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Phytochemical, Analgesic, Antioxidant and Antimicrobial Activities of *Calotropis procera* (Apple of Sodom) *L*eaves

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Abstract

With the emergence of new drug resistant microorganisms and current anti-oxidative drugs, many medicinal plants will continue to be the best source of drugs which could be administered as herbal supplements. The present study was designed to evaluate the phytochemical content, antimicrobial and analgesic activities of Calotropis procera leaf (methanol extract). Antimicrobial activities was carried out against selected clinical isolates by agar well diffusion method. The anti-oxidative activities of the extracts were determined by means of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, phenol, carbohydrate, steroids and triterpenes. Staphylococcus aureus was found to be the most susceptible bacteria at 500 mg/ml with inhibition zone of 17 mm followed by Escherichia coli, Streptococcus pyogenes, and Aspergillus niger, Staphylococcus aureus with 14 mm and MIC of 31.25 mg/ml each. The extract demonstrated dose-dependent varying degrees of anti-oxidative efficacy in the 2, 2-diphenyl-1-picrylhydrazyl radical scavenging assays. For the DPPH assay, IC_{50} values of 115.2 µg/mL. The analgesic effects observed at 20 mg/kg more than that of 40 mg/kg and 80 mg/kg of the extract. The results showed that Calotropis procera leaf extract possess compounds with antimicrobial, antioxidant as well as analgesic properties. The findings will add value to the traditional uses of Calotropis procera leaves and conservation is necessary due to the significant medicinal properties demonstrated by the plant. Further research on isolation and characterization of the active compounds from fractions that showed antimicrobial and analgesic activities is recommended.

Keywords: Analgesic properties, antimicrobial, antioxidant, *Calotropis procera,* Phytochemical.

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INTRODUCTION

Since ancient times, herbal drugs have been used as medicine for the treatment of a range of diseases (Dixit *et al.,* 2023). Herbal medicine is becoming popular all over the world than the Allopathic medicine for medication. Several medicinal plants have been screened based on the integrative approach on drug development. The use of the plants, plant extracts and pure compounds isolated from natural sources has always provided a foundation for modern drugs (Dixit *et al.,* 2023). The pharmacological evaluation of medicinal plants has promoted the use of herbal medicines through confirmation of their efficacy and safety (Teopolina, 2016). Only few plant species have been subjected to analysis and investigated biologically or pharmacologically (Teopolina, 2016). Research on antimicrobial, analgesic and oxidative stress drug from plants has gained much interest (Teopolina, 2016). This is because antibiotics have become ineffective since many microorganisms develop resistance against them. Therefore, more researches are needed on the isolation and identification of bioactive compounds from plant materials to develop new drugs with hopefully new mechanism of action and better activity profiles. The phytochemical, analgesic, antioxidant and antimicrobial analysis of extract from this plant species can serve as the baseline for identifying the active compounds responsible for this activity. These in turn can be used as substrates in the formation of new antimicrobial, analgesic and antioxidant agents. Validation of these plant species can also encourage the use of *Calotropis procera* leaves and this can benefit many communities because of their easy availability, simplicity and low cost.

In Nigeria traditional medicine, *C. procera* is either used alone or with other herbs to treat common diseases such as fevers, rheumatism, indigestion, cold, eczema and diarrohea (Dixit *et al.,* 2023). Antimicrobial, antioxidant and analgesic activity of *C. procera* have not been properly documented. Also, there is a need for safer and better antioxidants since current anti-oxidative drugs have safety concerns (Teopolina, 2016). In addition, oxidative stress is linked to cell damage which could lead to the development of various diseases such as cancer, autoimmune disorder, cardiovascular and neurodegenerative diseases (Teopolina, 2016). There are few results found in the literature for antimicrobial and analgesic studies done on the plant species, nor are there reports found on the study of antioxidant properties. The total phenolics and flavonoids content present in *Calotropis procera* is unknown and need to be determined, as these two chemical compounds are known to possess good antioxidant and antimicrobial properties.

