Evaluation of Bioactive Agents in *Calotropis Procera* Plant Parts Through Anti-Microbial, Proximate, and Antioxidant Studies.

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ABSTRACT

Successive extraction of the aerial plant parts of Calotropis procera using soxhlet extraction yielded the phytochemicals; saponins, alkaloid, tannins, phenol, flavonoids, anthraquinones, and glycosides. Bioassay analysis using standard procedures like Mueller Hinton dilution experiment, demonstrated antibacterial potentials against microbes. E. coli, C. albicans, S. aureus, S. pyogenes, P. aeruginosa, S. typhi, and C. stellatoidea. 27 mm to 41 mm indicated zones of inhibition range. The MBC and MFC for all the microorganisms were at 25 mg/ml except for S. pyogenes which was at 12.5 mg/ml. The MIC for the most sensitive organisms was 12.5 mg/ml with the exception of P. aeruginosa and S. pyogenes which had theirs at 6.25 mg/ml. Proximate analysis used standard AOAC method, and in antioxidant analysis, free radical scavenging method of 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) were employed. The results are as follows, proximate analysis revealed the percentage contents of fat, crude fiber, protein, ash, and carbohydrates to be 2.93, 7.05, 1.68, 56.50, and 18.69, respectively. In the antioxidant studies, free radical scavenging technique employing 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) was used. Samples and standards were determined using the IC50. The active sample was hexane extract, with an IC50 of 0.0878, followed by ethyl acetate (0.8811) and methanol (0.9215).

Keywords: *Calotropis procera,* Phytochemical, Antimicrobial, Proximate, Antioxidant,

INTRODUCTION

A medicinal plant contains physiologically active compounds that have therapeutic effects, such as saponins, tannins, essential oils, flavonoids, alkaloids, and other molecules[1]. These intricate chemical compounds with various compositions can be discovered in one or more of these plants as secondary plant metabolites. The antibacterial properties of several crude plant extracts, either alone or in combination, have been described in several published papers.

Drug resistance to human pathogenic microorganisms has recently been widely

reported worldwide[2]. However, the expensive use of antibiotics makes the situation worrisome in both developed and developing nations. Alternative antimicrobial techniques are thus urgently required, and as a result, the therapeutic use of traditional cures such plants for which *C. procera* has been documented has been reevaluated[3]. Such chemicals might not have the concern of microbial resistance because they are novel. A sizable undiscovered source of antimicrobials is derived from plants. A medicinal plant was also reported by some researcher's to stop the growth of bacteria[4-5]. According to reports, medicinal plants have a considerable amount of macronutrients[6]. The potency and effectiveness of therapeutic herbs are increased by this characteristic. Additionally, herbal medicine takes into account the sociocultural history of every nation. Despite the focus lately being on the study of synthetic medications, people are once again interested in therapeutic plants.

MATERIALS AND METHODS

The aerial plant parts of *Calotropis procera* were collected locally in Agbaroh in Ughelli local government area of Delta State, Nigeria. It was identified and authenticated at environmental management and toxicology department by Dr. Gloria Omoriege. The fresh plants were collected in bags to the laboratory. The plant *Calotropis procera* was thoroughly washed with distilled water to ensure no dirt is on them and allowed to drain. The plant material was dried under shade at an ambient temperature of about 25^0C for two weeks in the laboratory. The dried plant material was then crushed in a mortar using pestle into a powder. To enable a good extract and high yield, the powdered plant material was sieved over a 600-mesh screen. The ground sample was neatly labeled, stored at room temperature.

Extracting solvents

In this study, the soxhlet extraction method was employed. n-hexane, ethyl acetate, and methanol were used to extract 300 g of the air-dried, ground-up aerial plant parts of *Calotropis procera* in the order of their polarities. The cellulose thimble containing the powdered plant material was placed in the extraction chamber, which is situated above a collecting flask and below a reflux condenser. The setup was heated for 12 hours under reflux after solvents has been added to the flask. Each time, the powdered fruit and leaf material was air dried before extraction using the following solvent. The solvent was

then distilled, and the extracts were all concentrated by evaporating them at low temperatures until they were completely dry.

