Wiley
Journal of Chemistry
Volume 2024, Article ID 2012983, 23 pages
https://doi.org/10.1155/2024/2012983

WILEY

Research Article

In Vitro Biological and GC-MS Analysis of Whole Plant *Calotropis procera*

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Received 6 June 2023; Revised 9 January 2024; Accepted 22 April 2024; Published 28 May 2024

Academic Editor: Fabio Polticelli

Calotropis procera is a medicinal, traditional, and therapeutic plant in Pakistan. In this research study, the biological activities, for instance, anticancer, antileishmanial, antibacterial, antifungal, anti-inflammatory, and brine shrimp lethality assay and GC-MS are studied. MTT assay, antileishmanial bioassay, microplate Alamar Blue assay, agar tube dilution method, oxidative burst assay using chemiluminescence technique, B-hatching techniques, and triple quadrupole acquisition method MS parameters were the methods used for anticancer, antileishmanial, antibacterial, antifungal, anti-inflammatory, brine shrimp lethality assay, and gas chromatography-mass spectrometry. Whole plant methanol extract of *Calotropis procera* (WMECP) inhibited 69.1% of the growth of HeLa cell line with an IC_{50} value of 3.1 ± 0.4 and whole plant n-hexane fraction of *Calotropis procera* (WHFCP) and whole plant and aqueous fraction of *Calotropis procera* (WAFCP) inhibited the growth by 70.2% and 65.2% with IC_{50} values of 5.0 ± 0.3 and 17.1 ± 1.0. Whole plant methanol extract of *Calotropis procera* (WMECP) inhibited 70.1% of the growth of the PC3 cell line with an IC_{50} value of 5.1 ± 0.3 and whole plant n-hexane fraction of *Calotropis procera* (WHFCP) and whole plant aqueous fraction of *Calotropis procera* (WAFCP) inhibited 61.6% and 59.7% with IC_{50} values of 3.7 ± 0.5 and 16.4 ± 1.0. None of the extract and fractions of *Calotropis procera* showed anticancer activities against the 3T3 cell line. None of the extract and fractions of *Calotropis procera* showed antileishmanial, antibacterial, antifungal, and anti-inflammatory activities. Whole plant methanol extract of *Calotropis procera* (WMECP) exhibited lethality at the highest concentration while other fractions did not exhibit lethality. GC-MS studies revealed that the whole plant methanol extract of *Calotropis procera* (WMECP) consists of 11 compounds, whole plant n-hexane fraction of *Calotropis procera* (WHFCP) consists of 9 compounds, and whole plant aqueous fraction of *Calotropis procera* (WAFCP) consists of 7 compounds.

1. Introduction

Calotropis procera is a traditional, medicinal, and therapeutic plant in Pakistan and native people of Pakistan as well as all over the world use it for health-related properties. This is a flowering plant and belongs to *Apocynaceae* family [1]. In different regions of the world, the common name of *Calotropis procera* is dead sea, Calotrope and Calotropis in English, madar and oak in India and Pakistan, isipekag in Turkish, extranjero, bomba, algodon, and cazuela in Spanish, while kisher and oshar in Arabic language [2]. The different parts of *Calotropis procera,* for instance, stem, root, flowers, latex, and leaves are used for therapeutic purposes [3].

Calotropis procera is present in different parts of the world, for instance, in Asian countries such as Afghanistan, Nepal, China, Malaysia, Indonesia, India, and Pakistan, in African countries such as Niger, Nigeria, and Kenya, and in the Australian continent, this plant is distributed widely [4].

Calotropis procera consists of a variety of phytochemicals, for instance, calotropenyl acetate and multiflavenol in flowers, calotropin and calotropagenin in leaves, uzarigenin and terpineol ester in latex, while benzoylinesolone and benzolisolinelone in root [5]. Procerleanol, calopfriedelenyl, triterpenoids, procerudenyl acetate, proceranol, monooleolyl-2-phosphate, N-doctriacont-6-ene, methyl behenate, and methyl myristate are the compounds present in *Calotropis procera* [6].

The family *Apocynaceae* comprises of 180 genera and 2000 species of which the medicinal plant, *Calotropis procera* belongs to. This plant is distributed in tropical and subtropical regions of the world and is grown in deserts with evergreen nature. The leaves of *Calotropis procera* are flat, fluffy, and soft and with single branches or some stem. The large leaves of this traditional plant touch the lower region of growth. A light grey color bark covers the stem. Sap is also present in this plant and is released by cutting the stem, flower, and leaf. The root of this medicinal plant reaches up to 1.7–3.0 meters at the depth of the soil. The length of leaves is up to 7–18 cm and breadth is up to 5–13 cm. The color of the leaves is dark green with the white color veins inside the leaves. On the surface of leaves, minor hair-like structures are present which by pressing or rubbing the leaves can be felt. The color of the flower is white to pink. Sepals are usually 5 in number with a length of 5-4 cm. A green color fruit is also present in this plant with enclosed brown color seed inside the fruit. A bunch of white hairs is present on the seed which help to establish the floral part of the plant with the help of water, animal, and wind and also helps to transport the seed at long distances [7].

Calotropis procera has the ability to tolerate the condition of drought for longer periods, grow in saline environments and sandy soil, ability for fighting diverse changes in the environment, adaptability to numerous environmental conditions, for instance, cold, heat, drought, and salinity [7]. The part of a rich source of ethnomedicines is latex which is very important [8]. At the age of 3–5 years, the height of the plant reaches from 2.5 to 6 m. Ropes, papers, nets and bags can be made from this plant [7].

The medicinal plant *Calotropis procera* is used for the treatment of diverse form of diseases in the entire world, for instance, boils, indigestion, eczema, tooth decay, diarrhea, paralysis, fever, hair fall, cold, rheumatism, joint pain, and jaundice. The root is used to cure dysentery, cough, eczema, leprosy, tumors, paralysis, bronchitis, digestion, inflammation, abdominal pain, skin diseases, kill worms in the intestine, body pain, enlarged abdominal viscera, headache, enlargement of spleen and liver, diarrhea, and asthma, while the stem is used to treat skin diseases and to kill intestinal worm [9–13].

The latex is used to cure the disease of leprosy, wounds, boils, to stop bleeding in the area of bleeding, painful joints, swelling, skin burn, snake bites, intestinal worms and splenic and hepatic enlargement, abortion of babies, and contraction of muscle of uterus during baby birth. Leaves are used for the treatment of joint pain and swelling and the body with paralyzed parts is treated with the oil extracted from leaves. Flowers are used to treat asthma, loss of appetite, stomachic, digestive problems, and cough [14–18]. In countries such as India, the leaves are used for worshipping purposes [19]. The picture of *Calotropis procera* plant is shown in Figure 1.

2. Materials and Methods

2.1. Plant Material. The traditional, medicinal, and therapeutic plant *Calotropis procera* (*Linn*) was analyzed for biological and GC-MS studies at the University of Turbat. Dr. Shazia Saeed, Department of Botany, University of Balochistan, Quetta, Pakistan identified *Calotropis procera* (*Linn*) and the voucher number is QUETTA 000230.

2.2. Extraction. Calotropis procera (*Linn*) was dried in its entirety for a month in the shade. This is caused by solar radiation, which eliminates bioactive chemicals found throughout the entire *Calotropis procera* (*Linn*). *Calotropis procera* (*Linn*) was dried and the whole plant was ground into a powder using a mechanical grinder. The plant powdered 0.4 kg was then macerated in 3 L of methanol for just one week, and the mixture was then concentrated at reduced pressure and below 55°C while being filtered using Whatman filter paper No. 1. This crude whole plant methanol extract of *Calotropis procera* (WMECP) was 37.1 g [20–23].

2.3. Fractionation of Crude Extract. Crude extract with nonpolar solvent such as n-hexane and polar solvent such as aqueous was fractionated into whole plant n-hexane fraction of *Calotropis procera* (WHFCP) 5 g and whole plant aqueous fraction of *Calotropis procera* (WAFCP) 14 g [20–23].

2.4. MTT Assay (HeLa, 3T3, and PC3 Cell Lines). The American Type Culture Collection was used to purchase the cell lines of HeLa, 3T3, and PC3 (ATCC). A medium resembling Dulbecco's Eagle-modified media was used. It contained 10% FBS and 2% antibiotics such as penicillin and

Figure 1: *Calotropis procera* plant.

streptomycin at 100 IU/mL and 100 μg/mL and then this overall is preserved in 5% CO_2 which is at 37°C in this assay to create a culture of cancer cell line. Cell lines were extracted once confluence had formed. In a 96-well flat, 5, 104 cells were seeded in each well. Extracts and fractions containing 50 μg/ml were added after 24 hours. Cell lines and a sample were grown in a 96-well plate for 48 hours. Fractions and extracts containing 50 μg/mL were added after 24 hours. Cell lines and a sample were grown in a 96-well plate for 48 hours. The decrease of MTT led to the formation of formazan crystals. Crystals were dissolved by adding 100 μL of DMSO, and then, in order to measure using a microplate reader, at 570 nm, the absorbance was measured. The standard drug for HeLa, 3T3, and PC3 cell lines during the MTT assay was Doxorubicin.

In order to calculate the IC_{50}, a stock solution of the fraction or extracts at a concentration of 20 mM is diluted into a working solution at a concentration of 50 μM. The working solution is then serially diluted to produce less than 50% inhibition. With the aid of the EZ-fit5 programme, the IC_{50} is determined [24].

2.5. Antileishmanial Activity. The fractions and extracts of therapeutic plants were evaluated against Leishmania major using microplates for culture (promastigotes). Leishmania major promastigotes (MHOM/PK/88/DESTO) were cultivated using bulk normal physiological saline in an NNN biphasic medium. The mixture was then concentrated at reduced pressure and below 55°C while being filtered using Whatman filter paper No. 1. There were 37.1 g of this whole plant methanol extract of *Calotropis procera* (WMECP). The leishmanial parasites were centrifuged for 10 minutes at 2000 rpm after the promastigotes were extracted at the log phase. At the same speed and timing, leishmanial major was washed three times in saline.

180 μL of the culture medium was added to each of the 96 wells of a microtitre plate. Fractions and extracts of therapeutic plants were dissolved in PBS with a pH of 7.4 and 0.5% DMSO and 0.5% MeOH to create a 1000 mg/mL stock solution. In order to create a working solution with a concentration range of 1–100 μg/mL, the fractions and extracts with a 20 μL concentration were added to wells and serially diluted. 100 μL of the parasite culture was given to each well. In this experiment, two rows remained: one for the positive control, which received typical antileishmanial drugs such as amphotericin B (Fluka) and pentamidine, and the other for the negative control, which received medium (ICN).96-well plates were then incubated at 21-22°C for 72 h. To calculate the IC_{50} values of fractions and extracts with antileishmanial activities, a Neubauer chamber with EZ-Fit 5.03 (Percella Scientific, USA) was used to count motile cells. The culture was inspected for indications of cell viability, such as [25, 26].

2.6. Antibacterial Activity. *Pseudomonas aeruginosa* (ATCC 10145), *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (ATCC 25922), and *Salmonella typhi* (ATCC 14028) were the five types of microorganisms employed in this investigation as standard bacterial strains. ICCBS, University of Karachi, Karachi, Pakistan, provided the PCMD with access to its collection of common bacterial strains.

