

***Calotropis procera* Leaves and Fruits Phytochemical and GCMS Analysis and Evaluation of their Methanolic Extracts Antimicrobial effect on Isolated Bacteria**

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ABSTRACT

This study was conducted to investigate the bioactive and chemical components, ethnobotany and pharmacological activities of *Calotropis procera* leaves and fruits with a focus on its antimicrobial effect on isolated bacteria. To prepare the plant extract, methanol was used as the solvent, for evaluation of the bioactive compounds and their concentration in the plant extracts hence, qualitative and quantitative phytochemical analysis was conducted respectively. The GCMS analysis was conducted for further determination of the chemical composition of the plant parts. The antimicrobial impacts of the plant leaves and fruits methanolic extracts were carried out against assay organisms which were bacterial group and they include Standard and clinical strains of *Bacillus subtilis* ATCC 6633, *Bacillus subtilis* clinical, *Staphylococcus aureus* β -haemolytic strain and clinical, *Staphylococcus aureus*, *Salmonella typhi* clinical, *Klebsiella pneumonia* clinical, *Escherichia coli* and *Proteus sp.* Clinical strain. This study showed the presences of some phytochemical compounds such as tannin, alkaloid, saponin, flavonoid etc. in *C. procera* leaves and fruits at varying concentration. The GCMS analysis revealed that Pentadecanoic acid and n-Hexadecanoic acid were detected in both leaves and fruits of *C. procera* extracts. Furthermore, methanolic extract of the plant leaves had antibacterial effect potency towards *Bacillus subtilis* ATCC 6633, while the fruit extract had no antibacterial effect against the assay organism.

Keywords: Antimicrobial, *Bacillus subtilis* ATCC 6633, Bioactive compounds, *Calotropis procera*, *Staphylococcus aureus*

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INTRODUCTION

Most infections affecting humans over the years have been treated with natural substances or products that are plant extracts, of animal origin or mineral base (Lahlou, 2013). According to Dirir *et al.* (2017), *Calotropis procera* (as shown in Fig 1) belongs to Asclepiadaecae family that are also known as Ayurvedic plant with noticeable potential medicinal attributes. Its natural habitation is in warm climate areas of the world with soils that are sandy, alkaline and dry in nature (Attaelmanan and Kawam, 2012). The appellation "Calotropis" stands for "beautiful" or "flower" in Greek; and on the other hand, "procera" a Latin word stands for the cuticular wax on the stems and leaves (Hassan *et al.*, 2015). *C.alotropis procera* (Aiton) has been said to habit both semi-arid and arid region and can be characterized

as a perennial shrub and xerophytic plant that is soft-wooded found in arid and semi-arid habitats (Al-Rowaily *et al.*, 2020; Sharma *et al.*, 2012). According to Silva *et al.* (2010), *C. procera* various parts had been utilized in one therapeutic function or the other within traditional medicines practices. Around the globe, areas like North Africa, South-East Asia, Middle East etc., have been using the plant in traditional health practices (Al Sulaibi *et al.*, 2020). On a contrary according to Batool *et al.* (2020), the usage of the plant has been for timber, fodder and fuel. *Calotropis procera* latex has been reported to contain varieties of bioactive components such as alkaloids, cardiac glycosides and steroid which had been linked to its pharmacological values (Parihar and Balekar, 2016; Mohamed *et al.*, 2015).



Fig 1: *Calotropis procera* image
Source: Dirir *et al.*, (2017)

The flowers (as shown in Fig 2) of *C. procera* according to Upadhyay (2014) is small, characteristics of greenish white or creamlike color at the base, the lobe extreme are purple in color, with follicles that are recurved with 1 or 2 follicles which are 3-4 inch long (Upadhyay, 2014). The diameter of the fruit which is always fleshy and green in color is 10 cm although it could be more than

this in some cases (Orwa *et al.*, 2011). The fruit when matured do split in an inverted position to release its seeds that possess white hairs, flat, brown in color (Sharma *et al.*, 2012). The bark of *C. procera* produce strong fibers which are white silky, durable and strong in nature and used for producing ropes, nets for fishing, thread for sewing etc. (Upadhyay, 2014).



Fig 2: *Calotropis procera* with flowers
Source: Upadhyay (2014)

Calotropis procera possess very high pharmaceutical and ethnomedicinal properties (Upadhyay, 2014). And is used globally for treating conditions such as skin infection, asthma, rheumatism, elephantiasis etc. remediation (Sharma *et al.*, 2011). In a study carried out by Attaelmanan and Kawam (2012) to evaluate the elemental constituents of *C. procera* with the aid of X-ray analytical microscope, and it was revealed that major elements such as Cl, K and Ca were present with varying concentrations. In addition, Mg, Si, P, Fe, Sr, Mn, Br were presence at low concentration (Attaelmanan and Kawam, 2012).

