4.34	5.26	C15H13N	207.0
4.	5-Nonadecen-1-ol	33.40	7.62	C19H38	266.9
5.	9,12,15-Octadecatricenoic acid, methyl ester	52.82	2.78	C19H32O2	293.2
6.	Azulene	11.56	3.16	C10H8	125.4
7.	Benalaxyl	22.78	5.78	C20H23NO3	326.9
8.	Beryllium sulfate tetrahydrate	50.19	1.81	BeH8O8S	178.1
9.	Biphenyl	3.44	43.28	C12H10	154.7
10.	Butane-2,2-dimethyl	6.66	6.00	C6H14	87.0
11.	Campesterol	49.87	2.65	C28H48O	401.0
12.	Cholest-5-en-3 ol, 24, Propylidene(3.beta.)	41.79	3.56	C29H48O2	429.0
13.	Cis-vaccenic acid	22.78	5.78	C18H34O2	281.1
14.	Cyclohexane	3.46	43.28	C6H12	84.1
15.	Decane	46.98	2.17	C10H22	147.0
16.	D-Mannose-1-phosphate sodium salt	6.66	6.00	C6H13O9P	282.0
17.	Eicosane	46.98	2.17	C20H42	281.0
18.	Ethion	53.21	1.87	C9H22O4P2S4	385.0
19.	Guanidine nitriate	51.73	2.43	CH6N4O3	121.0
20.	Levomenol	49.87	2.65	C15H26O	222.8
21.	Pentacosane	33.40	7.62	C25H52	355.1
22.	Profenofos	22.78	5.78	C11H15BrClO3PS	372.2
23.	B-Tocopherol	22.78	5.78	C28H48O2	417.8
24.	β-Sitosterol	46.98	2.17	C29H50O	415.1

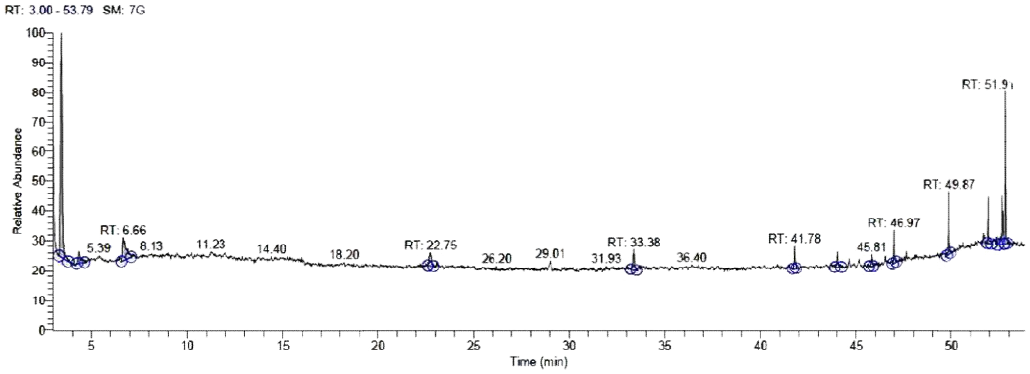


Fig. 4. GC-MS chromatogram of *Calotropis gigantea* latex extract

Table 4. GCMS analysis - bioactive compounds *Calotropis gigantean* latex methanolic extract

S. no.	Compound name	Retention time	Peak area (%)	Molecular formula	Molecular weight
1.	D-Mannose-1-phosphate sodium salt	46.98	2.74	C6H13O9P	282.0
2.	1-[(T-butyl) dimethyl silyl thin] butane	52.82	4.84	C7H15F3O3SSi	205.0
3.	1-Hexadecyne	51.92	3.32	C16H30	221.0
4.	2- Methoxy 4-vinyl phenol ethanone	6.71	5.86	C9H10O2	149.0
5.	5-Nonadecen-1-ol	33.40	4.64	C19H38	266.9
6.	9,12,15-Octadecatricenoic acid, methyl ester	52.82	4.84	C19H32O2	293.2
7.	Butane-2,2-dimethyl	6.71	5.86	C6H14	86.24
8.	Campesterol	49.87	3.50	C28H48O	401.0
9.	Cholest-5-en-3-ol,24,Propylidene(3.beta.)	41.79	3.69	C29H48O2	429.0
10.	Cyclohexane	3.45	51.52	C6H12	84.0
11.	Decane	41.79	3.69	C10H22	147.0
12.	D-Mannose	49.16	1.87	C6H12O6	180.1
13.	Eicosane	51.92	3.32	C20H42	281.0
14.	Guanidine nitrate	45.09	2.49	CH6N4O3	121.0
15.	Hexadecane	52.82	4.84	C16H34	227.2
16.	L-Glutamic acid	49.87	3.50	C5H9NO4	147.0
17.	Oxadiazon	41.79	3.69	C15H18	341.0
18.	Pentacosane	33.40	4.64	C25H52	355.1
19.	Phenol,2,5-bis (1,1-dimethylethyl)	49.16	1.87	C14H22O	204
20.	Phenol,3-isopropoxy-5-methyl	4.35	3.17	C10H14O2	166.9
21.	Tricosane	46.98	2.74	C23H48	326.9
22.	Z-1,6-Tridecadiene	49.57	3.19	C13H24	180.3