2.6.1. Microplate Alamar Blue Assay. The antibacterial activity is assessed using this test. The growing medium for organisms was Mueller–Hinton medium. A McFarland turbidity index with a value of 0.5 was employed to modify the inoculums. By dissolving extracts in DMSO, the stock solution was created. Media was moved to each well. Except for the control well, where extracts were not added, wells had extracts added to them. The wells were made into 200 μL in size. Last but not least, 5×106 cells were added to both the test and control 96-well plates. The plate was sealed with parafilm with the aid of parafilm before being placed in an incubator for 18–20 hours. Alamar Blue Dye was applied to each well, and the plate was shaken for two to three hours at an RPM of 80. The color of the dye, for example, Alamar Blue dye was turned pink to demonstrate the development of bacteria. For the measurement of absorbance at 570 nm, an ELISA reader was used [27].

2.7. Antifungal Assay. As a standard fungal strain, seven fungi were employed in this study: *Aspergillus niger, Candida albicans, Fusarium lini, Trichophyton rubrum, Microsporum canis, Candida glabarata, and Aspergillus fumigatus.* Seven fungi were acquired from the Northern Regional Research Laboratories or given as gifts by the Karachi University Culture Collection (NRRL).

2.7.1. Agar Tube Dilution Method. It was performed by dilution of agar tubes to assess the extracts' antifungal efficacy. Extracts were dissolved in 1 ml of DMSO at a concentration of 24 mg (Merck). SDA was made by dissolving Sabouraud, 4% glucose agar, and 4 g of agar-agar in 500 cc of distilled water (Sigma-Aldrich, Germany). Through a magnetic stirrer, mixture was then carefully stirred. This growth media was steamed until it totally dissolved, and then 4 ml was added to screw-cap tubes, which were subsequently autoclaved for 15 minutes at 121°C. After the tubes had cooled to 15°C and the SDA had not yet solidified, 66.6 μl of crude extracts were placed into them. At room temperature, tubes were allowed to harden in a slanting orientation. A

4 mm-diameter portion of the fungus was injected into the tubes. In other media, the negative control and the positive control, such as standard antifungal medicines, such as DMSO, were utilised. For 3–7 days, tubes were incubated at 27–29°C. Fungal cultures were checked twice a week while they were incubating [25, 28].

2.8. Anti-Inflammatory Assay

2.8.1. Oxidative Burst Assay Using Chemiluminescence Technique. Diluted whole blood in HBSS++ with the concentration of 25 μL including $CaCl_2$ and $MgCl_2$ was used (Sigma, St. Louis, USA) as well as the fractions and extracts of medicinal plants at a concentration of 25 μL were incubated at 37°C for 15 min. 96-well plates were then used to plate this mixture (CoStar, NY, USA). The unfinished wells were filled with HBSS++. Cells and HBSS++ were introduced to the control wells. 25 μl each of serum opsonized zymosan and luminol from the St. Louis-based Sigma Chemical Co., Missouri, the United States, was warmly received (Sigma Chemical Co., St. Louis, MO, USA). Relative light units were used to measure the ROS concentration in luminometers. Ibuprofen was used as a common treatment and had an IC_{50} of 11.2 1.9. [29].

2.9. Brine Shrimp Lethality Assay

2.9.1. B-Hatching Techniques. Using this approach, at 37°C for 2 days, 50 mg of shrimp eggs was incubated on a hatching tray with filtered brine solution, dissolved in 2 mL of a solvent, such as methanol, and in 20 mg of plant extracts and fractions. The concentration was increased to 10, 100, and 1000 μg/mL by dividing this solution into three vials and adding 5, 50, and 500 μl to each. The solvent was evaporated overnight. Each container received 30 larvae by Pasteur pipette addition. Seawater (5 ml) was then added. 24 hours of incubation at a temperature of 25–27°C with light was performed. Other vials included solvent-reference cytotoxic medication along with negative and positive controls. Etoposide, 7.4625 μg/mL, was the standard drug utilised in this investigation. The Finney computer programme was used to calculate the LD50 [30].

2.10. GC-MS Analysis

2.10.1. Triple Quadrupole Acquisition Method MS Parameters. We isolated and quantified chemicals from *Calotropis procera* in a column HP-5MS. 2 μL and mass spectrometer of the fraction or extracts were directly fed into the gas chromatograph mod using the 5973 Network Mass Selective Detector (Agilent Technologies Palo Alto, CA) 6890N Network GC System (0.25 mm interior diameter, 30 m length, and 0.25 μm film width; Agilent Technologies, Palo Alto, CA), and the incorrect variety of helium gas. A split-splitless injector at 250°C was injected using a 30 : 1 split ratio.

The oven's schedule was as follows: 70°C for three minutes, followed by six minutes at 180°C, five minutes at 280°C, and finally ten minutes at 290°C. 250°C was the MSD transfer line's temperature; the MSD quadrupole temperature was 150°C, the mass spectra were at 70 eV, the ionization temperature was 230°C, and the scan was successful in the series between 35 and 300 m/z. The identification of the components of the *Calotropis procera* extract or fraction was assigned by matching their mass spectra with those available in the libraries NIST 02 and WILEY [20, 21, 31].

3. Results and Discussion

In this research study, the medicinal plant of Balochistan, *Calotropis procera* was extracted and fractionated to form whole plant methanol extract of *Calotropis procera* (WMECP), whole plant n-hexane fraction of *Calotropis procera* (WHFCP), and whole plant aqueous fraction of *Calotropis procera* (WAFCP).

All the extracts and fractions of *Calotropis procera* exhibited significant anticancer activities against the HeLa cell line. Whole plant methanol extract of *Calotropis procera* (WMECP) inhibited 69.1% of the growth of the HeLa cell line with an IC_{50} value of 3.1 ± 0.4 and whole plant n-hexane fraction of *Calotropis procera* (WHFCP) and whole plant and aqueous fraction of *Calotropis procera* (WAFCP) inhibited 70.2% and 65.2% with IC_{50} values of 5.0 ± 0.3 and 17.1 ± 1.0. The standard drug, doxorubicin inhibited the growth of HeLa cell line 100% with an IC_{50} value of 0.9 ± 0.14. Anticancer activities (HeLa cell line) of extract and fractions of *Calotropis procera* are shown in Table 1.

All the extracts and fractions of *Calotropis procera* exhibited significant anticancer activities against the PC3 cell line. Whole plant methanol extract of *Calotropis procera* (WMECP) inhibited 70.1% of the growth of the PC3 cell line with IC_{50} value 5.1 ± 0.3 and whole plant n-hexane fraction of *Calotropis procera* (WHFCP) and whole plant aqueous fraction of *Calotropis procera* (WAFCP) inhibited 61.6% and 59.7% with IC_{50} values 3.7 ± 0.5 and 16.4 ± 1.0. The standard drug, doxorubicin inhibited the growth of the HeLa cell line 89.9% with IC_{50} 1.9 ± 0.14. Anticancer activities (PC3 cell line) of extract and fractions of *Calotropis procera* are shown in Table 2.

None of the extract and fractions of *Calotropis procera* showed anticancer activities against the 3T3 cell line. The standard drug doxorubicin inhibited 96.2% of the growth of the cell line with an IC_{50} value of 0.1 ± 0.02. Anticancer activities (3T3 cell line) of extract and fractions of *Calotropis procera* are shown in Table 3.

None of the extract and fractions of *Calotropis procera* showed antileishmanial activities and had an IC_{50} above 100. The standard drugs used for antileishmanial activities are amphotericin B and pentamidine with IC_{50} values of 3.41 ± 0.02 and 4.56 ± 0.01. Antileishmanial activities of extract and fractions of *Calotropis procera* are shown in Table 4.

The extract and fractions of *Calotropis procera* were inactive against bacterial strains. Ofloxacin was the standard drug used against bacterial strains with percent inhibitions

Table 1: Anticancer activities (HeLa cell line) *Calotropis procera* extract and fractions.

S. no.	Extract/fraction/std. drug	Conc. (μg/mL)	% inhibition/stimulation	$IC_{50} \pm$ S.D.
1	WMECP	30	69.1	3.1 ± 0.4
2	WHFCP	30	70.2	5.0 ± 0.3
3	WAFCP	30	65.2	17.1 ± 1.0
5	Doxorubicin	30	100	0.9 ± 0.14

Table 2: Anticancer activities (PC3 cell line) of *Calotropis procera* extract and fractions.

S. no.	Extract/fraction/std. drug	Conc. (μg/mL)	% inhibition/stimulation	$IC_{50} \pm$ S.D.
1	WMECP	30	70.1	5.1 ± 0.3
2	WHFCP	30	61.6	3.7 ± 0.5
3	WAFCP	30	59.7	16.4 ± 1.0
5	Doxorubicin	30	89.9	1.9 ± 0.14

Table 3: Anticancer activities (3T3 cell line) of *Calotropis procera* extract and fractions.

S. no.	Extract/fraction/std. drug	Conc. (μg/mL)	% inhibition/stimulation	$IC_{50} \pm$ S.D.
1	WMECP	30	12.4	Inactive
2	WHFCP	30	14.1	Inactive
3	WAFCP	30	3.5	Inactive
5	Doxorubicin	30	96.2	0.1 ± 0.02

Table 4: Antileishmanial studies of *Calotropis procera* extract and fraction.

S. no	Extract/fractions/standard drugs	IC_{50} (μg/mL) $\pm$ S.D.
1	WMECP	>100
2	WHFCP	>100
3	WAFCP	>100
9	Amphotericin B	3.41 ± 0.02
10	Pentamidine	4.56 ± 0.01

of 92.54%, 92.41%, 93.05%, 92.68%, and 92.37% against *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli,* and *Bacillus subtilis*. Antibacterial activities of extract and fractions of *Calotropis procera* are shown in Table 5.

All extracts and fractions of *Calotropis procera* exhibited no antifungal activities. Miconazole and amphotericin B were the standard drugs used against seven strains of fungi, for instance, *Aspergillus niger*, *Microsporum canis*, *Fusarium lini*, *Candida glabarata*, *Aspergillus fumigatus*, *Candida albicans,* and *Trichphyton rubrum*. Antifungal activities of extract and fractions of *Calotropis procera* are shown in Table 6.

None of the extract and fractions of *Calotropis procera* showed anti-inflammatory activities. The standard drug used for anti-inflammatory activity is ibuprofen with 73.2% inhibition and an IC_{50} value of $11.2 \pm 1.4\,\mu$g/mL. Anti-inflammatory activities of extract and fractions of *Calotropis procera* are shown in Table 7

The whole plant methanol extract of *Calotropis procera* (WMECP) exhibited lethality at the highest concentration, while other fractions, for instance, whole plant n-hexane fraction of *Calotropis procera* (WHFCP) and whole plant aqueous fraction of *Calotropis procera* (WAFCP) did not exhibit lethality. Brine shrimp lethality bioassay of extract and fractions of *Calotropis procera* are shown in Table 8.

GC-MS studies revealed that whole plant methanol extract of *Calotropis procera* (WMECP) consists of 11 compounds such as 12-methyloctadec-11-enoic acid trimethylsilyl ester, (1S.15S)-bicyclo[13.1.0] hexadecane-2-one, cholest-5-en-3-ol. (3@), melezitose, @-D-glucose, heptane. 1.7-dibromo, 1-decanol. 9-[(trimethylsilyl)oxy]-, trifluoroacetate, 15-tetracosenoic acid, methyl ester, isosorbide, 2TBDMS derivative, oleic acid, (Z)-TMS derivative, and 3.7.11.15.18-pentaoxa-2, 19-disilaeicosane, 2,2,19.19-tetramethyl. The chromatogram of whole plant methanol extract of *Calotropis procera* (WMECP) is presented in Figure 2. Name, molecular formula, molecular mass, RT, area, and % composition of compounds 1–11 of whole plant methanol extract of *Calotropis procera* (WMECP) are shown in Tables 9 and 10. Mass spectra of compounds 1–11 of whole plant methanol extract of *Calotropis procera* (WMECP) are shown in Tables 11 and 12. Structure and mass spectra of compounds 1–11 of whole plant methanol extract of *Calotropis procera* (WMECP) are shown in Figures 3–24.

