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ACUTE TOXICITY, PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL INVESTIGATION OF AQUEOUS EXTRACT OF SODOM APPLE TREE (*Calotropis procera Ait.F*) LEAF OBTAINED WITHIN ANCHOR UNIVERSITY, LAGOS

Olatunde A. Oseni[1,3], Hilda. A. Emmanuel-Akerele[2]*, Wuraola A. Adebayo[3]
[1]Department of Medical-Biochemistry, Faculty of Basic Medical Sciences, Ekiti State University, Ado-Ekiti, Nigeria.
[2]Department of Biological Sciences, Faculty of Science and Science Education, Anchor University, Lagos, Nigeria.
[3]Department of Chemical Sciences, Faculty of Science and Science Education, Anchor University, Lagos, Nigeria.
*Email: akatahhilda@gmail.com

ABSTRACT

Calotropis procera is a species of flowering plant, in the family Apocynaceae, the investigation of *Calotropis procera* for its medicinal usefulness has not been fully documented in the literatures. This study was therefore aimed at investigating the antimicrobial activity, phytochemical screening and acute toxicity of *Calotropis procera*. The antimicrobial activity, phytochemical screening and acute toxicity of the leaf was carried out using standard methods for the analyses. The antimicrobial activity of *Calotropis procera* leaf extract for bacterial showed that *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus candideus* were resistant to the plant extract, while *Penicillum corylophilum* showed susceptibility to the plant extract. The phytochemical screening showed the presence of flavonoids, saponins and reducing sugar in the aqueous leaf extract of the leaf, while alkaloids and phlobatannins were absent in the extract of *Calotropis procera*. The acute toxicity investigation showed that *Calotropis procera* concentration at 1424 mg/kg body weight resulted in scratching and shivering but was not toxic to cause death of the mice, while lower concentrations of 142, 352, and 712 mg/kg body weight did not cause any observable negative reactions. The plant of *Calotropis procera* therefore showed that the aqueous extract of the plant contained viable phytochemicals compounds, potent antimicrobial activity to some organisms while the studied concentrations for acute toxicity were observed to be safe.

Keywords: acute toxicity, antimicrobial activity, *Calotropis procera*, phytochemical screening

INTRODUCTION

Calotropis procera is a species of flowering plant, in the family *Apocynaceae* that is native to North Africa, tropical Africa, Western Asia, South Asia, and Indochina (Khairnar *et al.*, 2012). It is a spreading shrub or medium-sized tree reaching 2.5 to 6 m in height. It has a profound taproot, 3 - 4 m deep, and a secondary root structure with woody sidelong roots that may quickly restore unexpected shoots when the plant is harmed (Khairnar *et al.*, 2012). The Stems are crooked and covered with a fissured corky bark, the grey-green leaves are 15 - 30 cm long and 2.5 - 10 cm broad they are decussate, obviate, and acuminate (Khairnar *et al.*, 2012). The flowers are pentamerous, little, cream or greenish white at the base and purple violet at the edges of the lobes. The fruit is a fleshy and inflated, sub-globose, ellipsoid or ovoid, recurved follicle, 7.5 - 10 cm or more in diameter (Neto *et al.*, 2013). The Seed is light-brown, broadly ovate, flattened, 3.2 cm with silky hairs. A white milky juice is released from any cut on the plant.

Calotropis procera is a multipurpose tree, the stems yield a fiber valuable for making ropes, sacks, nets and paper (Neto et al., 2013). The silky sap (latex) is eminent for its ethno-restorative properties and as a food, especially as a coagulation factor for cheese making in West Africa. *Calotropis* yields 90 t of biomass two times per year and is a potential source of renewable energy. *Calotropis is also used as fodder*. Immature pericarp, old leaves and flowers can be fed to goats, camels, and

sheep in the midst of scarcity. The latex contains toxic components that may be harmful to livestock (Mainasara *et al*., 2011; Goyal *et al.*, 2013).

It has been observed that different degenerative diseases are rampant in the world these days, and many of the synthetic drugs are too expensive and not in the reach of the average human beings as a results of poverty level. Therefore, this study seeks to investigate the phytochemical constituents of the aqueous extract of *Calotropis procera* leaf and the acute toxicity effect on mice with the aim of establishing the plant viability for drugs development and human health benefits.

MATERIALS AND METHODS

Collection of Plant Materials

Leaves of *Calotropis procera* was obtained within Anchor University environment, Ayobo-Ipaja, Lagos State in October 2020. It was authenticated and identified by the Chief Technologist in Department of Plant Science and Technology, Faculty of Science, Ekiti State University, Ado-Ekiti, Nigeria with voucher number 2020083.