Calotropis procera Fruits and leaf powder adsorbed Acid red 73 and Congo Red dye respectively, these colorant dyes are malicious to aquatic life whenever they find their way into water bodies (EL-Adawy and Alomari, 2020; Kaur and Kaur, 2017).

The leaf, fruit and stem extracts of *C. procera* had been associated with antidepressant, antipyretic, and blocking of neuromuscular functionality (Garabadu *et al.*, 2019). Antihyperglycemic features of the *C. procera* leaf extracts had been reported (Nadeem *et al.*, 2019).

This study aimed at investigating the bioactive and chemical components, ethnobotany and pharmacological activities of *Calotropis procera* leaves

and fruits with a focus on its antimicrobial effect on isolated bacteria

Materials and Methods

Plant samples Collection

Calotropis procera fruits and leaves were obtained in Ojo Local Government Area (6°28'N 3°11'E), Lagos State, Nigeria. The Department of Botany, Faculty of Science, Lagos State University, Ojo, Lagos was consulted for the characterization and authentication of the obtained plant. The researcher aseptically plucked the leaves and washed away all debris on the leaves and fruits with tap water and subsequently used distilled water to rinsed them. Both the fruit and leaves were under room temperature shaded in order to air-dried them. In preparing the powdered samples of the dried leaves, Crest 5000W German Blender was used and pestle and mortar was used to crush the fruits. The samples were then subjected to extraction and bioassay

Test organisms

The bacterial isolates (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia clinical*, *Proteus sp* and *Salmonella typhi*) were obtained from the Microbiology department, Lagos State University, Ojo, Lagos State, Nigeria.



Extracts Preparation

The researcher embarked on slight modification to Akerele et al. (2008) method for the extraction of phytochemicals from the samples. Concisely, 100 ml of Ethanol and water individually were input into different sterile conical flask containing 10 g of the leaf and fruit sample individually, and left for 24 h at room temperature to soak. Whatman no1 filter paper was used in filtering the extracts, and rotary evaporator was used at 40°C to vacuo the filtrates concentrate (Nenaah and Ahmed, 2011; Doughari and Manzara 2008). The obtained residues were dried and preserved at 4°C for bioassay.

Determination of Bioactive constituents

The Mikail (2010) and Krishnaiah et al., (2009) chemical tests procedures were adopted for the bioactive constituent determination on the powdered and aqueous extract specimens.

Qualitative analysis

Test for availability of tannins: 0.5 g of the specimen was boiled in a 20 ml water in a test-tube and subsequently was filtered. A blue-black or brownish-green colouration was observed to signify the presence of tannins after adding drops of 0.1% FeCl₃.

Test for availability of phlobatannins: The extract of the specimen at 0.5g quantity was boiled along 2mL 1% HCl for 10 minutes. Phlobatannin was accentuate by red precipitate deposit.

Test for availability of saponin: 2 g of the specimen was boiled in a 20 ml water in a water bath and subsequently filtered. 10ml of the filtrate was added to 5 ml of distilled H₂O and shaken intensely for persistent froth. Olive oil (3 drops) was mixed the frothing and intensely shaken for emulsify formation.

Test for availability of flavonoids: Ammonia solution (5 ml) was added to some aqueous filtrate of the specimen extract then concentrated H₂SO₄ was added.

Test for steroids: Acetic anhydride (2 ml) was mixed to 0.5 g ethanolic extract of the specimen individually with 2 ml H₂SO₄. The presence of steroid is indicated with violet to blue or green colour change.

Test for availability of terpenoids using Salkowski test: In 2 ml of chloroform was added to each extract (5 ml) and 3 ml of concentrated H₂SO₄ was also added with the formation of a layer. Terpenoids presence is detected by reddish brown inter face colouration.

Test for availability of cardiac glycosides using Keller-Killani test: Individual extract (5 ml) was added glacial acetic acid (2 ml) with a drop of FeCl₃. This solution was then under laid with 1 ml of concentrated H₂SO₄. Deoxysugar feature of cardenolides is indicated with brown ring interface.

