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Antibacterial Activity and Phytochemical Components of *Calotropisprocera* (Ait) Used in Management of Measles Complications

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Abstract: *The study was conducted to determine the activity of Calotropisprocera in management of measles complications and phytochemical analysis using different solvents. Samples of the leaves and latex were collected in a sterile polythene bag and test tube which were extracted for antibacterial activity. The activity was determined against Escherichia coli, Staphylococcus aureus, Psedomonasaeruginosa, Salmonella typhiusing agar well diffusion method. Leaves extract of aqueous solvent was found to be effective against S.aureus while the latex of aqueous and chloroform solvents exhibited the same diameter zone of inhibition which have highest clearance against tested organisms. The preponderance of the organisms were susceptible to Calotropisprocera leaves and latex with varying degrees for aqueous extracts against S. aureus (leaves 12mm, and latex 7.5mm) S.typhi (leaves 8mm and latex 7mm) E. coli (leaves 10mm and latex 7.5mm), P aeruginosa (latex 8mm) for ethanol extract against S. aureus (leaves 7mm, and latex 5mm) S.typhi (leaves 13mm and latex 15mm) E coli (leaves 9mm and latex 12mm), P aeruginosa (leave 7.5mm and latex 5.0mm), and for chloroform extract against S. aureus (leave negative, and latex 10mm) S.typhi (leaves 9.1mm and latex negative) E. coli (leaves 9mm and latex 9mm), P aeruginosa (leave 12.5mm and latex 10mm). The phytochemical screening revealed there is presence of alkaloids, saponins, tannins, flavonoids, terpenoids and cardiac glycosides. The presence of these chemical constituents in this plant is an indication that plants when properly screened using additional solvents could yield drugs of pharmaceutical significance. The ethanol extract of Calotropis procera is significantly greater than the other two solvents, therefore the result obtain provide a support for the use of Calotropis procera leaves and latex in traditional medicine.*

Keywords: *Antibacterial, Measles, complications, phytochemicals, Caltropisprocera, management*

1. INTRODUCTION

Measles is contagious illness which can affect all age groups. However, children below 5 years of age and adults older than 20 years of age are more likely to suffer from measles complications. Common measles complications include ear infections and diarrhea and some people may suffer from severe complications, such as pneumonia (infection of the lungs) and encephalitis (swelling of the brain). Measles may cause pregnant woman to give birth prematurely, or have a low-birth-weight baby (CDC, 2018).

Plants serve as a source of proteins, carbohydrates (sugars) mineral, vitamins, fats and oils, which are all ingredient of food and complete balance diet must contain them all (Soforawa,1982). In Nigeria traditional medicine, *Calotropisprocera* either used alone or with other herbs to treat common diseases such as measles, diarrhea, cold, indigestion. In addition preparation from latex with honey are used as antirabies and also in the treatment of cough and toothache (Kew, 1985). However application of *Calotropisprocera* in management of measles complication and cytotoxicity have not been properly documented (Shittu,*et al.,* 2004) *Calotropisprocera* is an important plant with medicinal properties, (Gupta *et al.,* 2012). It is found in most parts of world especially in warm climate usually growing in dry, sandy and alkaline soils. *Calotropisprocera* extract has been reported to have antioxidant, antimicrobial and cytostatic properties (Kumar *et al.,* 2006). The leaf, stem and root are utilized in traditional medicine for treatments of diarrhea, stomach pain, and measles. The sap

is used for treating eye infections, and the bark of the plant is traditionally used for treatment of coughs, ulcers (Chandra *et al.*, 1990). Occasionally goats and sheep eat the leaves, but cattle and sheep eat the leaves, but cattle and other livestock avoid it because of pungent smell and toxicity (Murti*et al.*, 2010). The antibiotic resistant bacteria create life threatening infections danger that do not respond to the antibiotics. But not most herbal medicine are well tolerated by the patient, with fewer unintended consequences than pharmaceutical drugs. Herbs typically have fewer side effects than traditional medicine, and may be safer for use over time.

2. MATERIALS AND METHODS

2.1. Plant Identification

The *Calotropisprocera* leaves and latex were obtained from the surrounding Sokoto State University metropolis. The plant were identified and authenticated by the taxonomist in the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto where voucher specimens were deposited at the Herbarium.

2.2. Collection and Processing of Plant Samples

Leaves and latex of *Calotropisprocera* were collected from Sokoto State University. The latex was aseptically collected and centrifuged using a bench centrifuge at 1,500 rev/min for 5 minutes. The supernatant was discarded and the pellet was evaporated to dryness using water bath at 100 ^{o}C. *Calotropisprocera* leaves were washed properly with distill water. The leaves were shaded and dried at room temperature. Dried leaves were uniformly grinded using electric blender (Moulinex). Ten grams (10g) of plant powdered was then soaked in 100ml of distilled water and 60% ethanol and chloroform in a conical flask and loaded on orbit shaker at 120rpm for 24 hours. The mixture was then filtered using Whatman No 1 filter paper. The filtrate was concentrated using rotatory evaporator and dried using lypolizer. The dried extract was collected in an air tight container and stored at 4 ^{0} C. The mixture was used to perform antibacterial assay (Rios et al., 1998)

2.3. Extraction of Plant Extracts

Extraction of leaf and latex of *Calotropisprocera* was carried out with water, ethanol 60% and chloroform. The leaf powder and the latex (10g each) were dissolved in 100 ml of each solvent. The suspended solutions were left to stand for 5 days, and labeled accordingly. The extracts were filtered and stored at 4 ^{0}C. (Trease and Evans (1996) and Parekh and Chanda (2007).