The impacts of seasons upon the phytochemicals are diversified. About nine terpenes are common to all the seasons studied. The Pre-summer shows the highest quantity of terpenes i.e. about 65.11% and Southwest monsoon illustrates highest diversity of terpenes i.e. about 13 compounds. It shows that the season influence highly on the diversity and quantity of terpenes an economically important active compound [27]. *Calotropis gigantea* white flowers have identified by GC-MS analysis spectrums showed a

molecular ion peak at m/e 150.434, 167.490, 295.080, and 150.468 follow a molecular formulas of C9H10O2 (MW 150.00), C9H12O3 (MW 168.00), C19H36O2 (MW 296.00), C16H15BrN2O3 (MW 151.00) have identified three compounds in the chloroform extract namely (1) 2-methoxy-4-vinylphenol (RT 10.43), (2) Phenol-4-methoxy-3-(methoxy methyl) (RT 11.38), (3) 8-octadecenoic acid, methyl ester (E) (RT 19.00) and only one compound in the ethyl acetate extract namely (4) Benzhydrazide, 4-methoxy-N2-(5-bromo-2-methoxy benzylideno) (RT 2.37) [28]. The chemical compounds observed in only latex were 1-[(T-butyl) dimethyl silyl thin] butane, 1-Hexadecyne, Hexadecane, L-Glutamic acid, Phenol-3-isoproxy-5-methyl, Trocosane and Z-1,6-Tridecadiene [29]. Identified from leaf and latex of *C. gigantea* which supports that the plant have greatly and altered pharmaceutical value. Although, additional research is necessary to purify those compounds which are responsible for therapeutic activities.

Table 5. GC-MS analysis of activities/Uses of bioactive compounds methanolic extract *Calotropis gigantean*

S. no.	Compound name	Activity
1.	Biphenyl	Used in dye carriers, food preservatives, as fungicide.
2.	Cyclohexane	Used as solvent and paint remover.
3.	D-Mannose-1-phosphate sodium salt	Used in a study to assess *in vivo* targeting of alveolar macrophages and has also been used in a study to investigate genetic engineering of the phosphor carrier protein NPr.
4.	Azulene	Have anti-inflammatory, analgesic, antipyretic, and platelet-inhibitory actions.
5.	Cis-vaccenic acid	Confirm anti-carcinogenic properties, reserve of telomerase enzyme.
6.	Profenofos	Used as pesticide, toxic compound to human.
7.	β –Tocopherol	Act as antioxidant and vitamin.
8.	β-Sitosterol	Used for heart diseases and high colestrerol. Used for boosting the immune system and for preventing the common cold and flu (influenza), colon cancer, cervical cancer, HIV/AIDS, rheumatoid arthritis, tuberculosis, allergies, psoriasis, as well as for gallstones, fibromyalgia, migraine headache, systemic lupus erythematosus (SLE), asthma, bronchitis, hair loss and chronic fatigue syndrome.
9.	Decane	Second-hand used for industrial purpose or as a type of hydrocarbon solvent.
10.	Levomenol	Antimicrobial activity and wound healing
11.	Campesterol	Check the level of cholesterol in body
12.	Ethion	carry out as insecticide-affect a neural enzyme are acetylcholinesterase and stop it starting working
13.	Azulene	The used in treatment of ulcers, gastritis, athlete's foot, and vein struggle
14.	2- Methoxy 4-vinyl phenol ethanone	Used as flavoring agent
15.	L- Glutamic acid	Act as amino acid
16.	D-Mannose	Used for preventing urinary tract infections (UTIs) and treating carbohydrate-deficient glycoprotein syndrome, an inherited metabolic disorder
17.	Oxadiazon	Used as herbicide
18.	5-Nonadecen-1-ol	second-hand to make surfactants, lubricating oils, pharmaceuticals
19.	2- Methoxy 4-vinylphenol ethanone	Used as flavoring agent

4. CONCLUSION

The phytochemical screening indicated that alkaloids and phenolic compounds were present in large quantities. The leaves and latex *Calotropis gigantea* can be useful for the treatment of cancer due to the antioxidant activity of phenolic compounds. Isolation and identification of active compounds are important to discover new drug from this plant because little articles appear on this side. Further research is required for these parts of the plant in Iraq. The present study proved *C. gigantea* as restorative significant plant in light of the presence of different dynamic mixes. The investigation affirms the variety between the concoction constituent of leaf and latex, which shows their diverse capability of helpful exercises. The present preliminary research concludes that these chemical constituents can be used for the development of various traditional medicines. Further examinations needed to isolate the novel dynamic mixes from the leaf and latex methanolic remove which may make another approach to treat serious sicknesses. GC-MS is a highly reliable as it can extracts compounds in their pure form. It is the major of 46 bioactive compounds presents and leaves and latex of this tool as well as solvent that can give more products. Extraction of compounds using GC-MS can open a big platform for pharmacological companies to formulate various drugs from plants that can be good source of these drugs, but what we need in turn is also care and conservation for these plants.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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