Screening for phytochemicals

To determine the primary active groups present in the solvent extracts by their color reaction, the phytochemical screening of the extract was conducted using standard procedure[6]. Simple quantitative and qualitative techniques were used to test for the presence of saponins, alkaloids, terpenoids, flavonoids, glycosides, reducing sugars, and tannins[8].

Analyze flavonoids: Three milliliters of the extracts were mixed with a small amount of magnesium powder and a few drops of strong hydrochloric acid. The presence of flavonoids is indicated by a red or bright red coloring.

Antimicrobial Analysis

Standards procedures for microbiological antimicrobial analysis were followed[9].

Isolated microorganisms' source

Escherichia coli, staphylococcus aureus, candida albicans, methicillin-resistant staph aureus, and *vancomycin* isolates and previously known strains From the Medical Microbiology Department of the ABU Teaching Hospital in Zaria, slants of *resistant enterococci, Streptococcus pyogenes, Proteus mirabilis, salmonella typhi, Klebsiella pneumonia, Pseudomonas aeruginosa, , Candida krusei, candida tropicalis* and *Candida stellatoidea*, were obtained.

Preparing on a slant

For the inoculate to be stored at a specific temperature, agar-containing aslant was made. This was retained in the incubator for the study after being taken for inoculation. To enable the bacteria to reproduce, the media was changed every week**.**

Calculating the Minimal Inhibitory Concentration (MIC)

Using the broth dilution procedure, the extract's minimal inhibitory concentration was identified. After preparing the Mueller Hinton broth, 10 milliliters of it were distributed into test tubes, which were then sterilized at 121°C for 15 minutes before the broth was allowed to cool. The 0.5 on the Mc-farland standard scale was created to provide a turbid solution. The test microorganism was injected into 10 ml of prepared normal saline, which was then dispensed into a sterile test

tube. The sample was then incubated at for 6 hours at a temperature of at 37°C. The test microorganism was diluted in normal saline until its concentration reached 1.5 108 cfu/ml and the turbidity matched that of Mc-far land's scale, as determined by visual comparison.

The extract was serially diluted twice to achieve concentrations of 50 mg/ml, 25 mg/ml, 1.25 mg/ml, 6.25 mg/ml, 3.13 mg/ml, and 1.57 mg/ml. After obtaining the various extract concentrations in sterile broth, 0.1ml of the test microorganism in regular saline was then added to each concentration. After incubation for 6 hours at a temperature of at 37°C, the test tubes containing the broth were checked for turbidity (growth). The minimal inhibitory concentration was determined to be the extract's lowest concentration in sterile broth that exhibits no turbidity.

Establishing Minimal Bactericidal Concentration (MBC)

In order to establish if the test microorganisms were indeed killed or only had their growth slowed down, Minimum Bactericidal Concentration was used. In sterile Petri dishes, Mueller Hinton agar was prepared, sterilized at 370°C for 15 minutes, and then allowed to cool and harden. The serially diluted MIC contents were then subcultured onto the prepared medium. After a 24-hour period of incubation at 37°C during which the medium-coated plates were checked for colony formation, MBC was the plate with the lowest concentration of extracts that showed no growth.

Proximate analysis

The usual AOAC procedure was used to measure the samples' moisture, ash, crude lipids, proteins, and carbs[12]. The weight difference method was used to calculate the ash and moisture content. Using the Soxhlet system and petroleum ether (40 to 60°C) for 8 hours, crude fat was extracted. The defatted samples were successively digested with 1.25 percent sulphuric acid and 1.25 percent sodium hydroxide solutions to remove crude fibers. The micro Kjeldahl method modified, as described by the researcher[10]. This involves digestions, distillation, and lastly titration of the sample to estimate the nitrogen value, which is the precursor for the protein of a substance. Protein was created by multiplying a factor of 6.25 by the value of Nitrogen. A different approach was used to determine carbohydrate.