Test for availability of alkaloid: Some quantity of the individual extract samples (5 mg) was dissolved acidified ethanol (3 ml) and slightly warmed and subsequently filtered. To 1 ml of the filtrate, Mayer's reagent (few drops) and Dragendroff's reagent(1 ml) were mixed with the filtrate (1 ml) and turbidity was seen.

Quantitative determination

Quantification of tannins: 500 mg of each extract concentrate was weighed and dissolved in distilled water (50 ml), and agitated for 1 h. The filtrate (5 ml), was mixed with 0.1 M FeCl₃ (2 ml) in 0.1 N HCl and 8 x 10⁻³ M potassium ferrocyanide. The absorbance was read at 720 nm wavelength with Hitachi UH4150AD spectrophotometer within 10 minutes.

Total phenolic compound Quantification: 0.5g of each extract was weighed and dissolved in distilled water (50 ml). Take 0.5 ml add 0.1 ml of Folin- Ciocalteu reagent (0.5 N) (0.1 ml) was mixed with 0.5 ml of the extract solution which for 15 minutes was incubated at room temperature. Subsequently Na₂CO₃ solution (7.5% w/v) (2.5 ml) was added and for 30 minutes was incubated at room temperature. The absorbance was read at 760 nm wavelength with Hitachi UH4150AD spectrophotometer.

Total flavonoid Quantification: 1 ml of each extract solution (100 µg/ml) was added to methanol (3 ml), 10% AlCl₃ (0.2 ml), 1 M potassium acetate (0.2 ml) and distilled water (5.6 ml). The mixture for 30 minutes was incubated at room temperature and the absorbance was read at 415 nm wavelength with Hitachi UH4150AD spectrophotometer.



GC-MS Determination of phytochemical characterization

Before the analysis, the Mass Spectrometer was auto-tuned to perfluorotributylamine (PFTBA) utilizing already set principle to assess the bounty of m/z 69, 219, 502 and sensitivity and optimal of the various instruments to be used. Gas chromatography–mass spectrometry (GC-MS) was used for Bioactive levels determination in the sample via the operation of MSD in Scan mode in ensuring that all detection levels of the constituents. Gas chromatograph (Agilent 7820A) fixed to 5975C inert MS (Having detector with triple axis) with electron impact area (Agilent Technologies) was utilized in this study. Agilent Technologies HP-5 capillary column serves as the stationary phase where compounds are separated and this was coated with Phenyl Methyl Siloxane (5%) (30m (length) x 0.32mm (diameter) x 0.25 μ m (film thickness)). Helium was the carrier gas and it was utilized at .4871 mL/min constant flow with 1.4902 psi initial nominal pressure and 44.22 cm/sec mean velocity. At 300 °C injection temperature, injected into splitless mode was 1 μ L of the specimens. A15 mL/min purge flow to split vent at 0.75 min with a 16.654 mL/min total flow; the gas saver mode was off. Initially, the oven was set at 40 °C for 1 min and subsequently ramped to 300 °C (10 min) at 12 °C/min. With solvent delay of 5 min, 32.667 min was the Run time. The MS was used at 70eV electron-impact ionization mode with 230 °C ion source temperature, 150 °C quadrupole temperature and 280 °C transfer line temperature. Ion was acquired through Scan mode (at 2.0s/scan rate, m/z was scanned from 45 to 550 amu).

Antimicrobial Test**Sample Preparation**

There were three working samples of the concentrated extracts, 400g/ml, 200mg/ml, and 100mg/ml which were achieved by weighing 4g of extract and dissolving in sterile 5% solution of methanol as solvent and then double diluting to have other working values.

- 4g of extract in 10ml of preparation, concentration = 400mg/ml
- 2ml of A + sterile distilled water (2ml) (diluent), concentration = 200mg/ml
- 2ml of B + sterile distilled water (2ml) (diluent), concentration = 100mg/ml

Contents of bottles A, B, and C were the working samples.

Standard Preparation

Working standards of pure levofloxacin were of four concentration values, 50 μ g/ml, 25 μ g/ml, 12.5 μ g/ml and 6.25 μ g/ml solutions of the standard solutions were achieved by weighing, dissolving, and double diluting the standard as follows;

- 0.05g of the standard levofloxacin in 10ml of sterile distilled water(diluent), concentration =5000 μ g/ml solution
- 0.2ml of A + 19.8ml of sterile distilled water (diluent), concentration = 50 μ g/ml
- 2ml of B + sterile distilled water (2ml) (diluent), concentration = 25 μ g/ml
- 2ml of C + sterile distilled water (2ml) (diluent), concentration = 12.5 μ g/ml
- 2ml of D+ sterile distilled water (2ml) (diluent), concentration = 6.25 μ g/ml

Contents of bottles B, C, D and E were the working standards of levofloxacin.