Aqueous Extraction of Calotropis procera Leaves

After collection of plant, the leaves were hand plucked aseptically and was air dried in the laboratory for 3-4 days, then it was grounded using electric blender, for ratio 1 g of sample into 10 mL of distilled water, 20 g of powdered sample was weighed into 100 mL of distilled water which was mixed using mechanical shaker overnight, it was then filtered using muslin cloth and also filtered with No. 1 Whatman filter paper, then the extract was kept in a corked bottle for analysis.

Phytochemical Analyses

Test for Phlobatannins

4 drops of 1% solution of hydrochloric acid was added to 10 drops of *Calotropis procera* aqueous leaf extract, it was heated on hot plate. Formation of red colored precipitate indicates a positive result, and presence of phlobatannins (Goyal *et al*., 2013).

Test for reducing sugar

1ml of ethanol was mixed in 5ml of *Calotropis procera* leaf extract. After that, 1ml of Fehling solution A and 1ml of Fehling solution B were mixed in a test tube and boiled at 100^0C. It was then poured in the aqueous ethanol mixture. Presence of colour change shows a positive result (Adebayo *et al*., 2015).

Test for flavonoids

2.5 ml of ammonia and 1ml of concentrated sulphuric acid was added to 5ml of *Calotropis procera* leaf extract. Indication of yellow colour indicates the presence of flavonoid in the plant sample (Adebayo *et al*., 2015).

Test for alkaloids (Wagner's test)

5 drops of Wagner's reagent was added to 30 drops of *Calotropis procera* leaf extract. The formation of a reddish-brown precipitate indicates positive result (Mainasara *et al*., 2011).

Test for Saponins (Foam test)

10 drops of distilled water was added to 20 drops of *Calotropis procera* leaf extract, then it was vigorously shaken for persistent foam. The formation of foam indicates positive result (Adebayo *et al*., 2015).

Antimicrobial Activity

20 g of the leaf was weighed and blended with 100 mL of distilled water and filtered firstly with sterile muslin cloth, and also filtered with No. 1 Whatman filter paper to obtain the aqueous extract (Nenaah & Ahmed, 2011).

Microbial isolation

Standard clinical isolates of *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Aspergillus candideus, Aspergillus niger* and *Penicillum corylophilim* was obtained from the Nigerian Institute of Medical Research, Yaba, Lagos and the Department of Biological Sciences, Anchor University Lagos. Nutrient agar (NA) was used for the culture of bacteria at 37^oC and potato dextrose agar (PDA) was used to culture the fungi at 28^oC.

Introduction of the extract and antimicrobials into the isolates

Sterile discs were introduced into 2 ml of the extract, to be adsorbed/impregnated with the extract. Discrete colonies of the isolates were inoculated into 5ml of normal saline standardized with 0.5 McFarland standard suspensions. Sterile cotton wool swab was used for the inoculation of the bacterial suspension to freshly prepared Mueller-Hinton agar plates prepared according to manufacturer's instructions. The extract impregnated discs were placed on the agar plates and incubated at 37ºC and 28ºC for 18-24 hours for bacteria and fungi respectively.

The antibiotic discs used were: SXT; Septrin (30µg), R; Rocephin (25µg), AM; Amoxacillin (36µg); CN; Gentamycin (10µg), PEF; Pefloxacin (10µg), APX; Ampiclox (30µg), S; Streptomycin (30µg), E; Erythromycin (10µg) for Gram positive isolates. while SXT; Septrin (30µg), CH; Chloramphenicol (30µg), SP; Sparfloxacin (10µg), CPX; Ciprofloxacin (30µg), AM; Amoxacillin (30µg); AU; Augmentin (10µg), PEF; Pefloxacin (30µg), OFX; Tarivid (10µg) for Gram negative isolates. After incubation, the test plates were examined for confluent growth and zone of inhibition. The diameter of each zone of inhibition (clear zone) was measured in millimeter (mm). The interpretation of the measurement was recorded as sensitive (>16mm), intermediate (11- 15mm) and resistant(<11mm) (Mainasara *et al.,* 2011; Adebayo *et al*., 2015).

Acute Toxicity Investigation

Dilution was done on the extract of dried sample as described in the Table 1.

Sixteen (16) Swiss Albino mice were used in this experiment, they were housed in the Anchor University, Lagos Animal House, with average weight of 19.30 g, which were randomly assigned into four (4) groups. By oral administration, the control group received 0.2 % distilled water in the same volume, while the treated group received *Calotropis procera* leaf extract at 150, 350, 700 and 1400 mg/kg body weight using oral gavage feeding needle. The general physical observations started after the mice were given dosage, records were taken for at least 1 hour interval for 24 hours, physical observations like shivering, appetite, mortality and so on. Mortality of mice will show the toxicity of *Calotropis procera* leaf extract (Bagri *et al*., 2013).