Calculating the amount of moisture

Fresh plant material weighing two grams was put in the crucible and cooked at 105 degrees Celsius until a consistent weight was reached. The original sample's weight loss was used to compute the moisture content, which was then represented as a percentage [13].

Calculating crude protein

The Kjeldahl method was used to determine the crude protein with a small modification[11]. Digestion, distillation, and titration were the three procedures used to determine the amount of crude protein.

Digestion: A digestion flask was filled with one (1) g of the material. High quality lysine HCl and a reagent blank were used as checks to ensure that the digestion parameters were accurate. 16.7 g K_2SO_4, 0.01 g anhydrous copper sulfate, 0.6 g TiO2, and 0.3 g pumice were added along with 15 g potassium sulfate, 0.04 g anhydrous copper sulfate, and 0.5 to 1.0 g alundum granules, etc. 20 cc of sulfuric acid were then added.

White vapors became visible in the clear bulb of the flask after heating for some time it was gently stirred, and further heated for 90 minutes with copper catalyst on the prepared burner (set to bring 250 ml water at 25oC to a rolling boil in 5 minutes). After cooling the mixture, carefully add 250 ml of distilled water at room temperature.

***Distillation*:** To create an acid standard solution, 15 ml of hydrochloric acid and 70 ml of water (V HCl) were carefully measured and poured to the titration flask. 1 ml of acid, roughly 85 ml of water, and three to four drops of methyl red indicator solution were added to the reagent blank.

In order to lessen foaming, two to three drops of the antifoam chemical tributyl citrate were also added to the digesting flask. The next step was to add an additional 0.5 to 1.0 g of alundum granule. 80 ml of a 45 percent sodium hydroxide solution were gradually added to the flask bottom to make the combination sufficiently alkaline. When at least 150 mL of distillate had been collected in the titrating flask, the flask was connected to the distillation system and began to distill.

Titration: The volume of excess acid was measured to the nearest 0.01 mL and titrated with a standard sodium hydroxide solution of 0.1M to an orange endpoint (color changed from red to orange to yellow) (NaOH). Similarly, the reagent blank (B) was titrated.

Calculations were done as follows:

%N (DM basis) = [(V HCl x N HCl) - (V BK x N NaOH) - (V NaOH x N NaOH)]/1.4007 x W x Lab DM/100

Where DM – dry matter; V NaOH = volume in ml of standard NaOH needed to titrate sample; V HCl = volume in ml of standard HCl pipetted into titrating flask for sample; N NaOH = Normality of NaOH; N HCl = Normality of HCl; V BK = 1 ml of standard NaOH was titrated with 1 ml standard HCl minus B; B = 1 ml of standard NaOH was also used to titrate reagent blank carried through the method and distilled into 1 ml standard HCl; W = sample weight in grams; 1.4007 = milliequivalent weight of nitrogen multiplied by 100.

Calculating crude protein (CP) percentage: Dry Matter (DM) base = percent N X F, where F = 6.25 to calculate crude protein[11].

Calculating Crude Fiber

200 ml of 1.25 percent H2SO4 and five grams (5 g) of the plant material's powdered form were boiled for 30 minutes before being filtered through a Buchner funnel. With distilled water, the residue was cleaned until it was acid-free. The residue was boiled for 30 minutes in 200 ml of 1.25 percent NaOH, filtered, and washed numerous times with purified water until it was alkaline-free. Then it was cleaned twice with ethanol and once with 10% HCl. It was then three times washed with petroleum ether. The leftover material was placed in a crucible and baked overnight at 105° C. It was ignite for 90 minutes at a temperature of about 550° C in a muffle furnace. A desiccator was used to cool the sample after which the weight of the ash was determined [11].