Materials and Methods

Collection and Identification of *Calotropis procera*

The leaves of *Calotropis procera* were collected in March, 2023 at Bayero University, Kano, Kano state. The plant was identified and authenticated in the Herbarium of the Plant Biology Department of Bayero University, Kano and was compared with a voucher specimen number.

Experimental Animals
Mice of both sexes weighing 25-32 g were obtained from the Department of Pharmacology and Therapeutics, Bayero University, Kano. The animals were allowed free access to standard feed and water *ad libitum*. They were kept in clean cages filled with saw dust which was replaced every three days.

Preparation of Plant extracts
The *Calotropis procera* leaves were cleaned, air dried and ground to coarse powder using grinding machine. The powder was stored in air tight containers for further use. Two hundred grams (200 g) each of the powdered stem bark was soaked into 2 L of methanol. The mixtures were allowed to stand for 3 days at room temperature (28 ±2^{o}C) with hourly agitations. The extract was sieved through a muslin cloth, filtered through a Whatman (No.1) filter paper, poured unto a clean evaporating dish and placed on a water bath at 50^{o}C until all the solvent evaporated.

Qualitative Phytochemical of screening Methanolic extract of *Calotropis procera* leaves
The plant extract were subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the methods described below.

Tests for carbohydrates
Molish's (General) Test for Carbohydrates: To 1 ml of the filtrate, 1 ml of Molish's reagent was added in a test tube, followed by 1 ml of concentrated sulphuric acid down the test tube to form a lower layer. A reddish colour at the interfacial ring indicated the presence of carbohydrate (Evans, 2009).

Tests for Saponins
Frothing test: About 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 mins. A honeycomb froth that persists for 10-15 mins indicated presence of saponins (Evans, 2009).

Test for Flavonoids

Shinoda Test: A portion of the extract was dissolved in 1-2 ml of 50% methanol in the presence of heated metallic magnesium chips and a few drops of concentrated hydrochloric acid were added. Appearance of red color indicated the presence of flavonoids (Evan, 2009).

Test for Alkaloids

Wagner's Test: Few drops of Wagner's reagent was added into a portion of the extract, whitish precipitate indicated the presence of alkaloids (Evans, 2009).

Test for Steroids and Triterpenes

Liebermann-Burchard's test:

Equal volumes of acetic acid anhydride were added to the portion of the extract and mixed gently. Concentrated sulphuric acid (1 ml) was added down the side of the test tube to form a lower layer. A colour change observed immediately and later indicates the presence of steroid and triterpenes. Red, pink or purple colour indicated the presence of triterpenes while blue or blue green indicates steroids (Evans, 2009).

Test for Cardiac Glycosides

Kella-killiani's test:

A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Interphase for purple-brown ring was carefully observed, this indicates the presence of deoxy sugars and pale green colour in the upper acetic acid layer indicated the presence of cardiac glycosides (Evans, 2009).

Test for Tannins

Ferric chloride test:

3-5 drops of ferric chloride solution was added to the portion of the extract. A greenish black precipitate indicates the presence of condensed tannins while hydrolysable tannins give a blue or brownish blue precipitates (Evans, 2009).

Test for Anthraquinones

Borntrager's test:

Exactly 5ml of chloroform was added to the portion of the extract in a dry test tube and shaken for 5mins. This was filtered, and the filtrate shaken with equal volume of 10% ammonium solution, bright pink colour in the aqueous upper layer indicated the presence of free anthraquinones (Evans, 2009).

Microbiological analysis

Isolation of Bacteria Species

The specimens were cultured on sterile blood agar, chocolate agar and Mac-conkey agar plates at 37°C for 24 h in an incubator. Discrete colonies were picked based on their morphology and further sub-cultured on blood agar and chocolate agar to obtain pure strains. The isolated colonies were Gram stained and based on their Gram reactions were inoculated on different selective media — mannitol salt agar, cetrimide agar, eosin methylene blue agar. Different biochemical tests were conducted (catalase, coagulase, and oxidase tests). All the isolates that grew on selected agar media were then placed on nutrient agar and chocolate agar slants and maintained in a refrigerator at 4°C (Cheesbrough, 2010).