Table 1. Sample Dilution Concentration

Concentration	Destilled H_2O	Sample Extract
1	0.90	0.10
2	0.75	0.25
3	0.50	0.50
4	0	1.00

RESULTS AND DISCUSSION

Phytochemical Activity of *Calotropis procera*

Different chemical tests were carried out on the aqueous extract of *Calotropis procera* to screen for the phytochemical constituents.

Table 2. Phytochemical screening result

Parameters	Result
Alkaloids	-
Flavonoids	+
Saponins	+
Reducing Sugar	+
Phlobatannin	-

Antimicrobial activity (Antifungal and Antibacterial activity) was carried out on the aqueous leaf extract of *Calotropis procera* to determine the level of susceptibility or resistance of the microorganisms to the leaf extract (Nenaah &Ahmed, 2011).

Table 3. Antibacterial effect on aqueous extract of *Calotropis procera* compared with standard antibiotics disc for gram positive bacterial.

No	Organism	*Calotropis procera* extract	Gram positive antibiotic disc								
			PEF	CN	APX	AM	R	CP	S	SXT	E
1	*Streptococcus pyogenes*	*R*	*R*	*S*	*R*	*R*	*R*	*R*	*R*	*R*	*R*
2	*Staphylococcus aureus*	*R*	*S*	*S*	*S*	*R*	*S*	*S*	*S*	*S*	*S*

Key: SXT; Septrin (30µg), R; Rocephin (25µg), AM; Amoxacillin (36µg); CN; Gentamycin (10µg), PEF; Pefloxacin (10µg), R; Rocephin (10µg), (APX; Ampiclox (30µg), S; Streptomycin (30µg), E; Erythromycin (10µg) S; Susceptible, R; Resistant.

Antibacterial effect on *Calotropis procera* was carried out to determine if the aqueous extract of the leaf can have effect on the bacteria and act as an antimicrobial to cure or inhibit any bacterial diseases. This result shows that according to the standard gram positive antibiotic disc, for *Streptococcus pyogenes* it is resistant to the pefloxacin, amplicox, zinnacef, amoxacillin, rocephin, ciprofloxacin, streptomycin, septrin and erythromycin, but susceptible to gentamycin. *Staphylococcus aureus* was susceptible to pefloxacin, amplicox, gentamycin, rocephin, ciprofloxacin, streptomycin, septrin and erythromycin, but resistant to amoxacillin, and zinnacef. *Staphylococcus aureus* and *Streptococcus pyrogenes* were both resistant to the aqueous leaf extract of *Calotropis procera*.

Table 4. Antibacterial effect on aqueous extract of *Calotropis procera* compared with standard antibiotics disc for gram negative bacterial.

No	Organism	*Calotropis procera* extract	Gram positive antibiotic disc									
			SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
1	*Escherichia coli*	*R*	*R*	*R*	*R*	*S*	*R*	*R*	*R*	*R*	*R*	*R*

Key: SXT; Septrin (30µg), CH; Chloramphenicol (30µg), SP; Sparfloxacin (10µg), CPX; Ciprofloxacin (30µg), AM; Amoxicillin (30µg); AU; Augmentin (10µg), PEF; Pefloxacin (30µg), OFX; Tarivid (10µg); R= Resistance, S= Susceptible

This result shows that *Escherichia coli* was resistant to septrin, chloramphenicol, sparfloxacin, amoxacillin, augmentin, gentamycin, pefloxacin, tarivid and streptomycin, but susceptible to ciprofloxacin. *Escherichia coli* was also resistant to the aqueous leaf extract of *Calotropis procera.*

Table 5. Antifungal effect on aqueous extract of *Calotropis procera*

No	Organism	*Calotropis procera* extract
1	*Aspergillus candideus*	R
2	*Penicillum corylophilum*	S
3	*Aspergillus niger*	R

R= Resistance, S= Susceptible

Antifungal effect on *Calotropis procera* was carried out to determine if the aqueous extract of the leaf can have effect on fungi and act an antifungal to cure or suppress any fungal diseases. This result shows that *Aspergillus candideus* and *Aspergillus niger* was resistant to the leaf extract of *Calotropis procera*, while *Penicillum corylophilum* was susceptible to the aqueous leaf extract of *Calotropis procera*.

Acute Toxicity Investigation of *Calotropis procera*

Acute toxicity investigation was carried out to determine the toxic level/toxicity of the aqueous extract of *Calotropis procera* leaf on Swiss albino mice. After 24 hours investigation on Swiss albino mice, for the first 3 hours of the observation they were all fine without any physical changes, with continuous observation, the mice that were given the highest dose (1400 mg/kg BW) were scratching and shivering compared to the control that was given distilled water, they were fine and no physical changes, all other mice were shivering, weak and were not eating well (loss of appetite), and the last 2 hours during the 24 hours observation, they were all fine without any physical change, alongside with the control, they were all fine without physical changes throughout the 24 hours investigation. Finally there was no death recorded.

