Global Scientific JOURNALS

GSJ: Volume 11, Issue 8, August 2023, Online: ISSN 2320-9186
www.globalscientificjournal.com

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *Calotropis procera* (SODOM APPLE) LEAF AND FRUIT EXTRACTS AGAINST SELECTED CLINICAL ISOLATES

***Ogbeba J., Ebu B., Yakubu D.A., Wada N.M., Ahmed A., Isa A. S., Oladimeji R. U., and Okanlawon, M. E.**

Department of Science Laboratory Technology, Federal Polytechnic Bauchi, Bauchi state, Nigeria.

*Corresponding Author's Email: ogbebaj@fptb.edu.ng, jerrielife2012@gmail.com

ABSTRACT

Leaf and fruit extract against *Calotropis procera* were screened for its phytochemical and antimicrobial activities. Ethanolic and aqueous solvents were used for the leaf and fruit extraction. The extracts were tested against *Streptococcus pneumonia*, *Salmonella typhi* and *Tinea corporis* using agar diffusion method. The result revealed that the *C. procera* leaf and fruit aqueous extract had higher antimicrobial properties than the ethanol extract. The result of the antimicrobial activity also revealed that the leaf extracts had higher antimicrobial activity against the microorganisms than the fruit. The aqueous-leaf extract revealed highest inhibitory potency against *Streptococcus pneumonia* (17±4.1mm to 30±5.5mm) and *Salmonella typhi* (14±3.7mm to 28±5.3mm) while the aqueous- fruit, had *Streptococcus pneumonia* (13±3.6mm to 29±5.4mm) and *Salmonella typhi* (14±3.7mm to 28±5.3mm). This result also revealed that Gram-positive organism (*Streptococcus pneumonia*) was more susceptible to the extracts than the gram-negative (*Salmonella typhi*). The minimum inhibitory concentrations (MIC) of extracts on bacteria and fungi isolates were between 12.5mg/ml and 100mg/ml. Minimum bactericidal concentration (MBC) and fungicidal concentration (MFC) of the extracts were mostly at 50% on the employed test organisms. The result of phytochemical analysis revealed that the leaf extracts had higher quantities of bioactive constituents than the fruit. Alkaloids (44.03±0mg/g) and tannin (35.5±0mg/g) had the highest values in the leaf while alkaloids and saponin were absent in fruit which had cardiac glycosides (12.37±0.0mg/g) with the highest value. Flavonoid (28.0±0mg/g) was also higher in leaf than the fruit (3.34±0mg/g). However, saponin, alkaloids, tannins, steroids, terpenoids, phenol, cardiac glycosides and carotenoids were also qualitatively and quantitatively screened. The various phytochemical constituents analyzed could have contributed to the antimicrobial effects on the selected test organisms. The obtained result provides a support for the use of *Calotropis procera*, in traditional medicine and suggest its further advance investigation.

Keyword: *Calotropis procera,* Antioxidant, *Tinea corporis*, agar diffusion method and fungicidal concentration

INTRODUCTION

Many research efforts have been directed towards the provision of empirical proofs to back up the use of plants species in trade and medicinal practices in recent years (Ojo *et al*., 2005). It has been widely observed and accepted that the medicinal value of plants lies in the bioactive phyto-components present in the plant (Abubakar *et al*., 2011; Mandal *et al*., 2010; Veeramuthu *et al.*, 2006). Because of the side effect and the resistance that pathogenic microorganisms build against

antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicine (Nazma *et al*., 2008).

Many studies have examined the effects of plants used traditionally or by indigenous healers to support treatment of various diseases; scientific validations are being made globally to get evidence for traditionally reputed herbal plants. However, there still exist a large number of plants with tremendous medicinal potentials that have not been investigated (Ojo *et al*., 2005).

Calotropis procera also known as Sodom apple is a member of the plant family Asclepiadaceae; a shrub about 6m high and is widely distributed in West Africa and other parts of the tropics (Abbas *et al.,* 2012). *Calotropis procera*, a perennial, is a greyish-green, woody shrub with broad obovate fleshy leaves that grows wild in the tropics and in warm temperate regions. The plant is found in almost all parts of Nigeria but more abundant in the northern parts of the country (Aliyu, 2006). It is locally known as Tumfafiya in Hausa, Bomubomu in Yoruba and Kayou in Kanuri (Noatay, 2005).

Traditionally, *Calotropis procera* is used to treat common diseases such as fever, rheumatism, indigestion, cold, paralysis, eczema and diarrhoea (Kuta, 2006). The pungent sap latex is used to treat boils, infected wounds and other skin problems in people and to treat parasitic skin infestation in animals (Vohra, 2004).