Whole plant n-hexane fraction of *Calotropis procera* (WHFCP) consists of 9 compounds, for instance, 13-methyltetradec-9-enoic acid methyl ester, 4-methoxy-6-methyl-6.7-dihydro-4H-furo[3,2-c]pyran, propanoic acid. 2,2-dimethyl, 3,cis-(1,1-dimethylethyl)-4,cis-methoxycyclohexanol, 11-fluoroundecan-1-ol, TMS derivative, 3-nonen-1-ol, (Z, 3-methylpentan-1-yl trifluoroacetate, cholest-5-en-3-ol. (3a),

Table 5: Antibacterial studies of *Calotropis procera* extract and fractions.

S. no	Extract/fraction/standard drug	Inhibition % *E. coli* ATCC 25922	Inhibition % *B. subtilis* ATCC 23857	Inhibition % *S. aureus* NCTC 6571	Inhibition % *P. aeruginosa* ATCC 10145	Inhibition % *S. typhi* ATCC 14028
1	WMECP	NI	NI	NI	NI	NI
2	WHFCP	NI	NI	NI	NI	NI
3	WAFCP	NI	NI	NI	NI	NI
4	Ofloxacin	92.68%	92.37%	92.54%	92.41%	93.05%

Table 6: Antifungal studies of *Calotropis procera* extract and fractions.

S. no	Name of fungus	WMECP Inhibition (%)	WHFCP Inhibition (%)	WAFCP Inhibition (%)	Mic (μg/mol)	Linear growth (mm) of control	Linear growth (mm) of sample	Std. drug
1	*C. albicans*	0	0	0	110	100	100	Miconazole
2	*T. rubrum*	0	0	0	70	100	100	Miconazole
3	*A. niger*	0	0	0	20	100	100	Amphotericin B
4	*M. canis*	0	0	0	98.4	100	100	Miconazole
5	*F. lini*	0	0	0	73.25	100	100	Miconazole
6	*C. glabarata*	0	0	0	110.8	100	100	Miconazole
7	*Aspergillus fumigatus*	0	0	0	100	100	100	Amphotericin B

Table 7: Anti-inflammatory studies of *Calotropis procera* extract and fractions.

S. no	Extract/fraction/std. drug	Conc. (μg/mL)	% inhibition/stimulation	$IC_{50} \pm$ S.D.
1	WMECP	50	−20.1	—
2	WHFCP	50	12.8	—
3	WAFCP	50	−3.7	—
5	Ibuprofen	25	73.2	11.2 ± 1.4 μg/mL

Table 8: Brine shrimp lethality bioassay of *Calotropis procera* extract and fractions.

S. no	Extract and fractions	Dose (μg/mL)	No. of shrimps	No. of survivors	Mortality %	LD50 (μg/ml)	STD. Drug	LD_{50} (μg/ml)	Mortality%
1	WMECP	10	30	29	3.3		Etoposide	7.5	70
		100	30	26	13.3				
		1000	30	18	40				
2	WHFCP	10	30	30	0		Etoposide	7.5	70
		100	30	29	3.33				
		1000	30	29	3.33				
3	WAFCP	10	30	30	0		Etoposide	7.5	70
		100	30	29	3.33				
		1000	30	29	3.33				

and retinal. The chromatogram of whole plant n-hexane fraction of *Calotropis procera* (WHFCP) is presented in Figure 25. Name, molecular formula, molecular mass, RT, area, and % composition of compounds 1–9 of whole plant n-hexane fraction of *Calotropis procera* (WHFCP) are shown in Tables 13 and 14. Mass spectra of compounds 1–9 of whole plant n-hexane fraction of *Calotropis procera* (WHFCP) are shown in Tables 15 and 16. Structures and mass spectra of whole plant n-hexane fraction of *Calotropis procera* (WHFCP) are shown in Figures 26–41.

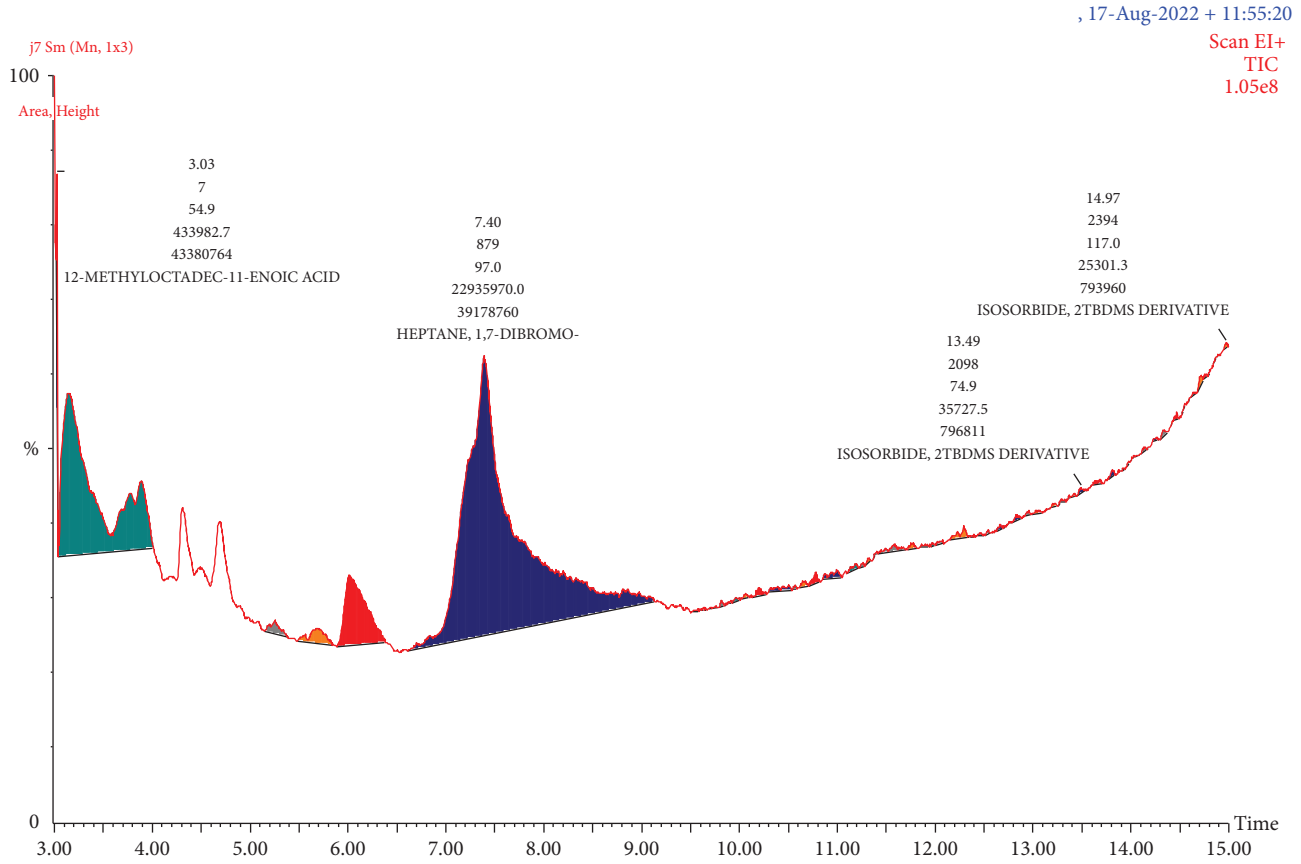

Figure 2: Chromatogram of whole plant methanol extract of *Calotropis procera* (WMECP).

Table 9: GC-MS of compounds 1–5 of methanol extract of *Calotropis procera*.

Compound	Name	Molecular formula	Molecular Mass	RT	Area	% composition
1	12-Methyloctadec-11-enoic acid trimethylsilyl ester	$C_{22}H_{44}O_2Si$	368	3.033	433982.7	1.167034018274278
2	(1S.15S)-Bicyclo[13.1.0]hexadecane-2-one	$C_{16}H_{28}O$	236	3.153	9035313	24.2970927834702
3	Cholest-5-en-3-ol, (3@)-	$C_{27}H_{46}O$	386	5.0259	222203.6	0.597533321427677
4	Melezitose	$C_{18}H_{32}O_{16}$	504	5.675	440087.3	1.18345020587543
5	@-D-Glucose	$C_6H_{12}O_6$	180	6.02	2432547	6.54142408623575

Table 10: GC-MS of compounds 6–11 of methanol extract of *Calotropis procera*.

Compound	Name	Molecular formula	Molecular Mass	RT	Area	% composition
6	Heptane. 1.7-dibromo-	$C_7H_{14}Br_2$	380	7.395	22935970	61.6777073654105
7	1-Decanol. 9-[(trimethylsilyl)oxy]-, trifluoroacetate	$C_{15}H_{29}F_3O_3Si$	342	9.586	7243.527	0.0194787549251
8	15-Tetracosenoic acid, methyl ester	$C_{25}H_{48}O_2$	380	10.577	14132.67	0.038004524023802
9	Isosorbide, 2TBDMS derivative	$C_{18}H_{38}O_4Si_2$	374	12.292	124957.5	0.336026451531588
10	Oleic acid, (Z)-. TMS derivative	$C_{21}H_{42}O_2Si$	354	13.443	893.462	0.002402631664504
11	3.7.11.15.18-Pentaoxa-2, 19-disilaeicosane, 2,2,19.19-tetramethyl-	$C_{17}H_{40}O_5Si_2$	380	13.558	972.03	0.002613910895872

Table 11: Mass spectra of compounds 1–5 of whole plant methanol extract of *Calotropis procera* (WMECP).

Compound	m/z (% relative abundance)
1	368(M^+), 353(438), 243(329), 129(300), 117(389), 83(300), 77(609), 75(999), 73(879), 69(558), 55(820)
2	236(M^+), 98(340), 98(370), 96(310), 95(460), 82(430), 81(620), 71(400), 69(580), 67(580), 55(999)
3	386(M^{+962}), 368(755), 275(414), 107(427), 95(449), 81(465), 71(609), 69(491), 57(999), 55(641)
4	504(M^+), 97(603), 73(999), 71(364), 69(383), 61(361), 60(705), 57(587), 55(353), 43(534), 29(451)
5	180(M^+), 73(999), 71(296), 61(603), 60(826), 57(244), 45(190), 44(196), 43(500), 31(391), 29(293)

Table 12: Mass spectra of compounds 6–11 of whole plant methanol extract of *Calotropis procera* (WMECP).