2.4. Test Organism

The microorganisms used in this study as test organisms comprising of clinical isolates of four bacteria (*Escherichia coli, Staphylococcus aureus, , Salmonella typhi ,Streptococcus pneumoniae)* were obtained from the Microbiology, Departmental laboratory of Veterinary College of Health Science Usmanu Danfodiyo University Sokoto. The typed cultures of bacteria was sub-cultured on nutrient agar respectively and stored at 4^{o}C until required for study.

2.5. Antimicrobial Test

The antimicrobial activities of aqueous, chloroform and ethanolic extracts were determined by filter paper disc and agar well diffusion methods as described by Omenka and Osuoha (2000).

2.6. Paper Disc Technique

Sterile filter paper discs (7.0 mm diameter) were soaked with the test extracts and dried at 40 ^{0}C for 30 minutes. The prepared Muller Hinton plates will be seeded with each of the test bacteria and the filter paper discs will be placed on each plate. The plates were incubated at 37^{o}C for 48 hours.

2.7. Agar Well Diffusion

The culture plates seeded with test organisms were allowed to solidify and punched with a sterile corkborer (7.0 mm diameter) to make open wells. (Kareem, Akpan and Ojo *et al.*,2008*).* Open wells were filled with 0.05 ml of the extract. The plates were incubated at 37 ^{0}C for 48 hours. The zones of inhibition were measured and recorded.

2.8. Secondary Metabolite of Plant (Bioactive Compounds)

Simple standard chemical tests were employed for detecting the presence of some phytochemicals components such as saponins, tannis, cardiac glycoside, flavonoids, phenol,terpenoids in the leaf extract of *Calotropisprocera* as described by (Treas and Evans, 2002; Sofowora, 1996.).

2.9. Test for Alkaloids

Two (2ml) of the aqueous extract of *Calotropisprocera* few drops of Wagner's reagent (a solution of potassium iodine) were added into the test tube. The formation of orange brown precipitate indicates the present of Alkaloids. (Sofowora, 1996).

2.10. Test for Saponins

Two (2mls) of the aqueous extract of *Calotropisprocera.* 3mls of distilled water were added and shaken vigorously for 5 minutes. The formation 2cm layer of foam which in turn persist for 10 minutes indicates the presence of saponins (Harborne, 1973).

2.11. Test for Tanins

Three drops of (0.1%) ferric chloride were added to two milliliters (2ml) of the extract. A brownish green indicates the presence of tanins (Treas and Evans, 2002)

2.12. Test for Flavonoids

To 3ml of the extract, 1ml of sodium hydroxide (NaOH) was added. A yellow coloration indicates the presence of flavonoids ((Treas and Evans, 2002)

2.13. Test for Phenols

To 2mls of the extract, 3 drops of ferric chloride solution were added (Harborne, 1973)

2.14. Test for Terpenoids

To 5mls of the extract, 2mls of chloroform and 3mls of concentrated H_2SO_4 were added carefully. (Harborne, 1998).

2.15. Test for Cardiac Glycosides

About 0.5g of dried extract was dissolved in 2.0ml of glacial acetic acid containing one drop of ferric chloride (Fecl3) solution. This was then under laid with 1.0ml of concentrated H_2SO_4 (Treas and Evans, 2002).

3. Result And Discussion

The results obtained showed that both the leaves and latex of *Calotropisprocera* have bactericidal effects on pathogenic microorganisms. Table I showed that ethanol was the best solvent for extracting antimicrobial substances from this plant compared to chloroform and water. However, the aqueous extract was not effective against *P.aeruginosa* and *S.typi*.). In this study ethanol was the best solvent for extracting antibacterial substance compared to aqueous and chloroform. The widest zone of inhibition (13.0mm) was demonstrated by the ethanolic extract for *C. procera* latex while the value dropped to 8.0mm and 10.0mm for chloroform and water extract respectively when tested against the same organisms. The result for antibacterial activity of plant against bacterial isolates were presented in table 1.