It has also been used to kill intestinal nematodes and filarial worms (Jain *et al.,* 2007). The root extract has been used to treat headache, eczema, leprosy, elephantiasis, asthma, cough (Sen *et al*., 2007) severe body pain, malaria fever and convulsion (Kayode 2006). Root extract is orally used by ladies in dysmenorrheal, protracted labour and dysentery (Showkat, 2007). It is also used as coagulation agent for cheese making in West Africa (Khan, 2009).

Antimicrobial drug resistance has recently been on the increase due to indiscriminate use of commercial antimicrobial drugs which are commonly used in the treatment of infectious diseases. This situation has brought about the need to search for new antimicrobial substances from various sources such as medicinal plants.

Since medicinal plants have been reported to contain a number of compounds that may be potential natural antimicrobial agents, they may serve as alternative, effective, cheaper and safe antimicrobial agents for the treatment of common microbial infections.

This study is to investigate the phytochemical and antimicrobial potentials of the leaves and fruits extracts of *Calotropis procera* against some clinically selected bacteria fungi in the north eastern zone of Nigeria

MATERIALS AND METHODS

Collections and Processing of Plant Samples

Fresh leaves and fruits of *C. procera* were obtained from Gwallameji market within Bauchi metropolis, Bauchi state, Nigeria in August, 2019 and were transported to Microbiology Laboratory of Science Laboratory Department (SLT), Federal Polytechnic Bauchi (FPTB). Botanical identification was determined by the descriptions given by Odugbemi (2006) and confirmation by native regular users of plant samples for traditional medicine at area of collection.

The leaves and fruits were sorted out to remove debris, washed thoroughly and air-dried at ambient temperatures for about 2-3 weeks under the shade. It was then ground into fine powder using pestle and mortars. The sample was then stored in air tight containers prior to use.

Collection and Confirmation of Test Microorganisms

Two clinical bacteria species (*Salmonella typhi, Streptococcus pneumoniae*) and one fungus (*Tinea corporis*) were obtained from the central diagnostic laboratory of National Veterinary Research Institute (NVRI), Vom, Plateau state.

These bacteria and fungi were then sub-cultured on nutrients agar and potato dextrose agar respectively and stored at 4^0C until required for study. The fungus species was confirmed by the

criteria of Barnett (1960) and bacteria species were studied through cultural, morphological, physiological and biochemical characteristics and were identified (Cheesbrough, 2006).

Preparation of Plant Extracts

Aqueous and ethanol extractions were carried out according to the method of Fatope *et al.* (1993).

Aqueous Extraction

150g of the fine ground powder of each plant parts (leaves and fruits) were weighed and suspended into 750ml-conical flask respectively. The suspended solutions were allowed to stand for two weeks. The aqueous extractions were subjected to agitation in a shaker bath. The percolates were then filtered using sterilized Whatman No. 1 filter paper. The aqueous solutions were then evaporated with water bath regulated at 55°C. The extracts were then stored at 4°C until required for use.

Ethanol Extraction

150g of fine ground powder of each of the plant parts was suspended into 800ml of 95% ethanol. It was then allowed to stand for two weeks with constant shaking at regular intervals under room temperature. It was then filtered using sterilized Whatman No. 1 filter paper and evaporated using water bath regulated at 55°C. All concentrated extracts were stored at 4°C until required for use.

Qualitative Phytochemical Analysis of the Extracts.

Specific qualitative tests were performed to identify bioactive compounds of pharmacological importance through standard method. Tannins, alkaloids, Saponins, flavonoids, terpenoids, and phenols were qualitatively determined using methods described by Mehta *et al. (*2013) Satheesh *et al., (*2013) and Soforowa (2003).

Test for alkaloids (Mayer's test)

Two milliliters of extract was measured in a test tube to which picric acid solution was added. The formation of orange coloration indicated the presence of alkaloids.

Test for phenolics

The extract mixed with 1% FeCl3 will form a blue, violet, purple, green or red brown. This indicates the presence of phenols.

Test for tannins

The extracts mixed with basic lead acetate solution. Formation of white precipitate indicates the presence of Tannins.

Test for saponins

Froth test for saponins was used. 1 g of the sample was weighed into a conical flask in which 10 ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5 ml of the filtrate was added to 10 ml of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30
second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Test for flavonoids

Five milliliters of dilute ammonia solution were added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated H_2SO_4. Formation of yellow color observed in each extract indicated the presence of flavonoids.

Test for terpenoids (Salkowski test)

5 ml of each plant part extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. Formation of reddish-brown coloration at the interface shows the positive results for presence of terpenoids.