Minimum Inhibitory Concentrations

This was investigated by adopting the criteria of the Clinical and Laboratory Standard institute (CLSI). Thirteen working values were prescribed and were used in order to establish the minimum concentration that inhibited a particular responsive assay organism. The concentrations were 0.0025, 0.005, 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, 2.56, 5.12, and 10.24mg/ml. Agar dilution technique recommended by Hugo and Russel was used because the colour of the extracts would interfere with the readings. To achieve these working values, three different stock concentrations were prepared from which the other working concentrations were also prepared. These stock concentrations include 5%, 10%, 20%.

Minimum inhibitory concentration plates preparation procedure

- 0.01ml of stock 5mg/ml + 19.99ml of single strength Mueller Hinton Agar, concentration = 0.0025mg/ml.
- 0.02ml of stock 5mg/ml + 19.98ml of single strength Mueller Hinton Agar, concentration = 0.005mg/ml.



- C. 0.04ml of stock 5mg/ml + 19.96ml of single strength Mueller Hinton Agar, concentration = 0.01mg/ml.
- D. 0.08ml of stock 5mg/ml + 19.92ml of single strength Mueller Hinton Agar, concentration = 0.02mg/ml.
- E. 0.16ml of stock 5mg/ml + 19.84ml of single strength Mueller Hinton Agar, concentration = 0.04mg/ml.
- F. 0.32ml of stock 5mg/ml + 19.68ml of single strength Mueller Hinton Agar, concentration = 0.08mg/ml.
- G. 0.64ml of stock 5mg/ml + 19.36ml of single strength Mueller Hinton Agar, concentration = 0.16mg/ml.
- H. 1.28ml of stock 5mg/ml + 18.72ml of single strength Mueller Hinton Agar, concentration = 0.32mg/ml.
- I. 2.56ml of stock 5mg/ml + 17.44ml of single strength Mueller Hinton Agar, concentration = 0.64mg/ml.
- J. 5.12ml of stock 5mg/ml + 14.88ml of 11/2 strength Mueller Hinton Agar, concentration = 1.28mg/ml.
- K. 10.24ml of stock 5mg/ml + 9.76ml of double strength Mueller Hinton Agar, concentration = 2.56mg/ml.
- L. 10.24ml of stock 10mg/ml + 9.76ml of double strength Mueller Hinton Agar, concentration = 5.12mg/ml.
- M. 10.24ml of stock 20mg/ml + 9.76ml of double strength Mueller Hinton Agar, concentration = 10.24mg/ml.

Minimum bactericidal concentration

Plates from the results of minimum inhibitory concentrations were subcultured onto blank agar for growth recovery using 4% tween 20 as a deactivating agent for emulsification. Any plate showing no growth was observed and recorded for minimum bactericidal concentration.

Media preparation

The media were prepared following the inscriptions by the manufacturers. 37g portion of Mueller Hinton Agar

was weighed and dispersed in the recommended volume of distilled water. It was heated to melt in the water bath at 100°C. The molten agar gel so formed was dispensed in 25ml portions into sample bottles for potency test and autoclaved at 121°C for 15 minutes. The 25ml portion of the agar gel in each of the sample bottles was the working volume of the agar for potency assay. Agars for minimum inhibitory concentrations were measured according to the working volumes. The agar strengths were also prepared accordingly for minimum inhibitory concentrations determinations.

The assay organisms

The assay organisms were bacterial group and they include Standard and clinical strains of *Bacillus subtilis* ATCC 6633, *Bacillus subtilis* clinical, *Staphylococcus aureus* β -haemolytic strain and clinical, *Staphylococcus aureus*, *Salmonella typhi* clinical, *Klebsiella pneumonia* clinical, *Escherichia coli* and *Proteus* sp. Clinical strain all obtained from the Laboratory stock cultures of Microbiology Laboratory. So, they were all standard and clinical isolates that were primarily isolated on various diagnostic and selective media to suppress other contaminants.

To remove the impacts of suppressive and indicator chemical related agents, the Isolates were subsequently subcultured on Mueller Hinton Agar and subsequently into sterile nutrient broth for optical density adjustment. Incubation periods were 24 hours for all the bacteria at 37°C.