Table1. *Anti – bacterial screening of aqueous, chloroform, ethanol extracts of Calotropisprocera and latex.*

Diameters of zones of Inhibition(mm)								
	250mg/ml		500mg/ml		750mg/ml		1000mg/ml	
Plant extract	*S. aureus*		*S. typhi*		*E.coli*		*P.aeruginosa*	
C.procera extract	Leave	latex	Leave	latex	Leave	latex	Leave	latex
Aqueous	12.0	7.5	8	7	10	7.5	-	8.0
Ethanol 60%	7.0	5.0	13	15	9	12	7.5	5.0
Chloroform	-	10	9.1	-	9	9	12.5	10.0

Key: *Absence of bacterial growth*

The result of the minimum inhibitory concentration for aqueous, chloroform and ethanol extract are presented in table 2. Leaves extract of aqueous solvent were found to be more effective against *S. aureus* while the latex of aqueous and chloroform exhibited the same zone of inhibition. The result of aqueous and ethanolic extracts of leave and latex of *Calotropisprocera* showed antibacterial activity against the tested organisms. It has been showed that there is great activity against the tested bacterial pathogens. The similar results was reported by Kawo *et al*., (2009) which revealed ethanol as the best solvent for extracting the antimicrobial active substances compared to other solvent.

The result of minimum inhibitory concentration (MIC), the least MICs (5.0 mg/ml) was recorded for latex and while the highest MICs 913.0 mg/ml) for leaves. Agar well diffusion method allows better

diffusion of the extracts into the medium, thus, enhancing contact with the organisms. Paper discs may act as a barrier between the extract and the organisms, thus, preventing total diffusion of active compounds absorbed by the discs in the medium and may be responsible for the observed differences (Omenka and Osuaba, 20002).

Table2. *Minimum inhibitory concentration of aqueous extract, chloroform, ethanol extract of Calotropis procera and latex.*

Diameters of zones of Inhibition(mm)								
	2.5mg/ml		5.0mg/ml		7.5mg/ml		10.0mg/ml	
Plant extract	*S. aureus*		*S. typhi*		*E.coli*		*P.aeruginosa*	
C.procera extract	Leave	latex	Leave	latex	Leave	latex	Leave	latex
Aqueous	12	10	9	8	11	10	8	7
Ethanol 60%	7.0	5.0	13	15	9	12	7.5	5.0
Chloroform	-	10	9.1	-	8	8	12.5	10.0

Key: *Absence of bacterial growth*

The result of the phytochemical screening of the aqueous extract of the leaves of the plant revealed different bioactive compounds in the plants which includes saponins, alkaloids, tannis, flavonoids cardiac glycoside, phenols and terpenoids. The result for phytochemical components were presented in Table 3. Most of the compound reported were found to have bioactivity. The presence of these chemical constituents in this plant is an indication that plants when properly screened using additional solvent could yield drugs of pharmaceutical significance**.** This is probably the secondary metabolites identified in the study have one or more therapeutic application. This is in agreement with the work of Rangiaah *et al*, (2010) and Patra *et al*. (2008) which indicates the presences of plants constituents in the plant extracts that can be used in pharmaceutical industry. Previous studies report the presences of phytochemicals like flavonoids, tannin, saponnin, cardiac acid and alkaloids as the major constituents in *Calotropisprocera* which may acknowledge the medicinal properties of plant (William *et al.*, 2015). Tannins have been reported to possess antibacterial properties due to their inherent character that allows them to react with proteins to form stable water soluble compounds thereby killing the Bacteria by directly deteriorating its cell membrane (Elmarie and Johan, 2001).

Table3. *Phytochemical (Qualitative) constituents of aqueous extract of ethanolic, chloroform extract of Calotropis procera leaves*

Phyto constituents	Chloroform	Extract	Ethanolic	Extract	Aqueous	Extract
Alkaloids	+	+	++	++	+	+
Saponins	-		+	++		
Tannis	+		++	++	+	+
Flavonoids	+	+	++	++		+
Terpenoids	-		-	-		
Cardiac glycoside	-		-	-		

Key: *+++ = present in high concentration, ++ = moderately present, + = trace amount, = not detected*

Medicinal plants have been used as sources of medicine in virtually in all cultures, of late; the used of traditional medicine has increased globally and is gaining popularity. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low-cost. Therefore, investigation of traditional medicinal plants offered exciting opportunities as it offers unlimited access or resources for the discovery of new drug which could be used for the benefit of mankind. In this context, *Calotropisprocera* leaves and latex could be used for drug formulations. Gomah and Essam (2011). Also reported that the antibacterial and antifungal of *C. procera* extracts showed considerable activities against the tested microorganisms with significant differences in their activities depending on the microorganism tested and the solvent used with diameters zones of inhibition ranges between 9.0 and 26.5 mm

4. CONCLUSION

The antibacterial study of the *C. procera* extracts revealed the presence of secondary metabolites which were found to be active ingredients against the diseases causing agents. It also shows that the plant latex is effective against bacterial pathogens which are frequently found to cause diseases associated with measles. Theactivity of leaves and latex using water, ethanol and chloroform against the tested *E. coli, P. aeruginosa, S. typhi* and *S. aureus,* shows that the leaves and latex exhibit high

inhibition at varying concentrations. Hence, the plant can be good source of alternative for drug formulation. Further research is recommended to investigate the toxicity and side effect of the plant.

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