Ash Content Determination

The percentage of inorganic residue left over after organic matter has been burnt is the substance's total ash content. The plant samples 2 g of it was poured in to a crucible and heated to a temperature of 550°C for about 6 hours in a muffle furnace. A desiccator was used to cool the sample after which the weight of the ash was determined[11].

Calculating Carbohydrates

By deducting the total percentage compositions of moisture, protein, fiber, and ash from 100, the carbohydrate content was calculated.

Screening for antioxidants

Using a slightly modified version of the 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging method reported by Onocha *et al*., 2011, the antioxidant potential of the extract of the aerial plant portions of *Calotropis procera* was investigated. Different quantities of the extract (1.0, 0.5, 0.25, and 0.125 g/mL) were combined with a 100 M methanol DPPH solution (2.0mL). The mixture was briskly agitated before being allowed to incubate for 30 minutes at room temperature and in the dark. Using a GS UV-12 and UV-Vis spectrophotometer, the absorbance at 517 nm was recorded as As and Ac for the sample and blank, respectively. The same approach was used to conduct a control experiment devoid of essential oils (DPPH + methanol), and the absorbance was noted as Ac.

As reference points for comparison, butylated hydroxyl anisole (BHA) and ascorbic acid's antioxidant capacities were used. The free radical scavenging abilities of the essential oils were estimated as a percentage inhibition for each trial in triplicate using the following formula:

(Absorbance control-Absorbance sample)/(Absorbance control) 100 is the formula for percentage inhibition.

RESULTS AND DISCUSSION

Plant-based chemical analysis

Table 1 below shows the results of the phytochemical screening. 2.9 %, 3.1 %, and 3.0 %, respectively, are the yields from the extract of *Calotropis procera* using n-hexane ethyl acetate and methanol. In accordance with their polarities, n-Hexane, ethyl acetate, and methanol were used in succession for the extraction. For the n-Hexane extract, the phytochemical screening of the extracts revealed the presence of alkaloids, terpenoids, and saponins. For ethyl acetate extract, the ingredients include saponins, reducing sugar, steroids, alkaloids, phenolic compound, carbohydrates, tannins, glycosides, and protein. proteins, carbohydrates, steroids, tannins, glycosides, alkaloids, phenolic substances, flavonoids, saponins, and glycosides for methanol extract.

Table 1: lists the various extracts' phytochemical screening results.

Phytochemicals	n-Hexane	Ethyl acetate	Methanol

Saponin	+	+	+
Alkaloids	+	+	+
Terpenoids	+	-	-
Flavonoids	+	+	+
Carbohydrate	-	+	+
Steroids	-	+	+
Tannins	-	+	+
Glycosides	-	+	+
Protein	-	+	+

Key: S= sensitive R= Resistant.

Antimicrobial analysis of the extract of Calotropis procera

Tables 2, 3, and 4 show the outcomes of the extract's antimicrobial testing. The zones of inhibition of the microorganisms ranged from 17 to 28 mm, whereas the zones of inhibition of the conventional antibiotics ranged from 27 to 41 mm. With the exception of P. aeruginosa and S. pyogenes, whose MIC values were 6.25 mg/ml and 12.5 mg/ml, respectively, respectively, respectively, an n-Hexane extract's MIC value was attained at a concentration of 12.5 mg/ml. The corresponding MBC and MFC values for all the microorganisms were 25 mg/ml. The microorganisms' entire MIC for ethyl acetate extract was 25 mg/ml, and a corresponding with the exception of *P. aeruginosa* and *S. pyogenes*, all microbes showed MBC/MFC at 50 mg/ml. All microorganisms in the methanol extract had MICs of 25 mg/ml, although *P. aeruginosa* had its MIC at 12.5 mg/ml with a corresponding MBC/MFC of 50 mg/ml. Extracts from *C. procera* shown significant antibacterial activity against Klebsiella pneumonia[5]. The host population employs Klebsiella pneumonia for typhoid and other bacterial and fungal illnesses, hence it was not taken into consideration in our research work.

Table 2 shows the extract's zone of inhibition against the test microorganism.