Identification and Characterization of Test Organism using Rapid Test Kits

Identification and characterization of the bacteria were carried out using Microgen Identification Kit (XYZ). The test was performed according to the manufacturer's specifications (API biomerieux). It was performed by adding saline suspension of the test organisms to each of the wells, and appropriate wells (1, 2, 3 and 9) were overlaid with sterile paraffin oil. After overnight incubation (18-24 hours) at 37°C, suitable reagents such as Nitrate A and B, Kovacs, Typtophan deaminase (TDA), Voges-proskauer (VPI and II) were added to wells 8, 10 and 12 for additional tests and colour changes of the different tests recorded. The results were converted into four to eight digits codes depending on the organisms being tested and interpreted using the Microgen Identification Software Package (MID-60) (Sylvester, 2016).

Antimicrobial Susceptibility Test

Preparation of Extract Concentration

This was carried out according to the method described by Srinivasan *et al.,* (2009). Stock solution of the plant extracts were prepared by adding 0.5g of each crude plant extract in 1ml dimethyl sulphuroxide (DMSO). From each of the stock solutions, 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml concentrations were prepared using Two-fold serial dilution method (Srinivasan *et al.,* 2009).

Standardization of bacterial Inoculum.

Using inoculum loop, over-night grown agar culture (bacteria and fungi) were transferred into a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard as described by the National committee for clinical laboratory standard (NCCLS, 2008).

Susceptibility Test of Bacterial and Fungal isolates to Different Concentrations of the Extracts

The antimicrobial activity of *Calotropis procera* leaf crude extract (Methanol) against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Streptococcus pyogenes, Pseudomonas aeruginosa, Aspergillus flavus, Candida albicans,* and *Aspergillus niger* were evaluated using agar well diffusion method of susceptibility test (Srinivasan *et al.,* 2009*)*. Mueller-Hinton agar and Sabouraud Dextrose agar plates were inoculated with 0.1ml of standardized inoculum of each bacterium (in triplicates) using 0.1ml pipette and spread uniformly with sterile swab sticks. Three wells of 6mm size were made with sterile cork borer (6 mm) into the inoculated agar plates. Using micropipette, 0.1ml volume of the various concentrations; 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml each of the crude extracts were dispensed into wells of inoculated plates. . DMSO was used as negative control. Commercially available standard antibiotic, ampicillin and fluconazole were used as positive control parallel with the extract. The prepared plates were then left at room temperature (37 ^{o}C) for 10 minutes, allowing the diffusion of the extracts into the incubation at 37 ^{o}C for 24 hrs in an incubator. The diameter of inhibition zones (DIZ) were measured and expressed.

Determination of Minimum Inhibitory Concentration (MIC)

The method used was the tube dilution method (Adesokan *et al*., 2007). Thus, the plant extracts were serially diluted from 500 mg/ml solution to obtain varying concentration. The concentrations were; 250 mg/ml, 125 mg/ml, 62.5mg/ml, and 31.25 mg/ml. Doubling dilutions of the extract were incorporated in Muller Hinton broth (Oxoid, UK) and Sabouraud Dextrose broth, and then inoculated with 0.1ml each of standardized suspension of the test organisms into the various test tube containing varying concentrations. Another set of test tubes containing only Mueller Hinton broth were used as negative control, and another test

tube containing Mueller Hinton broth and test organisms were used as positive control. All the test tubes and controls were then incubated at 37 ^{o}C for 24hrs. After incubation period, the presence or absence of growth on each tube was observed. A loop full from each tube was further sub cultured onto nutrient agar to confirm whether the bacterial growth was inhibited.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC and MFC were determined by collecting 1ml of broth culture from the tubes used for the MIC determination and sub culturing into fresh solid nutrient agar plates. The plates were incubated at 37 ^{0}C for 24 h. The least concentration that did not show any growth after incubation was regarded as the MBC and MFC (Adesokan *et al*., 2007).