Calibration of assay organisms

The bacterial load of the assay organisms was adjusted using a reference standard suspension. The bacterial liquid cultures were added drop by drop onto sterile normal saline to achieve turbidity reading of, 0.5 densometer according to McFarland turbidity standard. The adjusted suspension was used for the assay. The bacteria suspension for the minimum inhibitory concentration determination was adjusted further using serial and decimal dilution and plating out to achieve a colony forming unit of about 10^6 which was selected and used for evaluation of the minimum inhibitory concentration.



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Seeding and inoculation of assay organisms

The assay medium which was prepared and maintained at 45°C until required for use. The cultures were then homogenized by vortexing prior to conducting the assay. 1ml of calibrated organisms was seeded into the warm agar and mixed thoroughly using the roll-palm method before pour-plating. They were left to solidify under sterile conditions before cork boring after which 10µl of the test organism was added to the wells to determine the minimum inhibitory concentration (MIC).

Cork boring and dispensing of standards and samples

After allowing all the seeded agars to set a sterile, cork borer, size 10mm cross-section was used for boring the wells. 150 µl of various working concentrations was dispensed into the wells and allowed to stand for four hours to allow adequate diffusion before incubation.

Incubation of plates and readings

The plates for the antibacterial studies were incubated at 37°C lid up position and observed after 24 hours for zones of inhibition. Several measures were taken using a zone reader and average zone values determined and recorded. Plates for minimum inhibitory concentrations were incubated lid down and were observed daily for 72 hours

RESULTS AND DISCUSSION

Phytochemical Screening Test

Secondary metabolites in their thousands had been reported to be generated by plants (Quint *et al.*, 2016; Verma *et al.*, 2016; Zhu, 2016). *Calotropis procera* has oxypregnanes triterpenoids, flavonoids, glycosides, cardenolides, sterols, and other components (Ranjit *et al.*, 2012). In this study, *C. procera* leaf and fruit extracts had the presence of phenol in them as shown in Table 1 and at a concentration of 47.23 and 36.92 mg/100g respectively as illustrated in Table 2.

Table 1: Qualitative Phytochemical Screening of *Calotropis procera*

	Tannin	Phenol	Alkaloid	Saponin	Phloba Tannin	Steroid	Reducing sugar	Cardiac glycoside	Terpernoid	Flavonoid
<i>C. procera</i> fruit	+	+	+	+	-	+	+	+	+	+
<i>C. procera</i> leaves	+	+	+	+	+	+	-	+	+	+

+: Presence -: Absence

Phenolic compounds are one of the groups of bioactive components that are present everywhere simultaneously in plant (Singh *et al.*, 2007). They can act as suppressor to aging, atherosclerosis, apoptosis, carcinogen,

cardiovascular ailment, inflammation and endothelial dysfunction, as well as angiogenesis inhibitor and proliferation of cell (Han *et al.*, 2007).

Table 2: Quantitative Phytochemical Screening of *Calotropis procera* at mg/100g

	Tannin	Phenol	Alkaloid	Saponin	Steroid	Reducing sugar	Cardiac glycoside	Terpernoid	Flavonoid
<i>C. procera</i> fruit	26.86	36.92	38.85	34.69	27.72	33.40	26.55	20.80	50.23
<i>C. procera</i> leaves	34.36	47.23	40.62	41.00	26.16	-	31.05	24.82	53.50



Flavonoid was also present in both *Calotropis procera* leaf and fruit extract at a concentration of 53.50 and 50.23 mg/100g. The study by Morsy *et al* (2016) only detected flavonoid in the leaves of *C. procera*. Flavonoids have the potential to suppress microbial, allergic, inflammatory and cancer activities, while on the other hand, tannins do possess cytotoxic and anti-tumor activities (Joshi *et al.*, 2013).

Saponin was also present in the extract of the plants leaves and fruit at 41.00 and 34.69 mg/100g. Bashyal *et al.* (2017) also reported saponin in methanolic and acetone extracts of *C. procera* leaves.

Tannin, alkaloid, Cardiac glycoside and terpenoid were also present in the leaves of the plant extracts at 34.36, 40.62, 31.05 and 24.82 mg/100g respectively. The *Calotropis procera* fruits show the presence of Tannin, alkaloid, Cardiac glycoside and terpenoid at a concentration of 26.86, 38.85, 26.55 and 20.80 mg/100g. The presence of these compounds in *C. procera* were also reported by Goyal and Mathur, (2011).