Compound	m/z (% relative abundance)
6	380(M^+), 348(280), 97(260), 83(410), 74(500), 69(570), 67(250), 57(350), 55(999), 43(600), 41(650)
7	342(M^+), 117(534), 103(305), 97(498), 83(999), 75(377), 73(578), 69(550), 57(234), 55(649), 41(168)
8	380(M^+), 348(280), 97(260), 83(410), 74(500), 69(570), 67(250), 57(350), 55(999), 43(600), 41(650)
9	374(M^+), 317(184), 185(152), 133(214), 129(202), 117(999), 101(180), 75(400), 73(845), 69(753), 59(207)
10	354(M^+), 339(437), 145(299), 129(450), 117(634), 75(881), 73(999), 67(257), 55(549), 43(305), 41(404)
11	380(M^+), 131(461), 130(293), 129(178), 117(584), 116(277), 115(154), 103(350), 75(198), 73(999), 58(146)

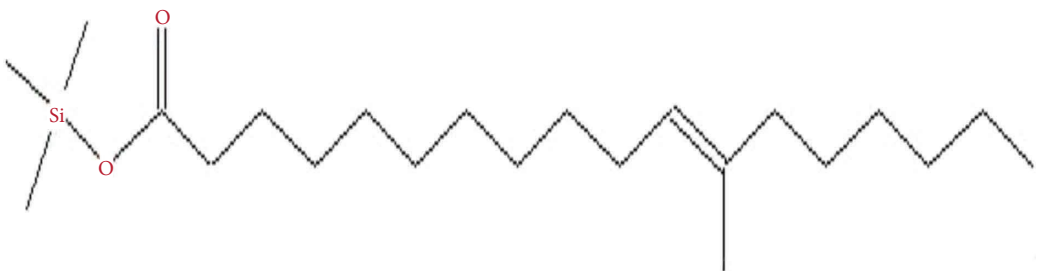

Figure 3: Structure of 12-methyloctadec-11-enoic acid trimethylsilyl ester of whole plant methanol extract of *Calotropis procera* (WMECP).

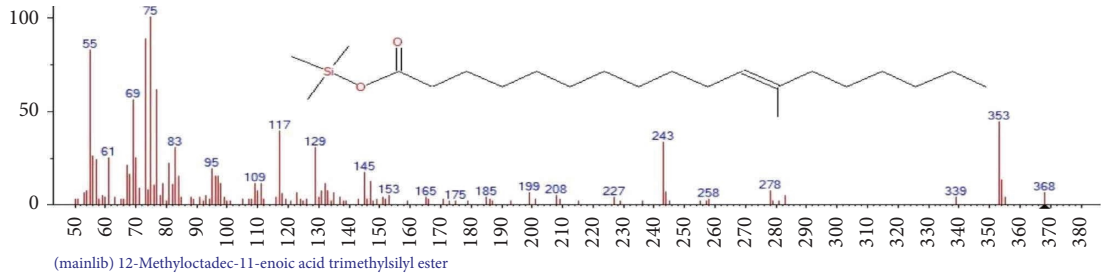

Figure 4: Mass spectrum of 12-methyloctadec-11-enoic acid trimethylsilyl ester of whole plant methanol extract of *Calotropis procera* (WMECP).

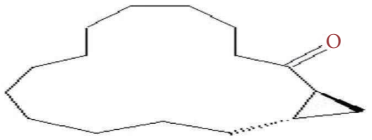

Figure 5: Structure of (1S.15S)-bicyclo[13.1.0]hexadecane-2-one of whole plant methanol extract of *Calotropis procera* (WMECP).

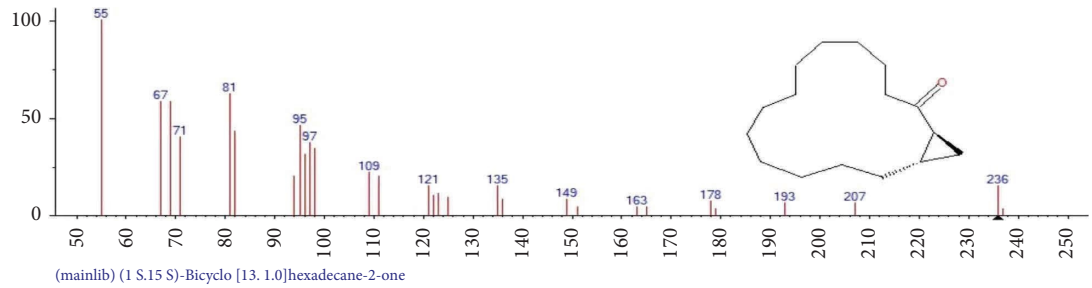

Figure 6: Mass spectrum of (1S.15S)-bicyclo[13.1.0]hexadecane-2-one of whole plant methanol extract of *Calotropis procera* (WMECP).

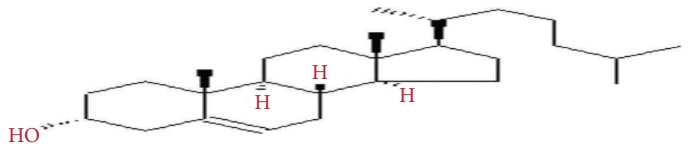

Figure 7: Structure of cholest-5-en-3-ol of whole plant methanol extract of *Calotropis procera* (WMECP).

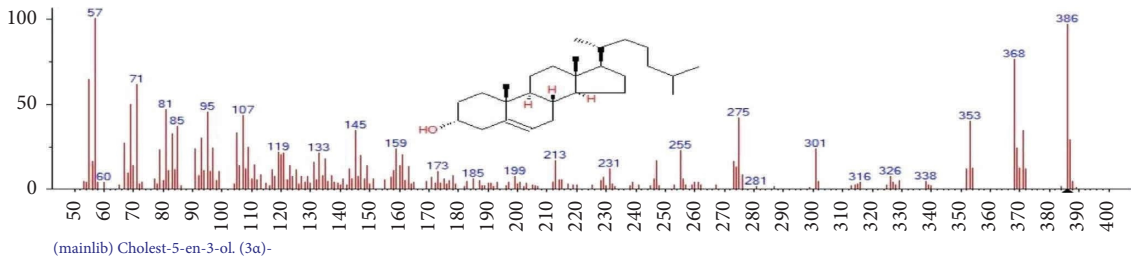

Figure 8: Mass spectrum of cholest-5-en-3-ol. (3@)- of whole plant methanol extract of *Calotropis procera* (WMECP).

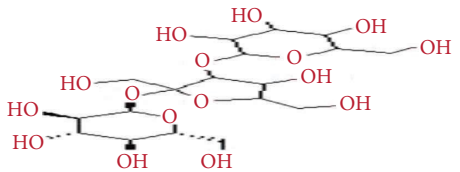

Figure 9: Structure of melezitose of whole plant methanol extract of *Calotropis procera* (WMECP).

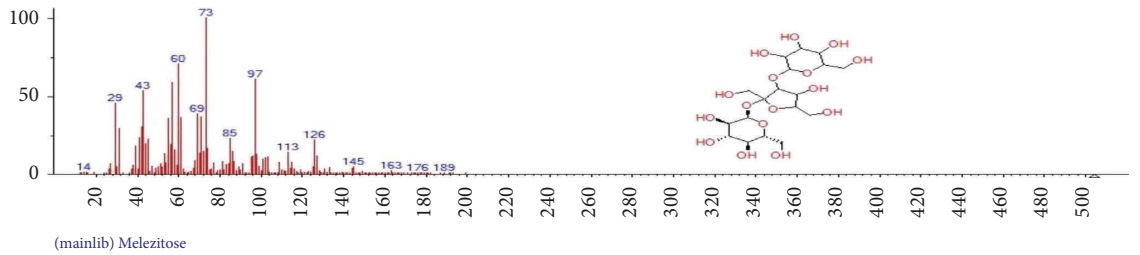

Figure 10: Mass spectrum of melezitose of whole plant methanol extract of *Calotropis procera* (WMECP).

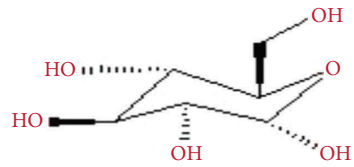

Figure 11: Structure of @-D-glucose of whole plant methanol extract of *Calotropis procera* (WMECP).

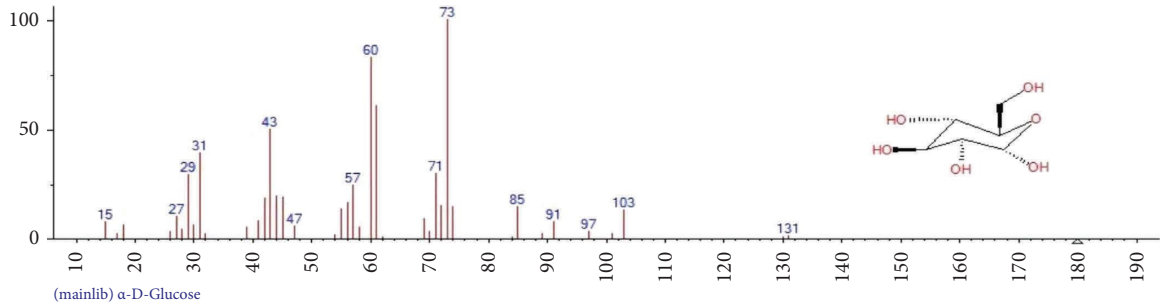

Figure 12: Mass spectrum of @-D-glucose of whole plant methanol extract of *Calotropis procera* (WMECP).

Figure 13: Structure of heptane. 1.7-dibromo- of whole plant methanol extract of *Calotropis procera* (WMECP).

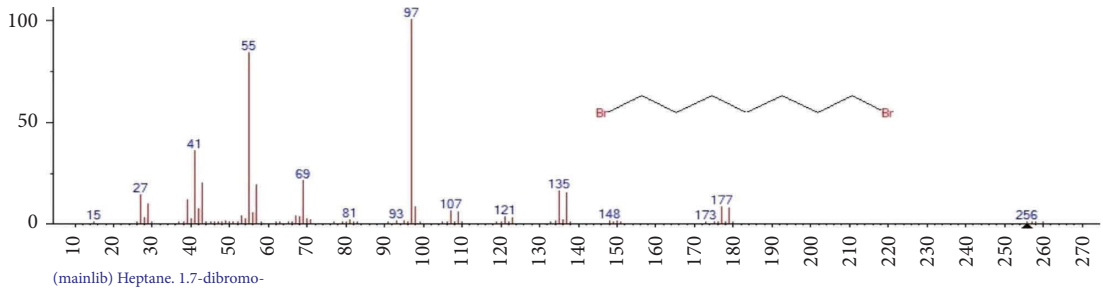

Figure 14: Mass spectrum of heptane. 1.7-dibromo- of whole plant methanol extract of *Calotropis procera* (WMECP).

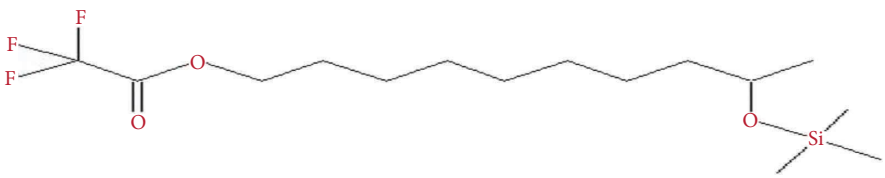

Figure 15: Structure of 1-decanol. 9-[(trimethylsilyl)oxy]-, trifluoroacetate of whole plant methanol extract of *Calotropis procera* (WMECP).