Analgesic studies of *Calotropis procera* leaves

Acetic acid induced writhing in mice

Acetic acid induced writhing method described by (Koster *et al*., 1959) was adopted for evaluation of analgesic activity. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, turning of trunk (Mishra *et al*., 2011). 30 Swiss albino mice of both sexes were divided into five groups, 1 and 5 served as negative control (distilled water 10 ml/kg) and positive control (Diclofenac 20 mg/kg), while groups 3, 4, and 5 received 250 mg/kg, 500 mg/kg, and 1000 mg/kg of the extract. All administrations were per oral.

Sixty minutes after treatment, the mice received 0.6% acetic acid (10ml/kg) interperitoneally to induce pain. 5minutes after acetic acid injection, the animals were observed and number of writhes by each mouse was counted for 15minutes. Percentage inhibition was calculated using the following formular (Mishra *et al*., 2011).

$$\%\ \text{inhibition} = \frac{\text{Average number of writhes (control)} - \text{Average number of writhes (test)}}{\text{Average number of writhes (control)}}$$

Antioxidant activity Procedure

The antioxidant activity of the plant extracts was measured in terms of radical scavenging ability, using a stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the modified method adopted from (Sani and Dailami, 2015). 200µl of 100µM methanol solution of DPPH were added to 100µL of various concentrations of the sample fractions in methanol (1000, 500, 250, 125, 62.5, 31.25, 15.63 and 7.8µg/ml) and made to react in dark for 30mins at room temperature. Absorbance of the blank, test and control were recorded at 517 nm. The experiment was performed in triplicate and scavenging activity was calculated by using the following formula and expressed as percentage of inhibition

$$\%\ \text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

The concentration corresponding to the 50%inhibition (IC_{50}) was determined using probit analysis by means of SPSS 16.0 software. The IC_{50}values obtained are compared with that of ascorbic acid as a standard antioxidant.

Results

The plant material was extracted with methanol to yield dark green extract (16.2 g) (Table 1).

Table 1. Mass and Percentage Yield of *Calotropis procera* leaf extract

Extract	Mass (g)	Percentage Yield (%)	Extract Appearance
Methanol	16.2	8.1	Dark green

Flavonoid, alkaloids, saponins, steroid, triterpenes, tannins, carbohydrate, phenols were detected in the extract while anthraquinones and glycosides were absent.

Table 2. Phytochemical screening of the *Calotropis procera* leaf extract

Metabolite	Inference
Alkaloid	+
Flavonoid	+
Saponins	+
Cardiac glycoside	-
Tannins	+
Steroid	+
Triterpenes	+
Phenol	+
Anthraquinones	-
Carbohydrate	+

The extract significantly decreased the number of writhes caused by acetic acid in a dose independent manner as shown in Table 3. The effects observed at 20 mg/kg more than that of 40 mg/kg and 80 mg/kg of the extract.

Table 3: Effect of methanol leaf extract of *Calotropis procera* on Acetic Acid Induced writhing in mice

Treatment	Dose (mg/kg)	Mean No. of Writhes ± SEM	Inhibition (%)
Distilled water	10ml/kg	46.0±0.33	-
Diclofenac	10	12.0±0.34	73.9
Extract	20	28.0±0.43	39.1
Extract	40	32.0±0.49	30.4
Extract	80	22.0±0.65	52.2

Table 4. Antimicrobial activity of Methanol extract of *Calotropis procera* leaves

Clinical isolates	**Concentration/Diameter zone of inhibition (mm)**						**MIC**	**MBC/MFC**
	500	250	125	62.5	CPR/FLC	DMSO		
S. aureus	17	14	12	10	32/*	06	31.25	62.5
E. coli	14	12	10	08	30/*	06	31.25	62.5
Klebsiella pneumoniae	10	08	06	06	34/*	06	-	-
P. aeruginosa	10	08	06	06	38/*	06	-	-
S. pyogenes	14	12	10	08	30/*	06	31.25	62.5
A. flavus	12	10	08	06	*/38	06	-	-
A. niger	14	12	10	08	*/37	06	31.25	62.5
C. albicans	12	10	08	06	*/38	06	-	-

Key: - Not tested for MIC and MBC/MFC because there was no inhibition at lowest concentration
*= Not test CPR (Ciprofloxacin was only used against bacterial isolates), FLC (Fluconazole was used against fungal isolates).