Comparing the concentration of each phytochemical present in the *C. procera* leaves and fruit, it shows from Table 2 that the leaves contain a higher concentration of the respective bioactive substance. In the study by Mainasara *et al* (2011), phytochemicals such as saponin, glycosides, flavonoids, tannin and alkaloids had been found in *C. procera* leaves methanol and ethanol extracts which were also found in the methanol extract in this study.

In identifying and quantifying the chemical constituents in the *C. procera* fruit and leaves, analytical GC-MS technique was adopted as shown in Table 3 and 4. Major components were identified in the *Calotropis procera* leaves methanol extract as indicated in Table 3 and Fig 1 are: formic acid, 1-methylpropyl ester (0.99%), 2-Pyrrolidinone, 1-methyl-(14.03%), Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynoxy)- (0.65%), 3-Heptene (0.31%), 9-Borabicyclo[3.3.1]nonane, 9-(1-ethylpropyl)- (0.33%), Cyclotetrasiloxane, octamethyl- (0.95%), n-Hexadecanoic acid (7.97%), 9,12-Octadecadienoic acid (Z,Z)- (8.12%) and others.



Table 3: GC-MS analysis of *Calotropis procera* leaves methanol extract

Peak No	Name of compounds	Retention time	Area %
1	Formic acid, 1-methylpropyl ester	3.642	0.99
2	2-Pyrrolidinone, 1-methyl-	4.920	14.03
3	Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-	5.587	0.65
4	3-Heptene	6.020	0.31
5	9-Borabicyclo[3.3.1]nonane, 9-(1-ethylpropyl)-	6.875	0.33
6	Cyclotetrasiloxane, octamethyl-	7.486	0.95
7	2-Ethyl-3-methoxypyrazine	9.497	0.75
8	Benzeneacetic acid, 2,5-dihydroxy-	11.475	3.29
9	2-Methyl-6,7-dihydro-5H-benzofuran -4-one	11.786	2.50
10	Propanoic acid, phenyl ester	12.230	0.71
11	Tricyclo[4.2.2.0(1,5)]decan-4-one	12.819	0.66
12	Pentadecanoic acid	13.841	0.60
13	n-Hexadecanoic acid	14.852	7.97
14	7-Chlorobicyclo[4.1.0]hept-3-ene	15.908	0.98
15	9,12-Octadecadienoic acid (Z,Z)-	16.463	8.12
17	1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy)-	18.652	1.18
18	2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-ol	19.852	1.34
19	Stigmastan-6,22-dien, 3,5-dedihydro-	20.829	3.23
20	Cholesta-3,5-diene	21.562	2.39



Table 4: GC-MS analysis of *Calotropis procera* leaves methanol extract

Peak No	Name of compounds	Retention time	Area %
1	Benzene, 1-ethyl-3-methyl-	3.409	0.84
2	Undecane	4.453	1.22
3	3-Methyl-2,3-dihydro-benzofuran	4.731	0.83
4	Dodecane	5.842	0.44
5	Phenol, 3,5-bis(1,1-dimethylethyl)	9.975	0.37
6	Cyclopentadecanone, 2-hydroxy-	10.886	0.57
7	Octadecanal	12.263	0.70
8	Tetradecanoic acid	12.863	2.24
9	Pentadecanoic acid	13.885	2.30
10	n-Hexadecanoic acid	14.919	35.93
11	Cyclopentadecane	16.752	9.59
12	2-Myristynoyl-glycinamide	19.807	0.55
13	1,2,5-Oxadiazol-3-amine, 4-(3-meth	20.251	0.90
14	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	21.262	0.29