Test Organisms	n-Hexane	Ethyl-acetate	Methanol	Sparfloxacin (mm)	Fluconazole (mm)
S. pyogenes	0	28	24	40	0
E. coli	17	25	22	29	0
K. pneumonia	0	0	0	32	0

P. mirabilis	0	0	0	28	0
P. aeruginosa	20	27	23	29	0
S. typhi	0	24	21	27	0
C. albicans	18	25	20	0	41
C. krusei	0	0	0	0	27
C. stellatoidea	17	24	22	0	40
C. tropicalis	0	0	0	0	38

Table 3 shows the extract's minimum inhibitory concentrations for the test microorganism in n-hexane, ethyl acetate, and methanol.

Test organism	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.13mg/ml	[illegible]	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.13mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.13mg/ml
S. pyogenes	-	-	-	o*	+	++	-	-	o*+	++	+++	-	o*	+	++	+++	++++
E. coli	-	-	o*	+	++	+++	-	-	o*+	++	+++	-	o*	+	++	+++	++++
P. aeruginosa	-	-	-	o*	+	++	-	-	o*	+	++	+++	-	-	o*	+	++ +++
S. typhi	-	-	o*+	++	+++		-	-	o*	+	++	+++	-	o*	+	++	+++ ++++
C. albicans	-	-	o*	+	++	+++	-	-	o*	+	++	+++	-	o*	+	++	+++ ++++
C. krusei																	
C. stellatoidea	-	-	o*	+	++	+++	-	-	o*	+	++	+++	-	o*	+	++	+++ ++++

Table 4 shows the minimum bacterial/fungal concentration of the extract for n-hexane, ethyl acetate, and methanol against the test microorganism.

Test Organisms	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.13mg/ml	1.57 mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.13mg/ml	1.57 mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.13mg/ml

S. pyogenes	- - o* + ++ +++	- o* + ++ +++ ++++	o* + ++ +++ ++++ +++++
E. coli	- o* + ++ +++ ++++	o* + ++ +++ ++++ +++++	o* + ++ +++ ++++ +++++
P. aeruginosa	- o* + ++ +++ ++++	- o* + ++ +++ ++++	o* + ++ +++ ++++ +++++
S. typhi	- o* + ++ +++ ++++	o* + ++ +++ ++++ +++++	o* + ++ +++ ++++ +++++
C. albicans	- o* + ++ +++ ++++	o* + ++ +++ ++++ +++++	o* + ++ +++ ++++ +++++
C. stellatoidea	- o* + ++ +++ ++++	o* + ++ +++ ++++ +++++	o* + ++ +++ ++++ +++++
C. tropicalis			

KEY: - No colony growth, o* MBC, + Scanty colonies growth, ++ Moderate,

Moderate colonies growth, +++ Heavy colonies growth

Proximate analysis of the extract of Calotropis procera

Tables 5, 6, 7, 8, and 9 show the outcomes of the proximal analysis. Crude fiber, protein, fat, moisture carbohydrate, and ash were all present in the sample, with values of 7.05 percent, 1.68 percent, 2.93 percent, 20.20 percent, 18.69 percent, and 56.50 percent, respectively, according to the proximate

analysis. They also have nutritional benefits as a source of amino acids, proteins have a significant influence in the organoleptic qualities of food[6]. It demonstrated how the plant under research can be used both as food and medicine. It was discovered that the crude fiber content was 8.22 percent, which is likewise within the range mentioned in the literature.