Table 5. Antioxidant activities of Methanolic extract of *Calotropis procera* leaves

Analyte	**Concentration (µg/mL) / % Inhibition**							
	1000	**500**	**250**	**125**	**62.5**	**31.25**	**15.6**	**7.8**
Extract	80.8	84.6	79.4	57.6	36.1	18.0	10.54	1.21
Ascorbic acid	91.5	88.5	89.6	89.4	89.2	82.8	46.6	38.7

The trend of the inhibition of DPPH radical by the extract was concentration dependent with respect to the IC_{50} values (concentration of the extract to cause 50% inhibition), the DPPH radical scavenging ability of the extract showed the following trend extract < Ascorbic acid. The IC_{50} values of the extract of *Calotropis procera* against DPPH free radical was 115.2 µg/ml. The high IC_{50} values obtained from this study, showed that extract had low antioxidants activities against DPPH radicals. The trend of the inhibition of DPPH radical by the extract was concentration dependent with respect to the IC_{50} values (concentration of the extract to cause 50% inhibition), the DPPH radical scavenging ability of the extract showed the following trend extract < Ascorbic acid. It is interesting to note that the lower the IC_{50} value, the higher the scavenging activity of the plant extract (Sowunmi and Afolayan, 2015).

Table 6: Antioxidant Activities of Methanolic extract of *Calotropis procera* leaves

Sample	IC_{50} (µg/mL)
Extract	115.2
Ascorbic acid	8.781

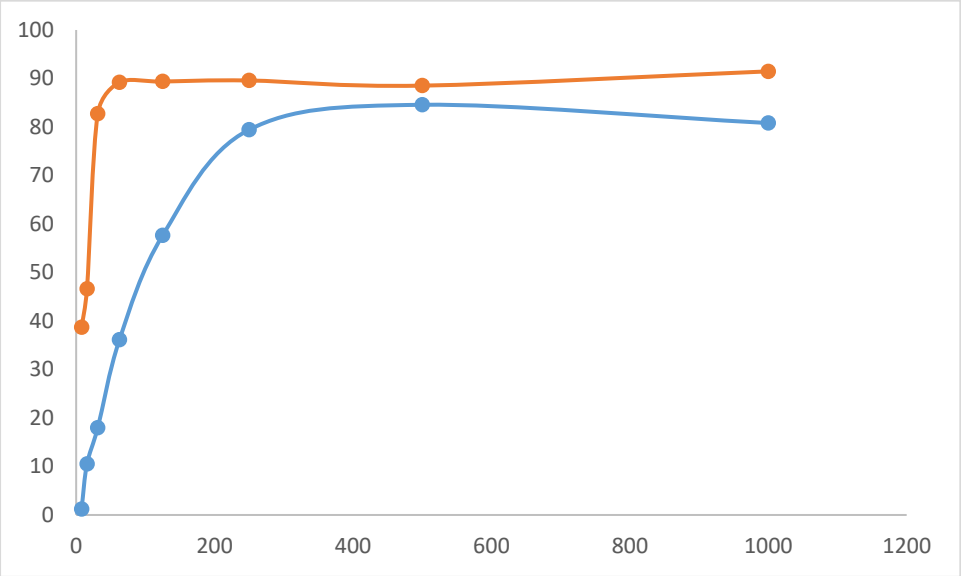

Fig. 1. Antioxidant Activities of Methanolic extract of *Calotropis procera* leaves