Quantitative Phytochemical Analysis of Extracts

The preliminary phytochemical analysis of the extracts of each plant parts were carried out to determine the quantity of tannis, flavonoids, saponins, alkaloids, phenol and steroid using standard procedures (Bohm and Kocipai-Abyazan, 1994; Obadoni and Ochuko, 2002; Harborne, 1998).

Determination of Saponin

Saponins were determined using the method of Obadoni and Ochuko (2002). 20g of each milled sample (leaf and fruit) was put into different conical flasks and 100 ml of 20% aqueous ethanol added. The sample was heated over a hot water bath for 4hours with continuous stirring at about 55°C. The mixture was then filtered and the residue again extracted with another 200ml of 20% ethanol. The combined extract was reduced to 40ml over a water bath at about 90°C. The concentrate was transferred to a 250ml separatory funnel and 20ml diethyl ether added with vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n-butanol was added. The combined n-butanol extract was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven (Gallenkamp, England) to a constant weight, and saponin content calculated as a percentage. The absorbance was read against blank at 380nm.

Determination of Tannin

Fine powdered ground plant sample (0.2g) was weighed into a 500ml sample bottle. Then, 100ml of 70% (v/v) aqueous acetone was added and properly covered with a stopper cork. The bottles were shaken for 2 hours at 30ºC. Each solution was then centrifuged at 1600rpm for 5 minutes and the sediment was stored on ice. Each solution 0.2ml was pipetted into test tube and 0.8ml of distilled water was added. Standard tannic acid solution was prepared from 0.5mg/ml of the stock and the solution was made up to 1ml with distilled water. Folin-ciocateau reagent (0.5ml) was added to both the sample and the standard tannic acid solution which were followed by the addition of 2.5ml of 20% (v/v) Na_2CO_3. The solution was then shaken vigorously and incubated for 40minutes at room temperature $28^{\pm}2\ ^0$C. The absorbance was read at 725nm against a standard tannic acid curve.

Determination of Alkaloid

Alkaloid determination using Harborne (1998) method. The sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added. Beaker was covered and allowed to stand for 4h. Then it was filtered and the extract was

concentrated on a water bath to one quarter of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to stand till its settlement. The precipitate was easily collected from the solution and was washed with dilute ammonium hydroxide and filtered. The residue was the alkaloid which was weighed after complete dryness and the percentage was calculated.

Determination of Flavonoid

Flavonoid determination was carried out by the method of Bohm and Kocipai-Abyazan (1994). 10 g of each plant sample was extracted with 100 ml of 80% aqueous methanol repeatedly at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath, the weight of the material and percentage quantity was calculated.

Determination of Terpenoid

2.0g of samples were soaked in 50ml of 95% ethanol for 24hours. The extracts were then filtered and the filtrates were extracted with petroleum ether (60 to 80^0C) and concentrated to dryness. The dry ether extracts were treated as total terpenoids.

Determination of Steroids

10ml of the solution water extract were pipetted into a 250ml conical flask. Chloroform was added and shaken in vortex mixer for 1 hour. The mixture was filtered into 100ml conical flask.

10ml of pyridine and 2ml of 29% sodium nitroprusside was added shaken thoroughly for 10 minutes. 3ml of 20% NaOH was then added to develop a brownish colour. Steroid standard (Digitoxin), concentrations which ranged from 0-50mg/ ml was prepared from stock solution and the absorbance was read at 510nm.

Preparation of McFarland Standard

0.5 McFarland equivalent turbidity standard was prepared by adding 0.5mls of 1% barium chloride ($BaCl_2$. $2H_2O$) to 99.5mls of 1% sulphuric acid solution (H_2SO_4) and mixed thoroughly. A small volume of turbid solution was transferred to a capped tube of the same type that was used to prepare the test and control inoculants. This was then stored in the refrigerator. 0.5 McFarland gave an equivalent approximate density of bacteria $1x10^8$cfu (Enos, 2016).

Inoculum Preparation by Colony Suspension Method

About 10ml of normal saline was poured into four different capped bijou bottle, to which colonies of each test organism was emulsified separately. The suspension was adjusted to match the 0.5 McFarland standards which had a similar appearance of an overnight broth culture.