Table 5: Moisture Content of the extract of *Calotropis procera*

Weight of crucible(g)	Weight of the crucible + sample before drying(g)	Weight of crucible + sample after drying(g)	Weight loss(g)	% moisture
30.51	32.52	32.11	0.41	20.20%

% moisture content = weight loss/ weight of sample× 100/1

% moisture content= 0.41 / 2.01 × 100 / 1 = 20.20%

Table 6: Ash content of the extract of *Calotropis procera*

Weight of crucible(g)	Weight of crucible + sample before ashing (g)	Weight of crucible+ sample after ashing(g)	Weight of ashed sample(g)	% ash
28.61	30.64	29.49	1.15	56.50

% Ash content = weight of the ashed sample/ weight of the sample × 100/1

% Ash content= 1.15/2.03×100/1 = 56.50

Table 7: Carbohydrate content of the extract of *C. procera*

% carbohydrate = 100 – (% protein+ % moisture+ % crude fat+% crude fibre

% carbohydrate = 100 – (1.68+20.20+56.50+2.93) = 18.69

Table 8: Crude fat content of the extract of *C. procera*

Weight of crucible	Weight of crucible+ sample	% fat content
27.71	30.64	2.93

Weight of the crucible= 27.71

Weight of crucible= 30.64

% fat content = weight of crucible+ sample/ weight of sample * 100/1= 2.93

Table 9: Results of the proximate analysis of the extract of *C. procera*

Test performed	Results.
Total weight of the sample	5.00g
% moisture	20.20
% fat content	2.93(from hexane extract)
Carbohydrate	18.69
% Ash	56.50
%protein	1.68
% crude fiber	7.05

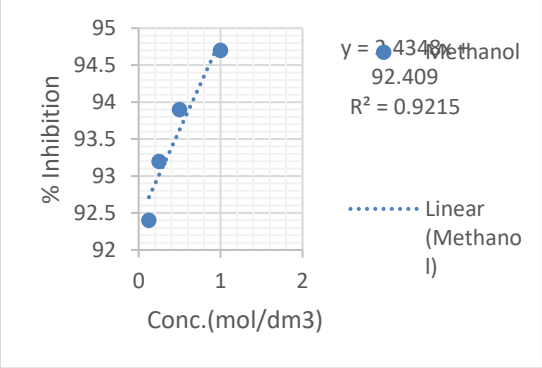
Antioxidant of the extract of C. procera

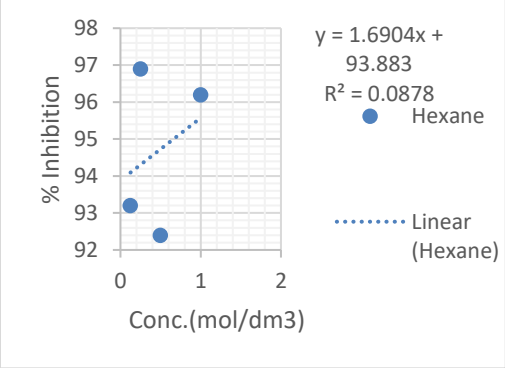
Table 10 shows the outcomes of the extract's antioxidant analysis. As the concentration dropped the methanol extract's percentage of inhibition also reduced. The antioxidant potency of the samples and standards were assessed using the IC50 (the concentration of the samples needed to scavenge 50% of the antioxidant). The total efficiency of the putative antioxidant is higher the lower the IC50. The most active field of research is finding natural antioxidants in plants since they typically have multiple uses, are safe, and have less adverse [15]. According to the findings, hexane extract demonstrated the best antioxidant properties. Efficacy with an indicated IC50 of 0.0878. Hexane therefore outperformed the other samples (ethyl acetate (0.8811) and methanol) in terms of effectiveness (0.9215). Hexane > Ascorbic acid > ethyl acetate > methanol > BHA was the general pattern when samples and

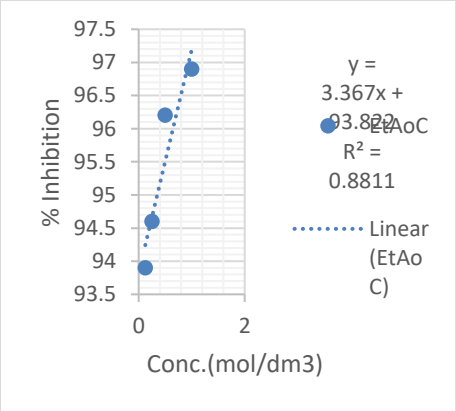
standards were compared (order of decreasing antioxidant effectiveness).