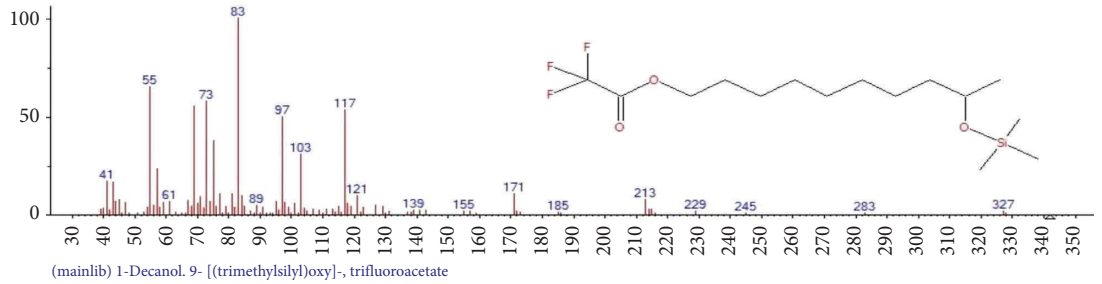

Figure 16: Mass spectrum of 1-decanol. 9-[(trimethylsilyl)oxy]-, trifluoroacetate of whole plant methanol extract of *Calotropis procera* (WMECP).

Figure 17: Structure of 15-Tetracosenoic acid, methyl ester of whole plant methanol extract of *Calotropis procera* (WMECP).

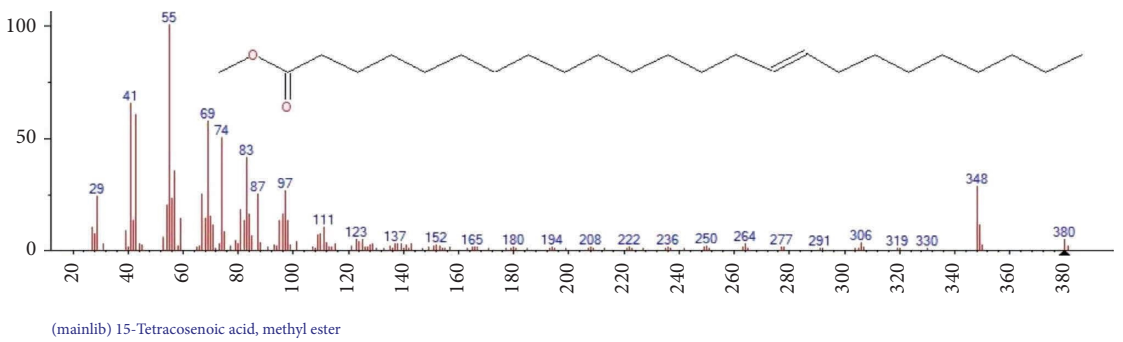

Figure 18: Mass spectrum of 15-tetracosenoic acid, methyl ester of whole plant methanol extract of *Calotropis procera* (WMECP).

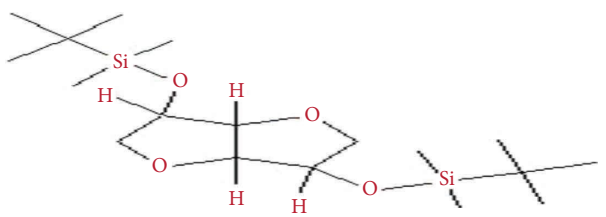

Figure 19: Structure of isosorbide, 2TBDMS derivative of whole plant methanol extract of *Calotropis procera* (WMECP).

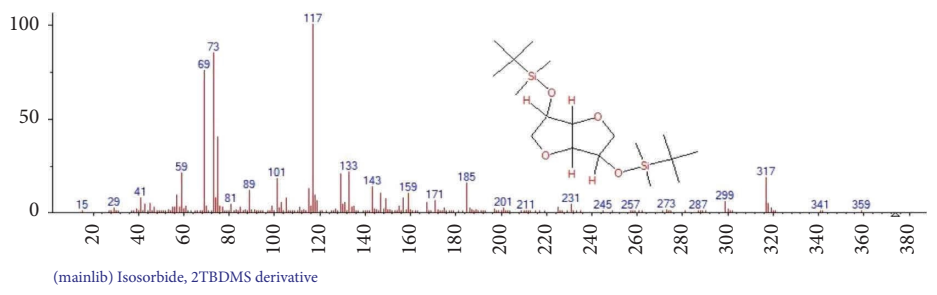

Figure 20: Mass spectrum of isosorbide, 2TBDMS derivative of whole plant methanol extract of *Calotropis procera* (WMECP).

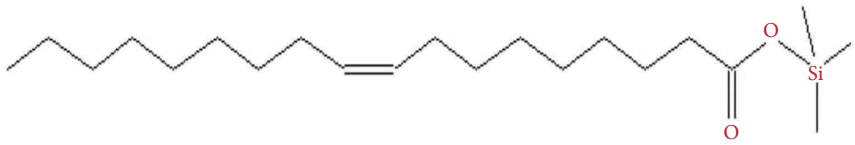

Figure 21: Structure of oleic acid, (Z)- TMS derivative of whole plant methanol extract of *Calotropis procera* (WMECP).

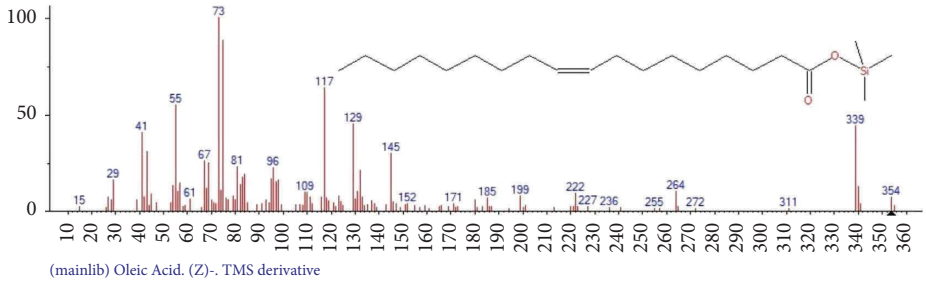

Figure 22: Mass spectrum of oleic acid, (Z)- TMS derivative of whole plant methanol extract of *Calotropis procera* (WMECP).

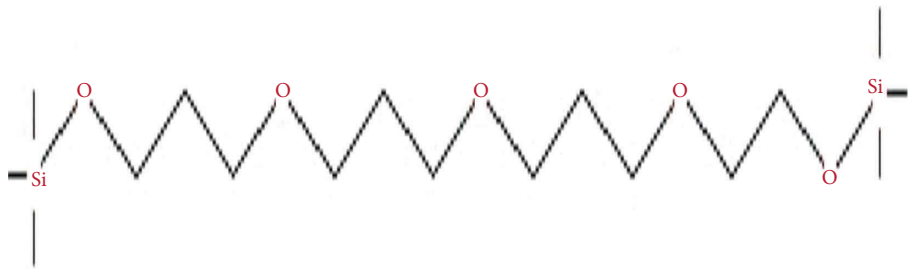

Figure 23: Structure of 3.7.11.15.18-pentaoxa-2, 19-disilaeicosane, 2,2,19.19-tetramethyl- of whole plant methanol extract of *Calotropis procera* (WMECP).

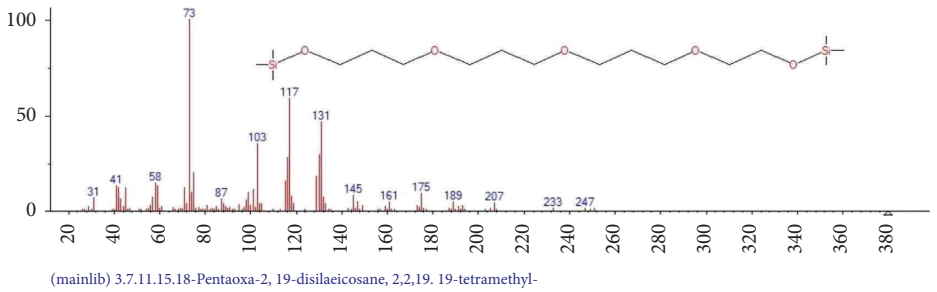

Figure 24: Mass spectrum of 3.7.11.15.18-pentaoxa-2, 19-disilaeicosane, 2,2,19.19-tetramethyl- of whole plant methanol extract of *Calotropis procera* (WMECP).

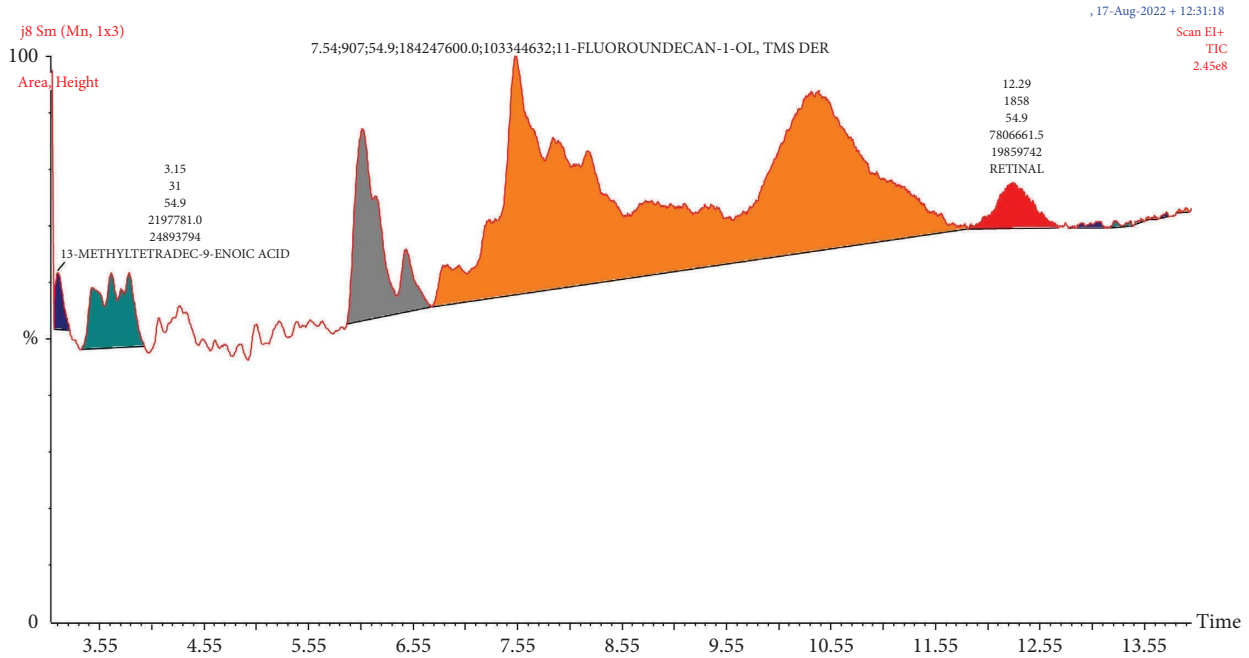

Figure 25: Chromatogram of whole plant n-hexane fraction of *Calotropis procera* (WHFCP).

Table 13: GC-MS of compounds 1–5 of n-hexane fraction of *Calotropis procera*.

Compound	Name	Molecular formula	Molecular mass	RT	Area	% composition
1	13-Methyltetradec-9-enoic acid methyl ester	$C_{16}H_{30}O_2$	254	3.153	2197781	0.945739463770572
2	4-Methoxy-6-methyl-6.7-dihydro-4H-furo[3,2-c] pyran	$C_9H_{12}O_3$	168	4.989	15017268	25.8524569440841
3	Propanoic acid. 2,2-dimethyl-	$C_5H_{10}O_2$	102	5.599	6589.031	0.389520015937791

Table 14: GC-MS of compounds 6–9 of n-hexane fraction of *Calotropis procera*.