Antimicrobial Test

1ml suspension of each test organism (*S. typhi*, *S. pneumonia*, *T. corporis*) was flooded into different already prepared media (NA and SDA respctively). The excess were decanted and it was allowed to dry for about 30 minutes. Cup of 7mm in diameter were made in the agar using sterile cork borer.1g of the extracts was dissolved in 10ml of dimethyl sulphur oxide to attain the required stock concentration. Four graded dilution of the plant extracts (100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) were prepared with the original concentration of the extract of the first, all in bijou bottles by pipetting 1ml from the stock and transferring to the second bijou bottle till the last bijou bottle serially. 0.01ml of Gentamycin and Fluconazole were prepared alongside to act as the control for bacteria and fungi respectively. Each concentration was introduced into each hole of the medium and allowed to stand on the bench for about one hour for diffusion. It was incubated at 37^0C for 24 hours for bacteria and at 25^OC for 3-5days for fungi. This was repeated for the entire prepared medium in duplicated. The diameter of zones of inhibition of different concentration of extracts of the organism was measured with a calibrated meter rule (Atlas 1995).

Determination of Minimum Inhibitory Concentration (MIC) of the Extracts

The minimum inhibitory concentration (MIC) was determined as the least concentration that showed an inhibitory effect on the test organisms using tube method as described by Atlas (1995). Seven-fold serial dilutions were made using nutrient broth for bacteria and Saboraud dextrose broth for fungi. 1ml of the solution of the test compounds (plants extract) of 1g/10ml was added to 10ml of double strength medium aseptically and was mixed thoroughly by shaking in a sterile test tube. The second test tube which contain 10ml single strength medium, 1ml of the double strength was transferred to it using Pasteur pipette and was mixed by shaking. This procedure was repeated up to 5 test tubes where 1ml was discarded from it after shaking. The 6^{th} test tube with no test compound served as control. To each test tube a drop of inoculum was pipetted and added aseptically. The tubes were covered with aluminum foil paper immediately and incubated at 37^0C for 24hours for bacteria and 25^0C for 3-5 days for fungi and observed for turbidity. The lowest concentration that inhibited the growth of the test organisms was recorded as the MIC (Atlas, 1995).

Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

Tubes that showed no visible growth from the MIC test tubes were sub-cultured onto nutrient agar for MBC and onto Saboraud Dextrose Agar for MFC and were incubated at 37^0C for 24-48hours and 25^0C for 3-5days respectively. The lowest concentration of the extract yielding no growth was recorded as the MBC and MFC respectively (Atlas, 1995).

RESULTS

The leaf extract revealed the presence of saponins, alkaloids, cardiac glycosides, steroids, carotenoids, terpenoids, cardiac glycosides and flavonoid while the fruit extract revealed the presence of tannins, flavonoids, carotenoids steroids, terpenoids, and cardiac glycosides. The concentrations of the various classes of secondary metabolite varied amongst the extracts evaluated. The concentration of the constituents is in the order of water >ethanol (Table 1).

The quantitative phytochemical constituents of the extracts revealed that the leaves had higher quantities of the bioactive constituents than the fruits extract (Table 2).

Table 3 shows the antimicrobial effect of the leaf aqueous extracts against *Streptococcus pneumoniae, Salmonella typhi, and Tinea corporis*. It shows the range of zones of inhibition for the leaf aqueous extract of *C. procera* against *S. pneumoniae* (17mm to30mm), *S. typhi* (14mm to 28mm), and *T. corporis* (12mm to 24mm).

Table 4 reveals the effects of fruits aqueous against the various microorganisms ranges from 13mm to 29mm for *S. pneumoniae*, 14mm to 26mm for *S. typhi*, and 15mm to 29mm *T. corporis.*

Table 5 shows the leaves ethanol extract of *C. procera* against *S. pneumonia* (17mm to 24mm), *S. typhi* (10mm to 20mm) and *T. corporis* (12mm to 21mm).

Table 6 shows the various zones of inhibition of each fruit's ethanol extracts against *S. pneumonia* (12mm to 27mm), *S. typhi* (16mm to 17mm) and *T. corporis* (11mm to 17mm).

Table 7 shows the result for the MIC, MBC and MFC for leaf aqueous extracts and fruit aqueous extracts against the microbial isolates. The MIC for *S. pneumoniae* and *S. typhi* were 12.5% and 50% respectively and *T. corporis* (100%). The MIC for the fruit aqueous extract against *S. pneumoniae*, and *S. typhi* were 25% and 50% respectively while *T. corporis* (50%).

Table 8 reveals the MIC for leaf ethanol extract against *S. pneumoniae* (25%), *S. typhi* (50%), and *T. corporis* (50%). The MIC for fruit ethanol extract was 50% against both *S. pneumonia* and *S. typhi* respectively while *T. corporis* (12.5%).

The MBC of the leaf aqueous extract against *S. pneumoniae* was 50mg/ml and *S. typhi* (100mg/ml) while MBC of fruit aqueous extract was *S. pneumoniae* (50mg/ml) and *S. typhi* (100mg/ml). However, the MFC of the leaf and fruit aqueous extracts against *T. corporis* was 100mg/ml for both extracts.