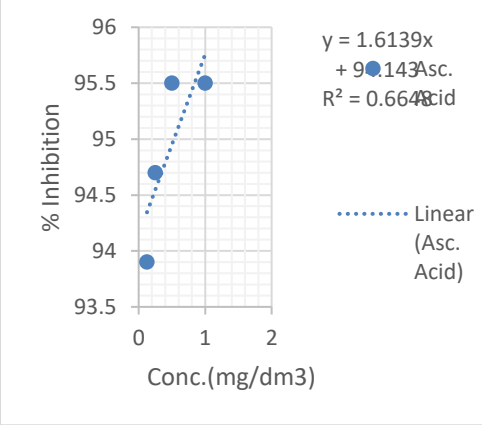
Table 10: Inhibition by Percentage

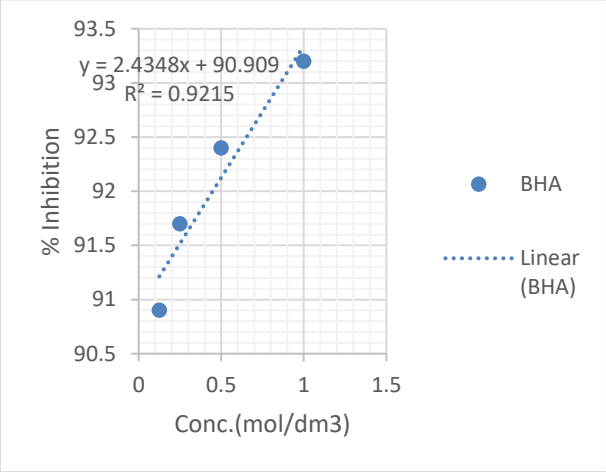
Conc.(mol/dm^3)	Methanol	EtAoC	Hexane	Asc. Acid	BHA
1.00	94.7	96.9	96.2	95.5	93.2
0.50	93.9	96.2	92.4	95.5	92.4
0.25	93.2	94.6	96.9	94.7	91.7
0.125	92.4	93.9	93.2	93.9	90.9











CONCLUSION

The current finding demonstrates that several physiologically and chemically active chemicals are found in the aerial plant portions of *Calotropis procera*. The traditional applications of *Calotropis Procera* as a medication are justified by the extract's effectiveness against the tested pathogens. As a result, *Calotropis procera* is useful in the treatment of fatal illnesses. The plant's high antioxidant activity indicates that it will be effective in combating ailments brought on by oxidative chemicals. Phytochemical A screening found components that can be used as medicines. The plant is abundant in fiber nutrients, according to the findings of the proximate analysis.

This study backs up claims made by the local population regarding the plant, offers proof of its safety when taken, and throws light on how medications are made. The current finding demonstrates that several physiologically and chemically active chemicals are found in the aerial plant portions of *Calotropis procera*. The traditional applications of *Calotropis Procera* as a medication are justified by the extract's effectiveness against the tested pathogens. As a result, *Calotropis procera* is useful in the treatment of fatal illnesses. The plant's high antioxidant activity indicates that it will be effective in combating ailments brought on by oxidative chemicals. Phytochemical screening uncovered components that can be used as medicines. The plant is abundant in fiber nutrients,

according to the findings of the proximate analysis. This research supports local community statements about the plant's safety and dependability for consumption, and also sheds light on medicine formulation.

COMPETING INTERESTS: According to Oluwakayode Odeja and Christiana Ene Ogwuche, there is no competing interests.

AUTHOR'S CONTRIBUTION: OCE carried out the sample collection. Both OCE and OO were involved in the different laboratory analysis and write up. OCE was directly involved in proximate analysis and antimicrobial. OO carried out the antioxidant analysis and phytochemical screening. Both OCE and OO were involved in writing and editing.

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