DISCUSSION

The results of this study showed the presence of carbohydrates, alkaloid, tannins, flavonoids, steroids, triterpenes, phenol and saponins in the methanolic leaf extract of *Calotropis procera* which might be responsible for its pharmacological actions. Anthraquinones and glycosides were however absent. Flavonoids have been used as effective constituents of several drugs used for treatment of capillary fragility and phlebosclerosis. (Musa, 2006). It has been suggested that flavonoids, which contain free hydroxyl groups at 3, 3′ and 4′ positions exert beneficial physiological effects on capillaries. Orally administered flavonoids have been observed to inhibit vascular permeability and prevent pulmonary haemorrhage. Flavonoids play this important role of maintaining capillary integrity by functioning as antioxidants (Musa, 2006). Several flavonoids isolated from medicinal plants have been shown to possess significant anti-nociceptive and/or antiinflammatory effects (Musa, 2006). This activity could

be linked to the presence of tannins and flavonoids that have been used in the treatment of diarrhea and dysentery (Musa, 2006).

Many naturally occurring compounds found in plants, have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against pathogens (Amponsah, 2012). Microbial infectious diseases represent an important cause of morbidity and mortality worldwide. Therefore, the development of new antimicrobial agents for the treatment of bacterial and fungal infections is of increasing interest. *Calotropis procera* leaf extract tested showed antibacterial and antifungal activities against *E.coli*, *S.aureus, S. pyogenes, Klebsiella pneumoniae, P. aeruginosa and Aspergillus niger* and *Aspergillus flavus, Candida albicans* at highest concentration of 500 mg/ml which may reflect the antimicrobial activity of plant active ingredients that inhibit bacterial growth. In the present study, the methanol extract of the leaves, inhibited the growth of all test organisms used at 250 mg/ml. It however, showed moderate activity against *Candida albicans*. It produced significant activities against *E. coli, S. aureus, A. niger* and *S. pyogenes* with respective MIC of 31.25 mg/ml. The methanol extract of *Calotropis procera* leaves showed good activity against *S. aureus (10 mm), E. coli, A. niger* and *S. pyogenes* with respective zones of inhibition of 8 mm each. The ethanol extract was inactive against *K. pneumoniae, P. aeruginosa, A. flavus and C. albicans at lowest concentration of 62.5 mg/ml* (Table 4). The considerable susceptibility of both Gram positive and Gram negative organisms to the extract at 500 mg/ml supported the folkloric use of the *Calotropis procera* leaves for wound healing, treatment of veneral diseases and other infections (Amponsah, 2012). Also the high susceptibility of *S. aureus* to the extracts further supports the use of the plant for the treatment of wounds since *S. aureus* is a common pathogen in most infected wounds (Amponsah, 2012).

The observed antimicrobial activities on the isolates is believed to be due to the presence of tannins, flavonoids and saponins which have shown to possess antimicrobial properties (Yagana *et al.,* 2012). The antimicrobial activities of *Calotropis procera* leaf extract have been attributed to the presence of these secondary metabolites (Jiyil, 2015). As shown by the phytochemical screening results, the extract of *Calotropis procera* leaves contain all these secondary metabolites. Some other researchers have identified, that tannins, flavonoids and alkaloids in the extracts of some medicinal plants possess antimicrobial activity (Yadav *et al*., 2011). thus, the growth inhibition effect of the extracts on the clinical isolates could be attributed to the presence of bioactive substances such as phenolic acids, tannins and flavonoids as reported by other workers. Phenolic acids are highly hydroxylated phenols, scientific evidence show that increase hydroxylation of phenol result to increased toxicity to pathogens (Yadav *et al*., 2011)

Saponins are surface active agents which alter the permeability of the cell wall of organisms thus facilitating the entry of toxic materials or leakage of vital constituents from the cell (Daniyan *et al*., 2010). In medicine, saponins are used as hypercholesterolemia, hyperglycemia, antioxidant, anti-cancer, anti- inflammatory agents due to their detergent property (Jiyil, 2015). These properties confirm saponins as potent antimicrobial agent. Tannins are polyphenols known to exhibit antibacterial, antiviral and anti-tumor activities. It was also reported that certain tannins are known to inhibit HIV replication selectively and is also used as diuretic (Jiyil, 2015). The leaves of *C. procera* also showed the presence of alkaloid which is known to possess anti-inflammatory and anti-asthmatic actions. This confirms the use of *C. procera* in folklore medicine for the treatment of malaria. Alkaloid is also one of the largest groups of phytochemicals that has amazing effect on humans which led to the development of powerful pain killer medications (Jiyil, 2015). Flavonoids have been referred to as nature's

biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergen, virus and carcinogens. Some flavonoids have also been reported to act like some coumarins in the inhibition of giant cell formation in HIV infected cell cultures (Jiyil, 2015).