The MBC of the leaf ethanol extract against *S. pneumonia* was 12.5mg/ml, *S. typhi* (50mg/ml), and the MFC for *T. corporis* (50mg/ml). The MBC of the fruit ethanol extract against *S. pneumonia* was 25mg/ml, *S. typhi* (25mg/ml), while the MFC for *T. corporis* (50mg/ml). The study revealed that antimicrobial activity increased with increase in extract concentration.

Table 1: Qualitative Phytochemical Constituents of Leaf and Fruit Extracts of *C. procera*

T1-Qualitatative Phytochemicals	**Aqueous**		**Ethanol**	
	Leaf	**Fruits**	**Leaf**	**Fruits**
Saponin	+	-	+	-
Tannins	+	+	+	+
Phenolics	+	-	+	-
Flavonoid	+	+	+	+
Carotenoid	+	+	+	+
Steroid	+	+	-	+
Terpenoid	+	-	+	+
Cardiac Glycoside	+	+	+	+
Alkaloid	+	-	+	-

Table 2: Quantitative Phytochemical Constituents of Leaf and Fruit Extracts of *C. procera*.

T2- Quantitative Phytochemical(mg/g)	Leaf	Fruits
Saponin	23.52±0.0	-
Tannins	35.5±0.0	0.65±0.0
Terpenoid	6.97±0.0	2.73±0.0
Flavonoid	28±0.0	3.34±0.0
Steroid	13.43±0.0	9.26±0.0
Alkaloid	44.03±0.0	-
Cardiac glycoside	28±0.0	12.37±0.0

Table 3: Mean zones of inhibition (mm) and concentrations of leaf aqueous extracts (LAE) of *Calotropis procera* against the microbial isolates

ap	Concentration of FAE (%)				
Test Organisms	100	50	25	12.5	Control
Streptococcus pneumoniae	29±5.4	22±4.7	17±4.1	13±3.6	41±6.4
Salmonella typhi	26±5.1	19±4.4	15±3.9	14±3.7	44±6.6

Key: Control- Gentamycin 100mg/ml for bacteria, Fluconazole 150mg/ml for fungi
Value expressed as Mean ± SEM of Three replicates

Table 4: Mean zones of inhibition (mm) and concentrations of fruit aqueous extracts (FAE)of *Calotropis procera* against the microbial isolates.

T3	Concentration of (LAE) (mg/ml)				
Test Organisms	100	50	25	12.5	Control
Streptococcus pneumoniae	30±5.5	25±5.0	19±4.4	17±4.1	41±6.4
Salmonella typhi	28±5.3	24±4.9	17±4.1	14±3.7	42±6.5
Tinea corporis	24±4.9	20±4.5	17±4.1	12±3.5	30±5.5

Tinea corporis	29±5.4	23±4.8	19±4.4	15±3.8	31±5.6

Key:Control- Gentamycin 100mg/ml for bacteria, Fluconazole 150mg/ml for fungi
Value expressed as Mean ± SEM of Three replicates

Table 5: Mean zones of inhibition (mm) and concentrations of leaf ethanol extracts (LEE) of *Calotropis procera* against the microbial isolates.

T5	Concentration of LEE (%)				
Test Organisms	100	50	25	12.5	Control
Streptococcus pneumoniae	21±5.1	19±3.6	19±7.8	12±4.3	41±6.4
Salmonella typhi	27±9.6	19±6.7	17±4.4	16±5.1	44±6.6
Tinea corporis	17±2.5	15±8.8	14±3.0	11±2.9	10±5.6

T6	Concentration of FEE (%)				
Test Organisms	100	50	25	12.5	Control
Streptococcus pneumoniae	24±5.4	20±0.0	18±6.0	17±2.1	41±6.4
Salmonella typhi	20±7.6	19±3.3	16±7.5	10±2.3	42±6.5
Tinea corporis	21±4.9	20±4.5	17±4.1	12±3.5	30±5.5

Value expressed as Mean ± SEM of Three replicates

Table 6: Mean zones of inhibition (mm) and concentrations of fruit ethanol extracts (FEE) of *Calotropis procera* against the microbial isolates.

Table 7: MIC, MBC and MFC of leaf and fruit aqueous extracts of *Calotropis procera* against the microbial isolates.