Compound	Name	Molecular formula	Molecular mass	RT	Area	% composition
6	3-Nonen-1-ol, (Z	$C_9H_{18}O$	142	9.986	7863172	13.5365710709783
7	3-Methylpentan-1-yl trifluoroacetate	$C_8H_{13}F_3O_2$	198	11.697	13496.69	0.02323476206308
8	Cholest-5-en-3-ol. (3a)-	$C_{27}H_{46}O$	386	13.118	549191.188	0.236325539099047
9	Retinal	$C_{20}H_{28}O$	284	12.292	7806661.5	3.35932827740724

Table 15: Mass spectra of compounds 1–5 of whole plant n-hexane fraction of *Calotropis procera* (WHFCP).

Compound	m/z (% relative abundance)
1	254(M^+), 96(348), 87(348), 84(399), 83(408), 74(588), 69(910), 67(327), 57(389), 56(700), 55(999)
2	168(M^+), 124(210), 137(160), 124(210), 109(240), 83(160), 81(160), 69(200), 59(270), 58(999), 55(260)
3	102(M^+), 87(47), 59(56), 58(44), 57(999), 56(60), 45(38), 41(436), 39(99), 29(292), 27(80)
4	186(M^+), 97(234), 95(192), 80(354), 72(999), 71(329), 70(235), 69(289), 58(260), 57(648), 55(311)
5	262(M^+), 107(321), 103(314), 97(598), 83(750), 75(247), 73(460), 69(926), 55(999), 43(300), 41(282)

Table 16: Mass spectra of compounds 6–9 of whole plant n-hexane fraction of *Calotropis procera* (WHFCP).

Compound	m/z (% relative abundance)
6	142(M^+), 95(407), 82(454), 81(707), 69(601), 68(779), 67(683), 57(327), 55(999), 54(466), 41(736)
7	198(M^+), 85(118), 84(262), 69(999), 57(623), 56(352), 55(799), 43(203), 41(480), 39(117), 29(206)s
8	386($M^{+,962}$), 368(755), 275(414), 107(427), 95(449), 81(465), 69(491), 71(609), 57(999), 55(641)
9	284(M^+), 119(690), 109(570), 105(730), 95(860), 91(999), 81(550), 79(530) 77(530), 69(730), 55(670)

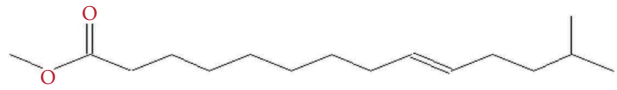

Figure 26: Structure of 13-methyltetradec-9-enoic acid methyl ester of whole plant n-hexane fraction of *Calotropis procera* (WHFCP).

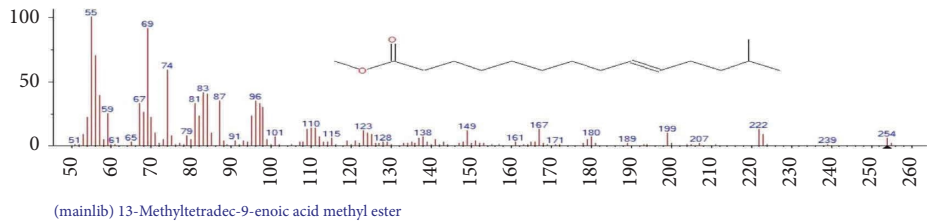

Figure 27: Mass spectra of 13-methyltetradec-9-enoic acid methyl ester of whole plant n-hexane fraction of *Calotropis procera* (WHFCP).

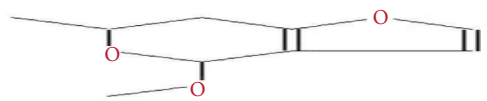

Figure 28: Structure of 4-methoxy-6-methyl-6.7-dihydro-4H-furo[3,2-c]pyran of whole plant n-hexane fraction of *Calotropis procera* (WHFCP).

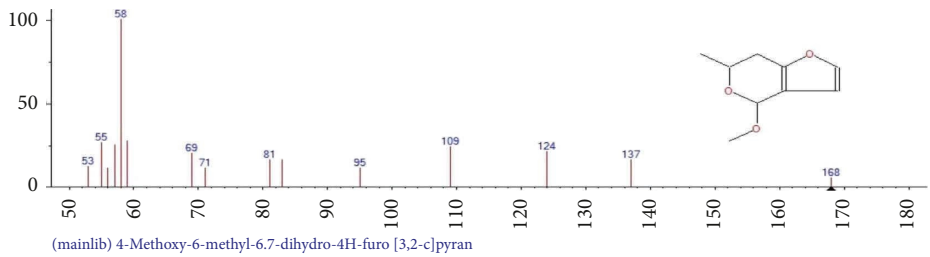

Figure 29: Mass spectra of 4-methoxy-6-methyl-6.7-dihydro-4H-furo[3,2-c]pyran of whole plant n-hexane fraction of *Calotropis procera* (WHFCP).

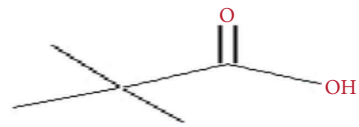

Figure 30: Structure of propanoic acid. 2,2-dimethyl- of whole plant n-hexane fraction of *Calotropis procera* (WHFCP).

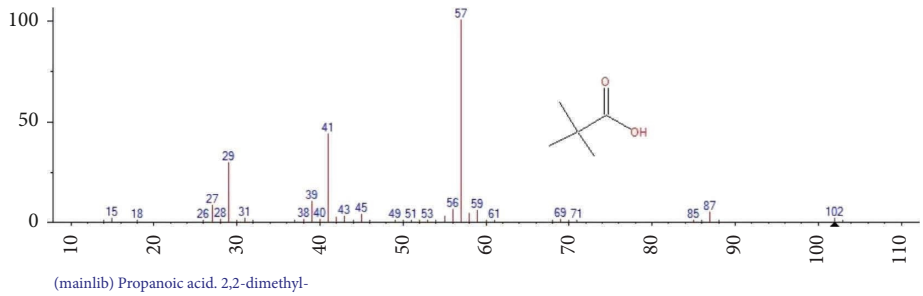

Figure 31: Mass spectra of propanoic acid. 2,2-dimethyl-of whole plant n-hexane fraction of *Calotropis procera* (WHFCP).

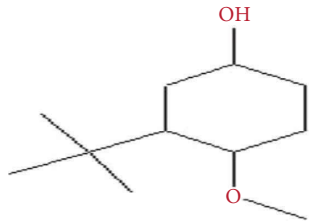

Figure 32: Structure of 3,cis-(1,1-dimethylethyl)-4,cis-methoxycyclohexanol of whole plant n-hexane fraction of *Calotropis procera* (WHFCP).

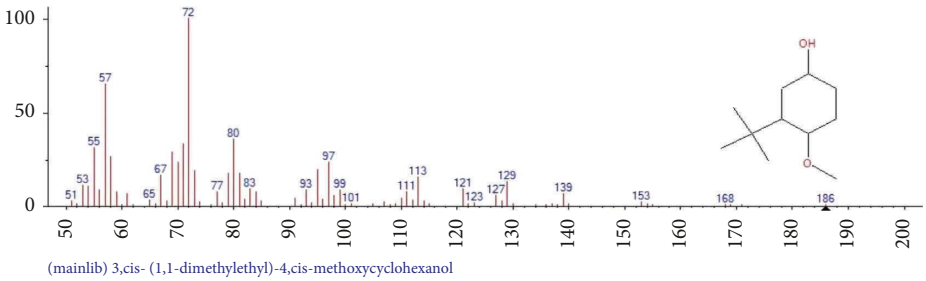

Figure 33: Mass spectra of 3,cis-(1,1-dimethylethyl)-4,cis-methoxycyclohexanol of whole plant n-hexane fraction of *Calotropis procera* (WHFCP).

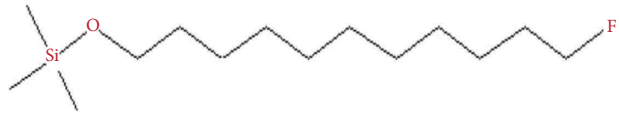

Figure 34: Structure of 11-fluoroundecan-1-ol, TMS derivative of whole plant n-hexane fraction of *Calotropis procera* (WHFCP).

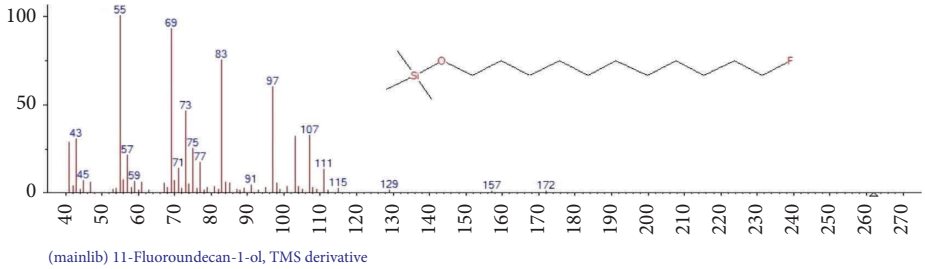

Figure 35: Mass spectra of 11-fluoroundecan-1-ol, TMS derivative of whole plant n-hexane fraction of *Calotropis procera* (WHFCP).

Figure 36: Structure of 3-nonen-1-ol, (Z of whole plant n-hexane fraction of *Calotropis procera* (WHFCP)).

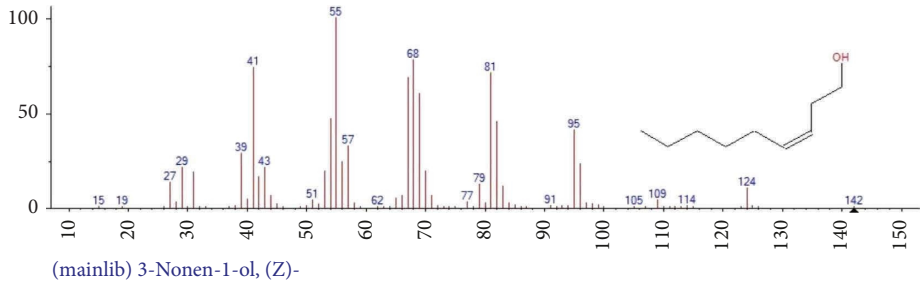

Figure 37: Mass spectra of 3-nonen-1-ol, (Z of whole plant n-hexane fraction of *Calotropis procera* (WHFCP)).

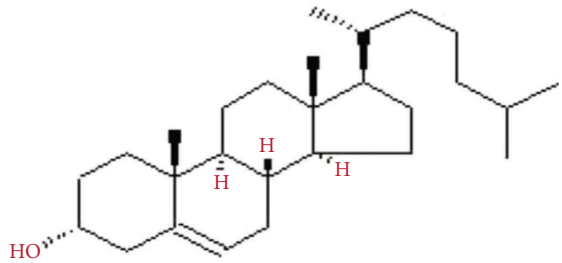

Figure 38: Structure of cholest-5-en-3-ol. (3a)- of whole plant n-hexane fraction of *Calotropis procera* (WHFCP).

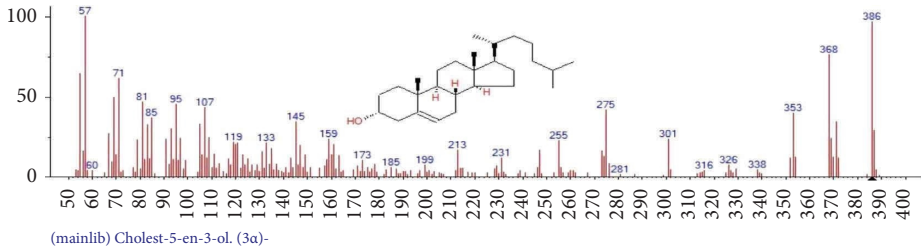

Figure 39: Mass spectra of cholest-5-en-3-ol. (3a)- of whole plant n-hexane fraction of *Calotropis procera* (WHFCP).