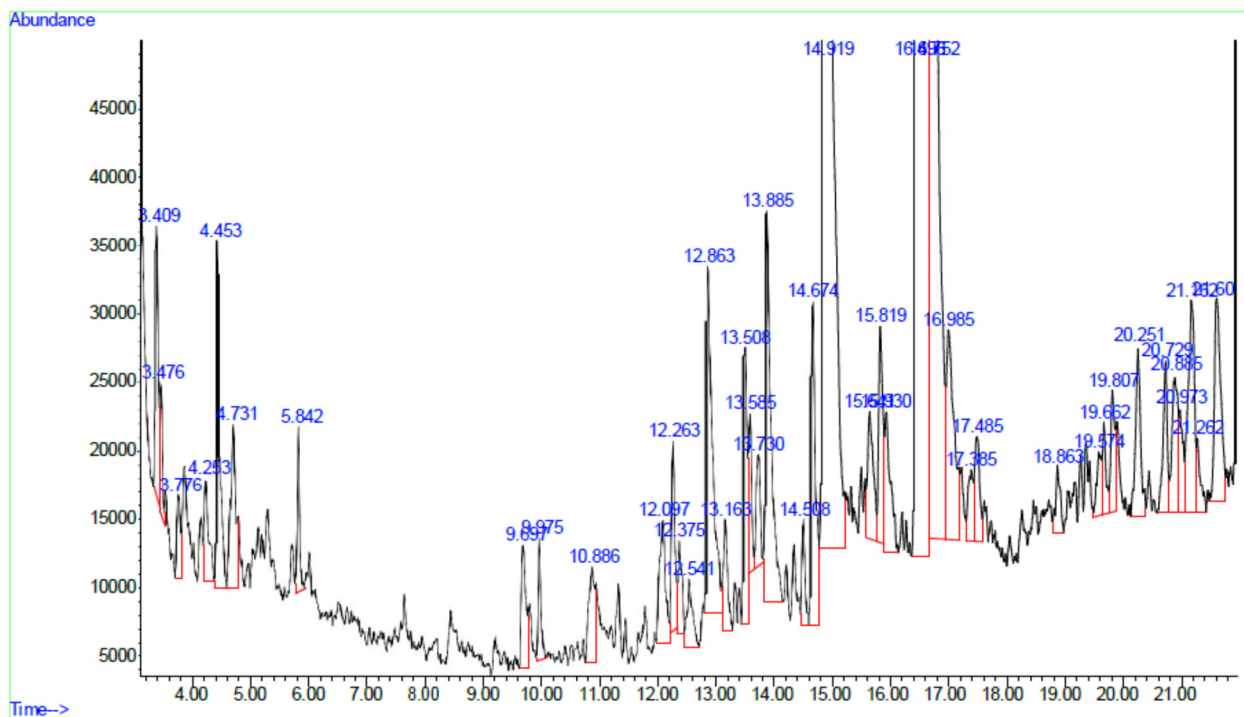


Fig. 2: GC-MS chromatograms of *Calotropis procera* fruits extract



Table 5: Inhibition zone diameter (mm) of methanolic *Calotropis procera* leaves extract on bacteria

Assay organism	75 mg/mL	150 mg/mL	300 mg/mL	Solvent control (5% methanol)
<i>Staphylococcus saprophyticus</i> DSM 18669	0.00	0.00	0.00	0.00
<i>Bacillus subtilis</i> ATCC 6633	0.00	0.00	16.50±0.50	0.00
<i>Staphylococcus aureus</i> ATCC 25923	0.00	0.00	0.00	0.00
<i>B- Haemolytic staphylococcus aureus</i>	0.00	0.00	0.00	0.00
<i>Salmonella typhi</i> Clinical strain	0.00	0.00	0.00	0.00
<i>Proteus sp.</i> Clinical strain	0.00	0.00	0.00	0.00
<i>Klebsiella pneumonia</i>	0.00	0.00	0.00	0.00
<i>Escherichia coli</i>	0.00	0.00	0.00	0.00

***Calotropis procera* methanolic extract Antibacterial activity**

Calotropis procera bark and leaves extracts anti-bacterial potency had been reported against *Pseudomonas aeruginosa*, *K. pneumoniae*, *E. coli*, and *B. subtilis* (Mehmood *et al.*, 2020). *Calotropis procera* leaves hydroethanolic extract has been reported by Saddiq *et al.* (2022) to show anti-bacterial engagement towards *S. aureus* (a Gram-positive bacteria) and *K. pneumoniae* (a Gram-negative bacteria) in comparison to antibiotics such as gentamycin. *Calotropis procera* leaves methanolic extract have been reported to exert antibacterial potency towards *S. aureus* and *S. typhi* (Kumar *et al.*, 2013). In this study, the methanolic extract of *C. procera* leaves and fruit from Table 5 and 6 respectively could not inhibit the growth of some of the assay organisms which and they include Standard and clinical strains of *B.s subtilis* ATCC 6633, *B. subtilis* clinical, *S. aureus* β -haemolytic strain and clinical, *S. aureus*, *S. typhi* clinical, *K. pneumonia* clinical, *E. coli* and *Proteus sp.* Clinical strain. The only exception case to the inhibitory effect of *C. procera* leaves and fruits was where the methanolic extract of leaves at 300 mg/mL was able to

prevent the growth of *B. subtilis* ATCC 6633 at a mean diameter of 16.50±0.50 mm as indicated in Table 5. In the study conducted by Mehmood *et al* (2020), *C. procera* leaves 50% methanol extracts generated the greatest inhibition zones (20 mm) and it was shown that *C. procera* extracts exhibited anti-bacterial effects towards *B. subtilis*, 50% methanol prevent the growth more than 100% methanol, while the *C. procera* leaves 100% methanol exhibit greatest inhibition with a zone of 21.66 mm diameter towards *K. pneumoniae* in the same study.