T7-Test organisms	MIC(%)	MBC/MFC(%)
Leaf Aqueous Extract		
Streptococcus pneumonia	12.5	50
Salmonella typhi	50	50
Tinea corporis	100	>100
Fruit Aqueous Extract		
Streptococcus pneumoniae	25	50
Salmonella typhi	50	100
Tinea corporis	50	100

Table 8: MIC, MBC and MFC of leaf and fruit ethanol extracts of *Calotropis procera* against the microbial isolates

T8-Test organisms	MIC (%)	MBC/MFC (%)
Leaf Ethanol Extract		
Streptococcus pneumonia	25	12.5
Salmonella typhi	50	50
Tinea corporis	50	50
Fruit Ethanol Extract		
Streptococcus pneumoniae	50	25
Salmonella typhi	50	25
Tinea corporis	12. 5	50

DISCUSSION

The organic solvent used for the extraction of bioactive molecules from *C. procera* were able to extract valuable chemical substances sufficient enough for wide inhibition of the selected microorganisms in the study. The increasing interest in natural products present in medicinal plants used in traditional medicine have placed medicinal plants on front line as one of the dependable sources of potential antimicrobial agents and possibly for discovery of novel drugs. The results of this study highlight the fact that the organic solvent extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and were extracted through the organic solvent medium as stated by Britto (2007). The present observation also suggests that the organic solvent extraction methods used are suitable to verify the antimicrobial properties of *C. procera,* which is also supported by many other investigators (Natarajan *et al*., 2005). However, the bioactive molecules extracted depend upon the type of solvent used in the extraction procedure (Shoba *et al*., 2014)

In this study, the phytochemical screening of the leaf and fruit extracts of *Calotropis procera* revealed the presence of saponins, alkaloids, cardiac glycosides, tannin, flavonoids and steroids. Kawo *et al*. (2009) also reported similar bioactive compounds in leaves and latex extract of *Calotropis procera.* This also correlate with the work of Asoso *et al.* (2018). where the fruits extracts show the presence of alkaloids, cardiac glycosides, steroids. This is in agreement with Mainasara *et al*. (2011), except saponin which didn't show its presence in fruits.

The presence of these components in the plant extracts is an indication of some medicinal potential. Plants rich in saponins have been reported to have immune boosting and anti-inflammatory properties (Kenner and Requena, 1996). Similarly, tannins have been reported to have antibacterial potential due to basic character that allows them to react with proteins to form stable water-soluble compounds thereby killing bacteria by directly damaging its cell membrane (Elmarie and Johan, 2001). The antibacterial activities of alkaloids and flavonoids have been reported by a number of authors (Aliero *et al*., 2008; Yesmin *et al*.,2008; Hassan *et al*., 2005).

The determination of the efficacy of leaf and fruit extracts of *C. procera* against *S. pneumoniae, S. typhi,* and *T. corporis* showed inhibition activity against all the microbial isolates. It was observed that susceptibility increased with an increase in the concentration of all the extracts. Leaf aqueous extract slightly showed more effect on all the test organisms compared to the fruits aqueous extract except for *T. corporis* which was more susceptible to the fruits aqueous extract compared to the leaf aqueous extract at all

concentrations. This may suggest that the plant latex is more concentrated in the fruits as stated by Kuta (2008).

It was also observed that *Salmonella typhi* was slightly more susceptible to fruit ethanol extract compared to leaf ethanol extract and fruit aqueous extract at lowest concentration of 12.5%. *Streptococcus pneumonia* revealed moderate susceptibility to leaf aqueous extract with *Tinea corporis* showing least susceptibility to same extract while *S. pneumonia* was however least susceptible to leaf ethanol extract and fruit aqueous extract. The ethanol and aqueous extracts of *C. procera* showed significant antibacterial activity against both the Gram positive and Gram-negative bacterial strain. Generally, this result is similar to that of Yesmin *et al.* (2008) who observed *Salmonella typhi* and *Streptococcus pyrogenes* to be the most susceptible organisms to *C. procera.* Both ethanol and aqueous extracts of *C. procera* revealed high antifungal effect against *Tinea corporis.*

The result of minimum inhibitory concentration (MIC) revealed that the leaf aqueous extract of *C. procera* at low concentration (12mg/ml) is capable of inhibiting the growth of *Streptococcus pneumonia*,and capable of inhibiting the growth of *Salmonella typhi* at low concentration (50mg/ml) unlike the fruits aqueous extract that inhibits at high concentration, except for *T. corporis* in which the fruits extract is capable of inhibiting its growth at a lower concentration (50mg/ml) compare to the leaf extract which inhibits at high concentration. This result is in contrast with the research of Asoso *et al.,* 2018 that worked on bacteria and fungi isolates and found MIC at 50-400mg/ml. However, the result agrees with the research of Nanaah (2013) who also worked on bacteria and fungi and found MIC at low concentration. But the fruits aqueous extract inhibited the growth of *T. corporis* at low concentration (50mg/ml) compared to the leaf aqueous extract that inhibit at high concentration (100mg/ml).