The extract exhibited analgesic potency. It significantly inhibited the abdominal constriction induced by acetic acid the mice. Acetic acid causes an increase in peritoneal fluids of PGF2 and PGF2α involving in part, peritoneal receptors and is very sensitive method of screening analgesic effect of compound (Mailafiya, 2014). The observed effect of the extract in this study suggested that postaglandins may be involved in the action of the extract (Mailafiya, 2014), the ability of the extract at 80 mg/kg and 20 mg/kg to produce comparable result to Diclofenac group in the study indicated moderate level of analgesic activity (Table 3).

Calotropis procera leaf extract displayed appreciable DPPH free radical scavenging activity. However, the scavenging activity was lower than that of ascorbic acid. Results obtained in the present study are a good indication that the extract possess proton-donating ability, and could serve as free radial inhibitors or scavengers. This signifies that leaf methanolic extract of *Calotropis procera* are capable of donating electrons that can react with free radicals to convert them into more stable products and thus inhibiting radical chain reactions. The total antioxidant capacity of the extract increased significantly with an increase in extract concentration. A relatively low total antioxidant capacity was observed for the leaf extract. Commercial antioxidants derived from plant sources are limited, with examples of curcumin, a phenolic compound and major component of *Curcuma longa* and resveratrol, a polyphenolic compound found in the skin of grapes (Teopolina, 2016). Thus, the findings of the study indicates the potential of the leaf extract of *Calotropis procera* as sources of antioxidant compounds. The DPPH assay has been a quick, reliable and reproducible parameter used to evaluate the in *vitro* antioxidant activity of plant extracts (Aliyu *et al.,* 2009). The decrease in absorbance by the DPPH radical with increase in concentration of the extract (Figure 1) which manifested in the rapid discolouration of the purple DPPH, suggested that the methanol extract of *C. procera* has antioxidant activity due to its proton donating ability. The extract was found to low scavenge free radicals activity when compared to standard antioxidants (Table 5). This indicates that there is no significant difference between the antioxidant activity of *C. procera* and those of standard ascorbic.

The trend of the inhibition of DPPH radical by the extract was concentration dependent with respect to the IC_{50} values (concentration of the extract to cause 50% inhibition), the DPPH radical scavenging ability of the extract showed the following trend extract < Ascorbic acid. The IC_{50} values of the extract of *Calotropis procera* against DPPH free radical was 115.2 µg/ml. The high IC_{50} values obtained from this study, showed that extract had low antioxidants activities against DPPH radicals. The trend of the inhibition of DPPH radical by the extract was concentration dependent with respect to the IC_{50} values (concentration of the extract to cause 50% inhibition), the DPPH radical scavenging ability of the extract showed the following trend extract < Ascorbic acid. It is interesting to note that the lower the IC_{50} value, the higher the scavenging activity of the plant extract (Sowunmi and Afolayan, 2015).

CONCLUSION

The study revealed that the leaf methanolic extract of *Calotropis procera* contains flavonoid, alkaloids, saponins, steroid, triterpenes, tannins, carbohydrate, phenols were detected in the extract and suggested that the plant could be a source of potential antimicrobial, antioxidant and analgesic agents. The observed appreciable antimicrobial, antioxidant and analgesic

activity of the leaf extract might be linked to the high phenolic and flavonoid contents of the extract. In addition, the findings from this study supported the traditional uses of *Calotropis procera* leaves. Moreover, the findings of this study add value to the traditional uses of *Calotropis procera*. Locally, *Calotropis procera* grows in the wild thus conservation of this plant is necessary due to the significant antimicrobial and analgesic activities demonstrated by *Calotropis procera* in this study.

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