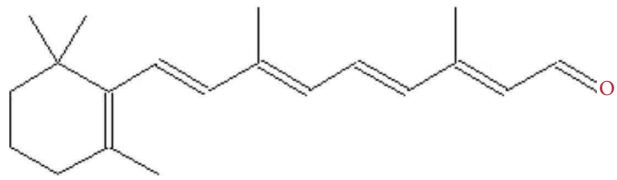

Figure 40: Structure of retinal of whole plant n-hexane fraction of *Calotropis procera* (WHFCP).

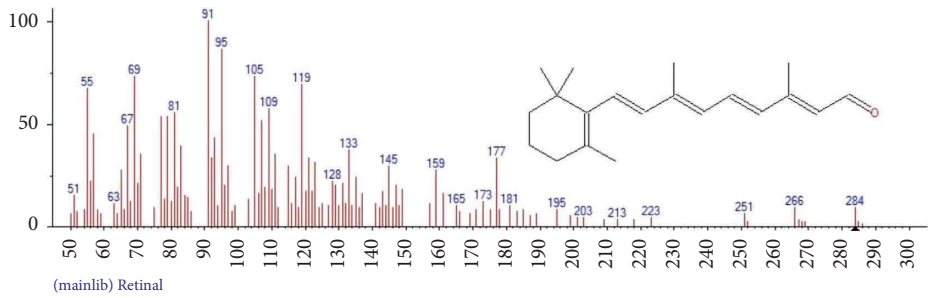

Figure 41: Mass spectra of retinal of whole plant n-hexane fraction of *Calotropis procera* (WHFCP).

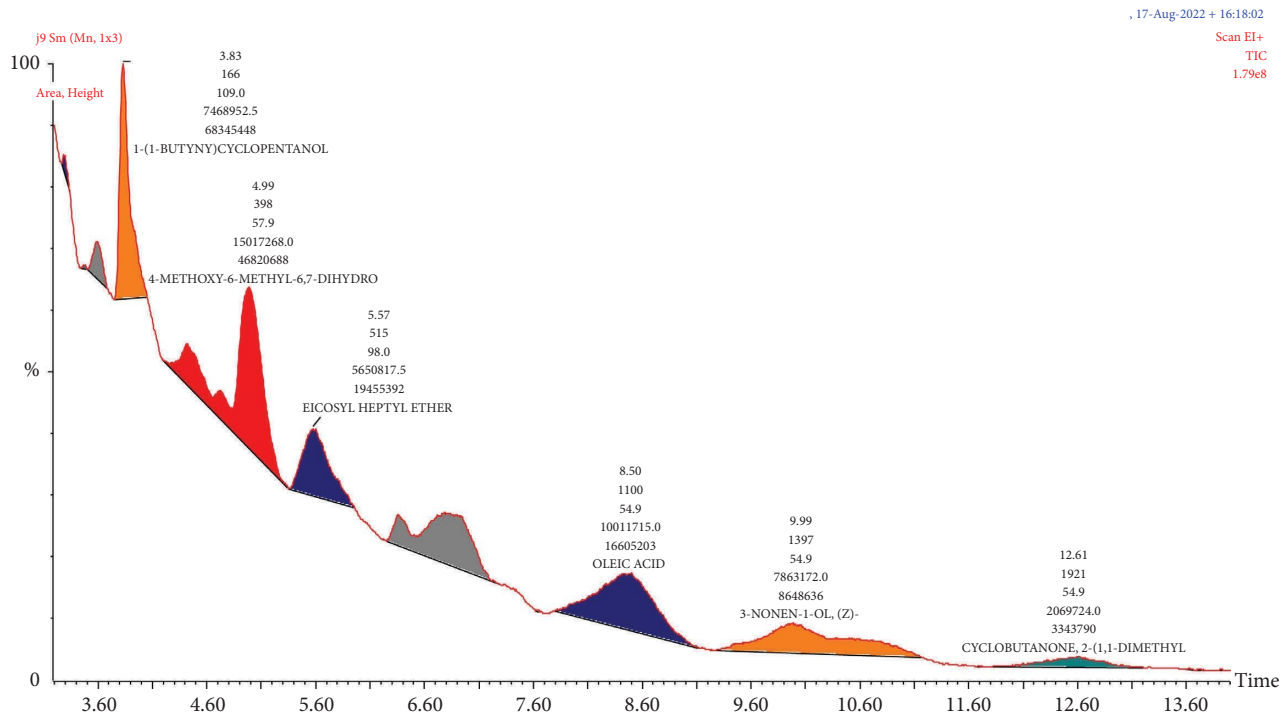

Figure 42: Chromatogram of whole plant aqueous fraction of *Calotropis procera* (WAFCP).

Whole plant aqueous fraction of *Calotropis procera* (WAFCP) consists of 7 compounds such as 1-(1-butynyl) cyclopentanol, eicosyl heptyl ether, 1,2-trans-1,5-trans-2,5-dihydroxy-4-methyl-1-(-1-hydroxy-1-isopropyl) cyclohex-3-ene, 3-methyl-2(2-oxopropyl)furan, oleic acid, 5,5-dimethyl-cyclohex-3-en-1-ol, and 1H-imidazole, 1-(-1 oxopentyl). A chromatogram of the whole plant aqueous fraction of *Calotropis procera* (WAFCP) is presented in Figure 42. Name molecular formula, molecular mass, RT, area, and % composition of compounds 1–7 of whole plant aqueous fraction of *Calotropis procera* (WAFCP) are shown in Table 17. Mass spectra of compounds 1–7 of whole plant aqueous fraction of *Calotropis procera* (WAFCP) are shown in Table 18. Structures and mass spectra of whole plant aqueous fraction of *Calotropis procera* (WAFCP) are shown in Figures 43–56.

Table 17: GC-MS of compounds 1–7 of aqueous fraction of *Calotropis procera*.

Compound	Name	Molecular formula	Molecular mass	RT	Area	% composition
1	1-(1-Butynyl)cyclopentanol	$C_9H_{14}O$	138	3.829	15017268	9.72796890337357
2	Eicosyl heptyl ether	$C_{27}H_{56}O$	396	3.574	5650818	9.72796890337357
3	1,2-trans-1,5-trans-2,5-dihydroxy-4-methyl-1-(-1-hydroxy-1-isopropyl) cyclohex-3-ene	$C_{10}H_{18}O_3$	186	6.22	2676.132	0.00460700223947
4	3-Methyl-2(2-oxopropyl)furan	$C_8H_{10}O_2$	138	6.78	8543020	14.7069398190436
5	Oleic acid	$C_{18}H_{34}O_2$	282	8.501	10011715	17.2353207636663
6	5,5-Dimethyl-cyclohex-3-en-1-ol	$C_8H_{14}O$	126	13.935	136.373	0.00023476820889
7	1H-Imidazole, 1-(-1 oxopentyl)	$C_8H_{12}N_2O_3$	152	13.973	921.289	0.00158601312872

Table 18: Mass spectra of compounds 1–7 of whole plant aqueous fraction of *Calotropis procera* (WAFCP).

Compound	m/z (% relative abundance)
1	138(M^+), 109(999), 95(454), 81(568), 79(297), 67(511), 55(397), 53(433), 41(354), 39(379), 27(402)
2	396(M^+), 99(193), 97(263), 85(168), 83(239), 71(253), 70(205), 69(235), 57(999), 55(243), 43(300)
3	186(M^+), 110(420), 109(470), 95(940), 81(270), 71(999), 70(860), 69(500), 67(310), 59(950), 55(350)
4	138(M^+), 109(240), 95(450), 71(450), 70(250), 69(680), 67(230), 65(210), 57(999), 56(260), 55(770)
5	282(M^+), 83(299), 69(425), 67(253), 57(241),55(824), 54(254), 43(595), 41(999), 29(504) 27(258),
6	126(M^+), 93(197), 85(817), 83(578), 82(932), 69(173), 67(767), 57(999), 56(360), 55(890), 53(240)
7	152($M^{+,137}$), 85(571), 69(809), 68(257), 57(999), 55(246), 41(593), 40(199), 29(413), 27(149)

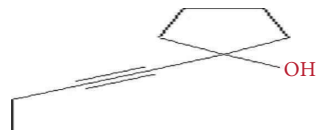

Figure 43: Structure of 1-(1-butynyl) cyclopentanol of whole plant aqueous fraction of *Calotropis procera* (WAFCP).

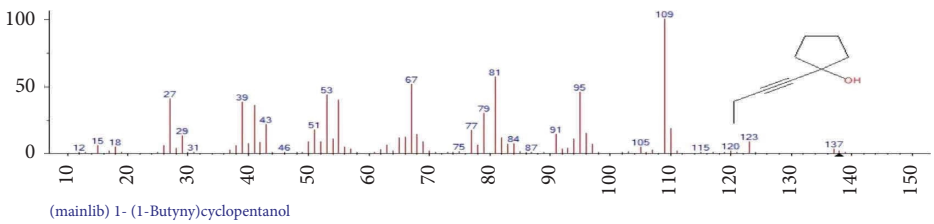

Figure 44: Mass spectrum of 1-(1butynyl) cyclopentanol of whole plant aqueous fraction of *Calotropis procera* (WAFCP).

Figure 45: Structure of eicosyl heptyl ether of whole plant aqueous fraction of *Calotropis procera* (WAFCP).

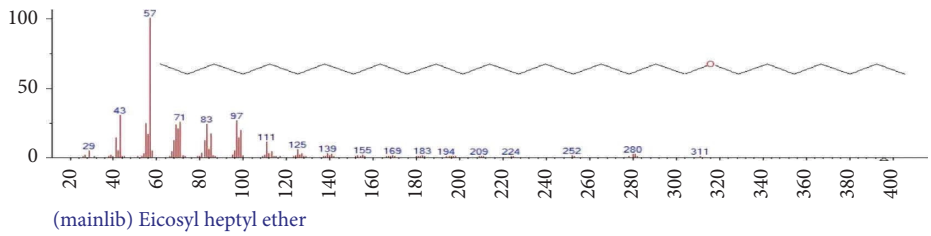

Figure 46: Mass spectrum of eicosyl heptyl ether of whole plant aqueous fraction of *Calotropis procera* (WAFCP).

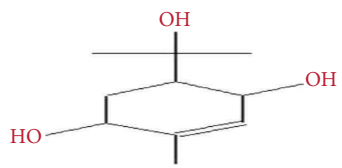

Figure 47: Structure of 1,2-trans-1,5-trans-2,5-dihydroxy-4-methyl-1-(-1-hydroxy-1-isopropyl)cyclohex-3-ene of whole plant aqueous fraction of *Calotropis procera* (WAFCP).

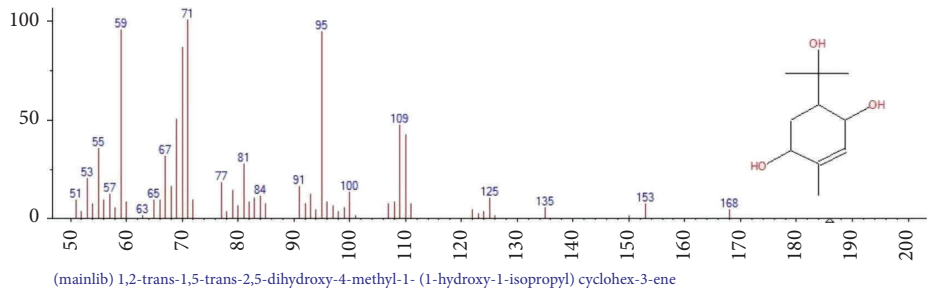

Figure 48: Mass spectrum of 1,2-trans-1,5-trans-2,5-dihydroxy-4-methyl-1-(-1-hydroxy-1-isopropyl)cyclohex-3-ene of whole plant aqueous fraction of *Calotropis procera* (WAFCP).