The result of minimum bactericidal concentration shows that the leaf and fruits aqueous extract are capable of inhibiting *S. pneumoniae* at a low concentration (50mg/ml), and also capable of inhibiting *S. typhi* at a low concentration (100mg/ml). This result shows that the extracts are bactericidal which agrees with the work of Nazma *et al.* (2008), Komathi *et al*. (2012), Asoso *et al*. (2018), and Mainasara *et al*.,2011) that reported on effect of the extracts as bactericidal.

However, the result of minimum fungicidal concentration (MFC) revealed that the leaf extract of *C. procera* is capable of inhibiting at a low concentration (100mg/ml) compared to the fruits extracts that inhibit at high concentration (>100mg/ml). This result agrees with Asoso *et al.,* (2018). The fruits aqueous extract inhibits *T. corporis* at a low concentration (100mg/ml) unlike the leaf extract that inhibit at high concentration (>100mg/ml).

The bioactive compounds showed higher quantitative values in the leaf extract of *C. procera* than in the fruits while steroid was of moderate quantity.

The *in-vitro* sensitivity of bacteria and fungi that are causative agents of cutaneous diseases, diarrhea and respiratory tract infection to the plant extracts suggests *C. procera* as a promising antimicrobial agent.

REFERENCES

Yesmin, M.N., Uddin, N.S., Sanzida, M., and Muhammad, A.A. (2008). Antioxidant and Antibacterial Activities of *Calotropis procera. American-Eurasian Journal of Agric and Environmental Science.* **4** (5): 550-553

Abbas, A.E., Tayeb, El, Suleiman, Y.R. (2012). *Calotropis procera*: Feed potential for arid zones. Veterinary Record. 6:131-132.

Abubakar S., Ahmed Q. U., Othman A.S., & Omar M. N., (2011). Bacteriostatic and Bactericidal Activity of the Polar and Non-Polar" extracts of *Andrographis paniculata* against skin disease causing pathogenic bacteria. *J. Med. Plant Res.***5**, 7-14.

Aliero B.L, Aliero A.A, & Yakubu M. (2008). Phytochemical and Antibacterial properties of *Caloptropisprocera* fruit and bark extracts. *International Journal of drug Discovery* and *Herbal Research.* **1.3**:138-143

Aliyu, B. S. (2006). Common Ethnomedicinal plants of the semi-arid regions of West Africa their description and phytochemicals, Triumph Publishing Company Limited Kano, Nigeria. **Pp** 193-196.

Asoso O. S., Oladunmoye M. K, & Ogundare A. O. (2018). Phytochemical analysis and antimicrobial activities of *Calotropis procera* extracts on selected pathogenic microorganisms. *Chemistry research journal.* **3**(1):14-22.

Atlas, R.M. (1995). Microbiology in our world. Mosby Publishers Inc Baltimore. **Pp** 785.

Barnet HL. Illustrated genera of imperfect fungi. 4th Edition; 1960.

Bohm BA, Kocipai- Abyazan R (1994). Flavonoid and condensed tannins from the leaves of *Vaccinum raticulation* and *Vaccinum calcyimium*. Pacific Sci., 48: 458-463.

Britto J.S. (2007). Comparative antibacterial activity study of *Calotropis procera*. *JournalSwamy Botany* Club. **18**:81-82

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries (2nd Edition). London English Language Book Society. pp. 100-194.

Elmarie S.K, & Johan P.K. (2001). Pharmacological characterization of different fraction of *Calotropis procera* (*Asclepiadaceae*). *Streptozotocini induced experimental model of diabetic neuropathy.* **152**(2): 349.

Enos K. (2016). Antibacterial activity of *Zingiber officinale* against three common upper respiratory tract pathogens. *Mekehere Pharmaceutical Journal.* **12**.

Fatope, M.O, Ibrahim H. and Takeda, Y. (1993): Screening of higher plants reputed as pesticides using the brine shrimp lethality assay. *International Journa lof pharmacognosy*. **31**:250- 254.

Harborne J.B. (1998) Phytochemical methods: A guide to modern techniques of plant analysis. (3rd edition). Chapman and Hall Company, New York.;1-302.

Hassan, S.W., Srivastava, A. & Kumar, V.L. (2005). *Calotropis procera* latex afford protection against carbon tetrachloride-induced hepatotoxicity in rats. *Journal of Ethnopharmacology.***113**(3): 498-502.

Jain A, Katewa S. S., Galav P. K., & Nag A. (2007) Unrecorded Ethnomedicinal Uses of Biodiversity from Tadgarh Raoli Wildlife Sanctuary, Rajsthan, India. *Acta Botanica Yunnanica*: **29**:337-344.