Ability of *C. procera* extracts to destroy the growth of microbes with greater efficiency could be attribute to availability of bioactive metabolites which have been reported to be antimicrobial substances (Adnan *et al.*, 2020). Thenmozhi and Sivaraj (2010) opined that plants with anti-bacterial potential exhibit this characteristic with the help of secondary metabolites formed by them in protecting themselves from pests and other agents that is against their existence. Also, α -amyrin had been associated with significant antimicrobial features of *C. procera* (Hernández-Vázquez *et al.*, 2012; Rodrigues *et al.*, 2012).



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Table 6: Inhibition zone diameter (mm) of methanolic *Calotropis procera* fruit extract on bacteria

Assay organism	100 mg/mL	200 mg/mL	400 mg/mL	Solvent control (5% methanol)
<i>Staphylococcus saprophyticus</i> DSM 18669	0.00	0.00	0.00	0.00
<i>Bacillus subtilis</i> ATCC 6633	0.00	0.00	0.00	0.00
<i>Staphylococcus aureus</i> ATCC 25923	0.00	0.00	0.00	0.00
<i>B- Haemolytic staphylococcus aureus</i>	0.00	0.00	0.00	0.00
<i>Salmonella typhi</i> Clinical strain	0.00	0.00	0.00	0.00
<i>Proteus sp.</i> Clinical strain	0.00	0.00	0.00	0.00
<i>Klebsiella pneumonia</i>	0.00	0.00	0.00	0.00
<i>Escherichia coli</i>	0.00	0.00	0.00	0.00

Minimum inhibitory concentration (MIC) determination

An extract least concentration value that was able to effectively inhibit the growth of pathogens is adjudged the minimum inhibitory concentration (MIC) (Ostrosky *et al.*, 2008). The MIC for the methanolic *Calotropis procera* leaves was determined and found to be 5.12mg/mL from

Table 7. Kuta (2008) in a study, subjected *C. procera* Linn leaves methanolic and water extracts to both antioxidant and anti-bacterial potency activity and the study revealed that the plant extracts have chemotherapeutic drugs potential in addressing epidermophyton and trichodermophyton linked to tinea.

Table 7: Minimum inhibitory concentration values (mg/mL) of methanolic *Calotropis procera* leaf extract on responsive organism

Assay organism	2.5 x 10 ⁻³ mg/mL	5 x 10 ⁻³ mg/mL	1 x 10 ⁻² mg/mL	2 x 10 ⁻² mg/mL	4 x 10 ⁻² mg/mL	8 x 10 ⁻² mg/mL	1.6 x 10 ⁻¹ mg/mL	3.2 x 10 ⁻¹ mg/mL	6.4 x 10 ⁻¹ mg/mL	1.28 mg/mL	2.56 mg/mL	5.12 mg/mL	10.24 mg/mL	MIC value (mg/mL)
Concentration code on sample bottle	A	B	C	D	E	F	G	H	I	J	K	L	M	
<i>Bacillus subtilis</i> ATCC 6633	+	+	+	+	+	+	+	+	+	+	+	-	-	5.12



CONCLUSION

This study had shown that some phytochemicals such as phenol, flavonoid, saponin, tannin, alkaloid, cardiac glycoside, terpenoid, Pentadecanoic acid and n-Hexadecanoic acid were present in both leaves and fruits are present in both the leaves and fruit of *Calotropis procera* at varying concentration. Also, the study shows that methanolics *Calotropis procera* leaves had antibacterial activity against only *Bacillus subtilis* ATCC 6633, while the fruit extract had no antibacterial effect against the assay organism.

AUTHOR'S CONTRIBUTION:

OLU, J.: Designed the study, wrote the protocol, managed the literature searches, wrote the first draft of the manuscript and interpreted the statistical analysis
YUSUF, H. O.: Worked on the initial draft and edited the manuscript and read through the phytochemical analysis
DANTANKO, F: Was involved in the drafting and Editing of the manuscript
ADETUNJI, O.: Was involved in the manuscript preparation and the interpretation of the findings
AJENIFUJAH-SOLEBO, S.O.: Was involved in the antimicrobial investigation aspect of the study and also involved in editing of the manuscript

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