Figure 49: Structure of 3-methyl-2(2-oxopropyl)furan of whole plant aqueous fraction of *Calotropis procera* (WAFCP).

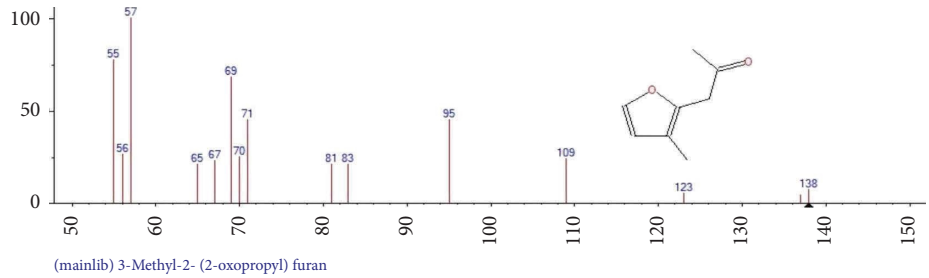

Figure 50: Mass spectrum of 3-methyl-2(2-oxopropyl)furan of whole plant aqueous fraction of *Calotropis procera* (WAFCP).

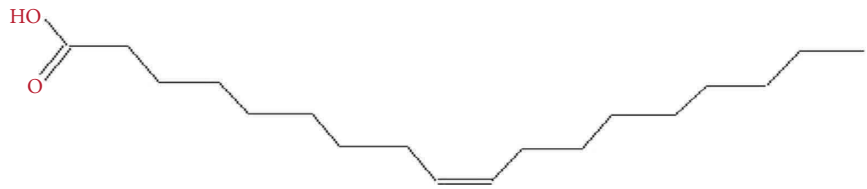

Figure 51: Structure of oleic acid of whole plant aqueous fraction of *Calotropis procera* (WAFCP).

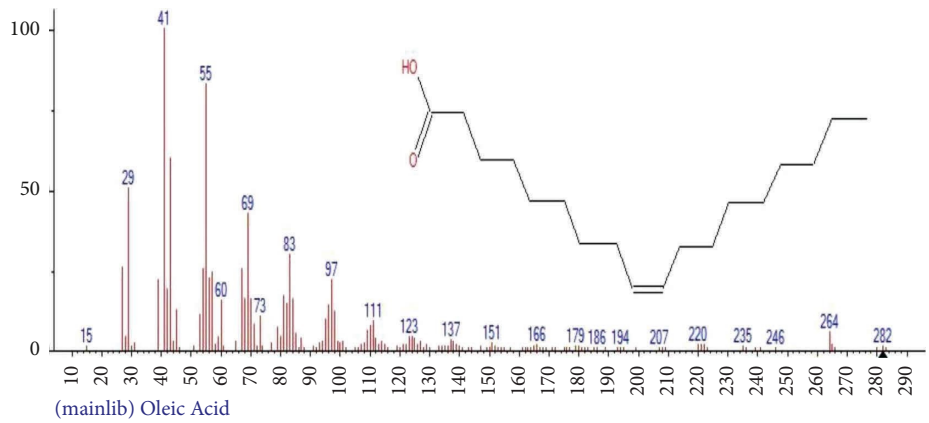

Figure 52: Mass spectrum of oleic acid of whole plant aqueous fraction of *Calotropis procera* (WAFCP).

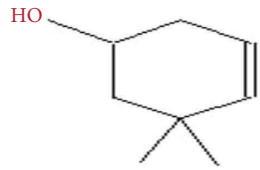

Figure 53: Structure of 5,5-dimethyl-cyclohex-3-en-1-ol of whole plant aqueous fraction of *Calotropis procera* (WAFCP).

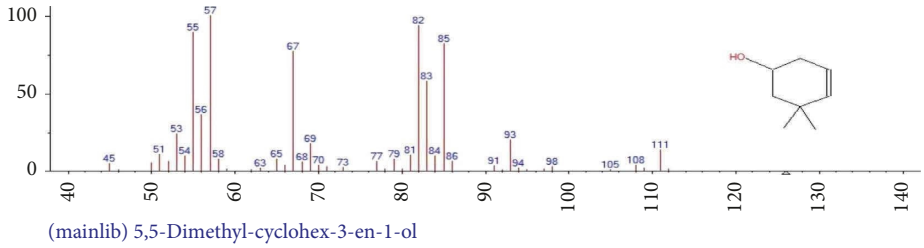

Figure 54: Mass spectrum of 5,5-dimethyl-cyclohex-3-en-1-ol of whole plant aqueous fraction of *Calotropis procera* (WAFCP).

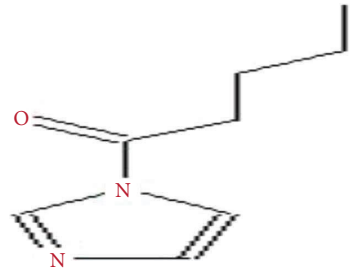

Figure 55: Structure of 1H-imidazole, 1-(-1 oxopentyl) of whole plant aqueous fraction of *Calotropis procera* (WAFCP).

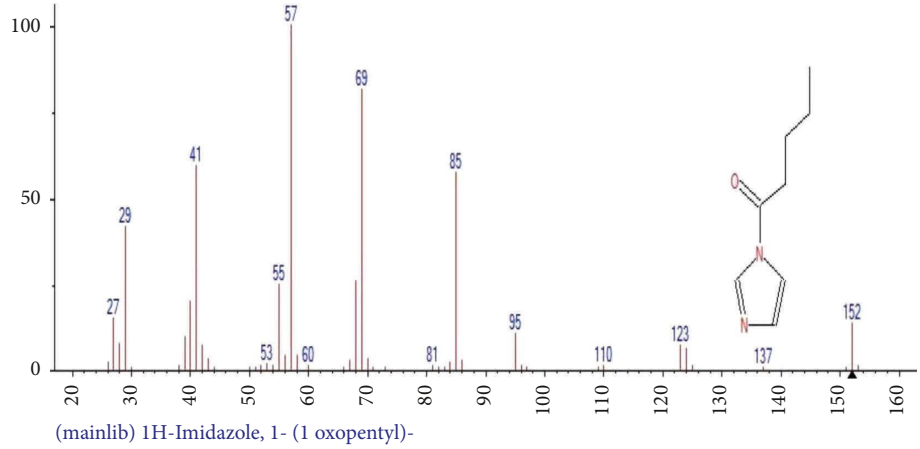

Figure 56: Mass spectrum of 1H-imidazole, 1-(-1 oxopentyl) of whole plant aqueous fraction of *Calotropis procera* (WAFCP).

4. Conclusion

None of the extract and fractions of *Calotropis procera* showed antileishmanial activities and had an IC_{50} above 100. The extract and fractions of *Calotropis procera* were inactive against bacterial strains. All extracts and fractions of *Calotropis procera* exhibited no antifungal activities.

All the extracts and fractions of *Calotropis procera* exhibited significant anticancer activities against the HeLa cell line. Whole plant methanol extract of *Calotropis procera* (WMECP) inhibited 69.1% of the growth of HeLa cell line with an IC_{50} value of 3.1 ± 0.4 and whole plant n-hexane fraction of *Calotropis procera* (WHFCP) and whole plant and aqueous fraction of *Calotropis procera* (WAFCP) inhibited 70.2% and 65.2% with IC_{50} values of 5.0 ± 0.3 and 17.1 ± 1.0. All the extracts and fractions of *Calotropis procera* exhibited significant anticancer activities against the PC3 cell line. Whole plant methanol extract of *Calotropis procera*

(WMECP) inhibited 70.1% of the growth of the PC3 cell line with an IC_{50} value of 5.1 ± 0.3 and whole plant n-hexane fraction of *Calotropis procera* (WHFCP) and whole plant aqueous fraction of *Calotropis procera* (WAFCP) inhibited 61.6% and 59.7% of the growth with IC_{50} values of 3.7 ± 0.5 and 16.4 ± 1.0.

None of the extract and fractions of *Calotropis procera* showed anticancer activities against the 3T3 cell line. None of the extract and fractions of *Calotropis procera* showed anti-inflammatory activities. Whole plant methanol extract of *Calotropis procera* (WMECP) exhibited lethality at the highest concentration, while other fractions, for instance, whole plant n-hexane fraction of *Calotropis procera* (WHFCP) and whole plant and aqueous fraction of *Calotropis procera* (WAFCP) did not exhibit lethality.

GC-MS studies reveal that whole plant methanol extract of *Calotropis procera* (WMECP) consists of 11 compounds such as 12-methyloctadec-11-enoic acid trimethylsilyl ester, (1S.15S)-bicyclo[13.1.0] hexadecane-2-one, cholest-5-en-3-ol. (3@), melezitose, @-glucose, heptane. 1.7-dibromo, 1-decanol. 9-[(trimethylsilyl)oxy]-, trifluoroacetate, 15- tetracosenoic acid, methyl ester, isosorbide, 2TBDMS derivative, oleic acid, (Z)-TMS derivative, and 3.7.11.15.18-pentaoxa-2, 19-disilaeicosane, 2,2,19.19-tetramethyl.

Whole plant n-hexane fraction of *Calotropis procera* (WHFCP) contains 9 compounds, for instance, 13-methyltetradec-9-enoic acid methyl ester, 4-methoxy-6-methyl-6.7-dihydro-4H-furo[3,2-c]pyran, propanoic acid. 2,2-dimethyl, 3,cis-(1,1-dimethylethyl)-4,cis-methoxycyclohexanol, 11-fluoroundecan-1-ol, TMS derivative, 3-nonen-1-ol, (Z, 3-methylpentan-1-yl trifluoroacetate, cholest-5-en-3-ol. (3a), and retinal.

Whole plant and an aqueous fraction of *Calotropis procera* (WAFCP) contains 7 compounds such as 1-(1-butynyl)cyclopentanol, eicosyl heptyl ether, 1,2-trans-1,5-trans-2,5-dihydroxy-4-methyl-1-(-1-hydroxy-1-isopropyl) cyclohex-3-ene, 3-methyl-2(2-oxopropyl)furan, oleic acid, 5,5-dimethyl-cyclohex-3-en-1-ol, and 1H-Imidazole, 1-(-1 oxopentyl).

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors are thankful to Hussain Ebrahim Jamal (HEJ), Research Institute of Chemistry, University of Karachi, Karachi, Pakistan, for providing antibacterial, antifungal, anti-leishmanial, and MCF-7 cell line facilities and GC-MS analysis and Institute of Biochemistry, University of Balochistan, Quetta, Pakistan, for providing lab facilities for extraction and fractionation. The study was funded by all authors.

Supplementary Materials

Figure 1: Structure of compound 1–11 of whole plant methanol extract of*Calotropis procera*(WMECP). Figure 2: Structure of compound 1–8 of whole plant n-hexane fraction of*Calotropis procera*(WHFCP). Figure 3: Structure of compound 1–7 of whole plant aqueous fraction of*Calotropis procera*(WAFCP). (*Supplementary Materials*)

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