This result shows that higher concentration of the dose will yield change in reaction physically, which can lead to cold or scratching of the body, and also shows that the leaf extract is not toxic because there was no

death recorded throughout the 24 hours investigation.

Phytochemical properties of *Calotropis procera*

Secondary metabolites are responsible for medicinal activity of plants. *Calotropis procera* is a potential medicinal plant which can be explored further for its various uses in pharmaceuticals, nutraceuticals, fiber, health, environment and many other areas. The results suggest that *Calotropis procera* possess phytochemical properties for curing various ailments and may lead to the isolation of novel compounds. Flavonoids are a diverse group of phytonutrients found in almost all fruits and vegetables. Along with carotenoids, they are responsible for the vivid colors in fruits and vegetables. Flavonoids are the largest group of phytonutrients, with more than 6,000 types. Some of the best-known flavonoids are quercetin and kaempferol. The findings in this report are in contrast with that of Shobowale *et al.,* (2013) who detected the presence of flavonoids in the leaf extract of *Calotropis procera* gotten from Khulais, Kingdom of Saudi Arabia. The report of Bruno *et al.*, (2013) agrees with the findings of this research, as flavonoids was absent in the aqueous extract of *Calotropis procera* leaf gotten from Fortaleza beaches, Ceara, Brazil.

Alkaloids are a huge group of naturally occurring organic compounds which contain nitrogen atom or atoms (amino or amido in some cases) in their structures. According to Bruno *et al.,* (2013) there was absence of alkaloids from the aqueous extract of *Calotropis procera* leaf gotten from Fortaleza beaches, Ceara, Brazil. The findings in this report disagrees with that of Shobowale *et al.*, (2013) who detected the presence of alkaloids in the leaf extract of *Calotropis procera* gotten from Agbado, Ifo Local Government Area of Ogun State, Nigeria.

Phlobaphens can be formed under action of acids or heating of condensed tannins or of the fraction of tannins called Phlobatannins. According to Shobowale *et al.,* (2013), tannins was present in the aqueous extract of *Calotropis procera* leaf, but according to the findings of this research, phlobatannins was absent in the aqueous extract of *Calotropis procera* leaf.

Saponins may help reduce cholesterol levels, strengthen the immune system, treat diabetes and inhibit tumor growth. They also improve lipid metabolism and may help prevent and treat obesity. The findings of this research agrees with the report of Morsy *et al.*, (2016) and Shobowale *et al.,* (2013) who detected saponnins was present in the aqueous extract of *Calotropis procera* leaf. *Calotropis procera* leaf helps to strengthen immune system, treat diabetes and inhibit tumor growth generally.

A reducing sugar is any sugar that is capable of acting as a reducing agent, because it has a free aldehyde group /a free ketone group. Reducing sugar was detected in the aqueous extract of *Calotropis procera* which shows there are presence of monosaccharides in the aqueous extract of the leaf.

Antimicrobial activity of *Calotropis procera*

Antimicrobial activity shows that the aqueous extract of *Calotropis procera* leaf, was not effective on *Streptococcus pyogenes*, *Staphylococcus aureus, Escherichia coli, Aspergillus candideus* and *Aspergillus niger.* Only *Penicillum corylophilim* was susceptible to the aqueous extract of *Calotropis procera* leaf.

Acute toxicity investigation of this research shows that the aqueous extract of *Calotropis procera* leaf is non-toxic to the mice, because there was no mortality recorded, only physical changes were observed to those that were given high dosage of 1400 mg/kg BW. They were shivering and scratching profusely, which shows that only high concentration of the extract given to the mice will yield physical changes.

CONCLUSION

Phytochemical screening, antimicrobial activity and acute toxicity investigation carried out on *Calotropis procera* aqueous leaf extract showed the presence of saponnins and reducing sugars and the absence of alkaloids, flavonoids, and phlobatannins in the leaf extract of *Calotropis procera*. It also showed that the aqueous leaf extract of *Calotropis procera* was not toxic because there was no mortality of mice recorded throughout the 24 h observation of the mice, only physical changes

were observed like scratching and shivering of the group that were fed with higher concentration of the dose (i.e aqueous extract of *Calotropis procera* leaf). Finally, the antimicrobial activity of the aqueous extract of *Calotropis procera* leaf showed that there was marked resistance by *E. coli, S. pyogenes, S.aureus, Aspergillus niger* and *Aspergillus candideus* to the leaf extract, while *Penicillum corylophilum* was susceptible to the leaf extract.

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