Kawo, A.H., Mustapha, A., Abdullahi, B.A., Rogo, L.D., Gaiya, Z.A. and Kumurya, A.S. (2009). Phytochemical Properties and Antibacterial Activities of The Leaf and Latex Extracts of *Calotropis procera*. *Bayero Journal of Pure and Applied Science*s, 2(1):34-40.

Kayode J., (2006). Conservation of Indigenous Medicinal Botanicals in Ekiti State, Nigeria. *J Zhejiang Univ Sci B.***7**:713-718.

Kenner, D & Requena, Y. (1996). Botanical medicine. A European professional perfective. Paradign publication Brookline, Massachusetts. **Pp** 7-12.

Khan, F.M., (2009). Ethno veterinary medicinal usage of flora of greater Cholistan Desert Pakistan, *Pakistan Vetinary Journal.* **29**:75-80.

Komathi, J. V and Rana, A. C. (2012). Preliminary study on antifertility activity of *Calotropis procera* roots in female rats. *Fitoterapia*. **73**(2): 111-115.

Kuta F. A., (2008). Antifungal effect of *Calotropis procera* Stem Bark on *Epidermorphyton flocosum* and *Trichonphyton gypseum. African journal of Biotechnology*. **7**(13):2116-2118.

Mainasara, M. M, Aliero, B. L, Aliero, A. A, & Yakubu, M. (2011). Phytochemical and Antibacterial properties of *Caloptropisprocera* fruit and bark extracts. *International Journal of drug Discovery* and *Herbal Research.* **1.3**:138-143

Mandal S, DebMandal M, Pal NK and Saha K (2010) Antibacterial Activity of Honey against Clinical Isolates of *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi. Asian. Pac. J. Trop. Med*. (**12**), 961-969

Mehta, B., Pramila K, Prerana A. (2013). Antimicrobial activity and phytochemical analysis of *Calotropis gigantea* root, latex extracts. *Journal of Pharmacy*. **4**:07-11.

Nanaah, G. (2013). Antimicrobial activity of *Calotropis procera* Ait. (*Asclepiadaceae*) and isolation of Saponin as the active constituents. *World Journal of Microbiology and Biotechnology*. **30**(8):1321-1356.

Natarjan, D. , Britto, J.S., Srinivasan, K.&Perumal, G. (2005). Antibacterial activity of *Euphorbia fusiformis*- a rare medicinal plant. *Journal of Ethno Pharmacology*. **102**:123-126.

Nazma Yesmin, Sarder Nasir Uddin, Sanzida Mubassara & Muhammad Ali Akond (2008). Antioxidant and Antibacterial Activities of *Calotropis procera* Linn. American - *Eurasian J. Agr. & Environ. Sci*. **4**(5), 550-553.

Noatay, K.L. (2005): Aak, wild plant that has medicinal value. The Tribune, Chandigarh, India. *http//www.tribuneindia.com/2003/2003:1215/agro.h*

Obadoni B.O and Ochuko P.O (2000). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigerian. *Global Journal of Pure and Applied Science*. **86**: 2003-2008.

Odugbemi T. (2006). Outlines and pictures of medicinal plants from Nigeria. University of Lagos Press. Pp 13-71.

Ojo O.O., Tella I.O & Ademola A.O.O., (2005). Effect of *Azaditactha indica, indoca* and *Eukalyptus camaldulensis* on paracetamol induced lipid perioxidation in rats. *Journal of sustainable development agricultural environment.***1**:755-760.

Satheesh H.A, Sanni S, Sandabe U.K. (2013). Phytochemical and elemental analysis of *Acalyphawilkesiana* leaf. *Journal of American Science.***6**(11):510-514.

Sen S, Niranjan P. S., & Mahato S.B., (2007). Flavonol glycosides from *Calotropis gigantea*. *Phytochemistry*, **31**:2919-2921.

Shoba F.G, Babu V.A, Parimua M, Sathya J. (2014). In vitro evaluation of antibacterial activity of *Moringa oleifera* and *Momordica charantia* seeds. *International Journal of Pharmaceutical sciences and Research*. **5**(5):988-1993

Showkat, R.M. (2007). Different system of classification of crude drugs, Alternate system of medicine. *Pharmacognosy*. **24**.

Sofowora, E.A., (2003). Medicinal and traditional medicine in Africa. Spectrum Book Ltd London. **Pp** 25-28.

Veeramuthu, D., Muniappan, A. and Savarimuthu, I., (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complement Altern. Med*. **6**, **pp**: 35.

Vohra R. (2004). *Calotropis* the medicinal weed. India: Online medicinal book store;

C.procera Leaf and Fruit and its Extract

Various Zones of Inhibition