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Full Length Research Article

Antimicrobial Activities of *Calotropis procera* on Selected Pathogenic Microorganisms

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ABSTRACT

The antimicrobial effect of ethanol, aqueous and chloroform extracts of leaf and latex of *Calotropis_procera* on six bacteria namely, *Escherichia coli, Staphylococcus aureus, Staphylococcus albus, Streptococcus pyogenes, Streptococcus pneumoniae* and three fungi: *Aspergillus niger, Aspergillus flavus, Microsporium boulardii and one yeast Candida albicans* were determined using agar well diffusion and paper disk methods. The results revealed that ethanol was the best extractive solvent for antimicrobial properties of leaf and latex of *C. procera* followed in order by Chloroform and aqueous ($P<0.05$). The ethanolic extracts of *C. procera* latex gave the widest zone of inhibition (14.1mm) against E-coli using agar well diffusion while 9.0 mm was recorded for the same organism in the disc plate method.The growth of six bacterial isolates were inhibited by the three extracts except *P.aeruginosa* and *S.pyogenes* that were not inhibited by the aqueous extracts of both leaf and latex of *C.procera*. Similarly, the growth of four test fungi were inhibited by ethanol and chloroform extracts while the aqueous extract was the least effective on the test fungi. The best antifungal activity was recorded in ethanol extract of *C.procera* latex against *Candida albicans*. The minimum inhibitory concentration (MIC) for the ethanol extract was between 5.0 and 20.0 mg/ml for fungi. This study revealed that the *C.procera* latex demonstrated strong inhibitory effect on the test organisms than *C.procera* leaf. The results therefore established a good support for the use of *C.procera* in traditional medicine

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INTRODUCTION

Calotropis procera (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 (Irvine, 1961). The plant is erect, tall, large, much branched and perennial with milky latex throughout. In India, the secretions from the root bark is traditionally used for the treatment of skin diseases, enlargements of abdominal viscera and intestinal worms (Parrotta, 2001). In Senegal, the milky latex is locally applied in the treatment of cutaneous diseases such as ringworm, syphilitic sores and leprosy (Kew, 1985). 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. In addition preparations from latex with honey are used as antirabies and also in the treatment of toothache and cough (Kew, 1985). Leaf extracts, chopped leaves and latex of *C.procera* have shown great promise as a nematicide *in vitro* and *in vivo* (Khirstova and Tissot, 1995). The potentials of *C.procera* leaves in water treatment and its ability to reduce total viable count have also been reported (Shittu, *et al*., 2004).

Traditional doctors in West Africa have claimed to have successfully used the plant to cure many diseases. However, antimicrobial activities of *C.procera* have not been properly documented. In this report, we provide new information on the antimicrobial activities of *C.procera* using known microbial pathogens as test organisms.

MATERIALS AND METHODS

Collection and Processing of Plant Samples

Leaves and latex of *Calotropis procera* were collected from University of Agriculture, Abeokuta. 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. *C.procera* leaves were sundried for 4 – 6 days and blended into powder using an electric blender (Moulinex). The samples were stored in air tight containers for further analysis.

Test Organisms

Ten microorganisms used in this study as test organisms comprising of clinical isolates of six bacteria (*Escherichia coli, Staphylococcus aureus, Staphylococcus albus, Streptococcus pyogens, Streptococcus pneumoniae)* and four fungi *(Aspergillus niger, Aspergillus flavus, Candida albicans, Microsporioum boulardii*) were obtained from the Microbiology Department of Sacred Heart Hospital, Abeokuta. The typed cultures of bacteria and fungi were sub-cultured on Nutrient agar (Oxoid) and Saboraud dextrose agar (Oxoid) slants respectively and stored at 4^{o}C until required for study.

Extraction of Plant Extracts

Extraction of leaf and latex of *C.procera* was done 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^{o}C.

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}

Paper Disc Technique

Sterile filter paper discs (7.0 mm diameter) were soaked with the test extracts and dried at 40^{o}C for 30 minutes. The prepared Nutrient agar plates were seeded with each of the test bacteria and the filter paper discs were placed on each plate. The plates were incubated at 37^{o}C for 48 hours. The fungal isolates were similarly cultured on SDA plates and incubated at 30^{o}C for 72 hours.

Agar Well Diffussion

The culture plates seeded with test organisms were allowed to solidify and punched with a sterile cork borer (7.0 mm diameter) to make open wells. The

open wells were filled with 0.05 ml of the extract. The plates were incubated at 37°C for 48 hours. For the fungi, the test was carried out on SDA plates and incubated at 30°C for 72 hours. The zones of inhibition were measured and recorded.

Minimum Inhibitory Concentration

Different concentrations of the leaves and latex extract of *C.procera* were prepared to obtain 2.5 mg/ml, 5.0 mg/ml, 7.5 mg/ml. Three drops of overnight broth culture of the test organisms were inoculated into the dilutions and incubated at 37°C for 24 hours. The lowest concentration of the extracts that inhibited the growth of the test organisms was recorded as the minimum inhibitory concentration (MIC).

Kinetic Study of the Extracts

An overnight broth culture of *E.coli* (5ml) was mixed with fresh nutrient broth (45ml) followed by the addition of 2ml of the ethanol extracts of *C.procera* leaf and latex (10mg/ml). For *Candida albicans*, yeast extract dextrose broth was used. The mixture was thoroughly shaken on a mechanical shaker. The optical density (427nm) was determined at 30 minutes intervals for four hours using spectrophotometer[JENWAY UK] .

RESULTS AND DISCUSSION

The results obtained showed that both the leaves and latex of *Calotropis procera* have bacteriocidal 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. The widest zone of inhibition (9.0 mm) was demonstrated by the ethanolic extract of *C.procera* latex while the value dropped to 8.5 and 6.0mm for chloroform and water extract respectively when tested against the same organism (Table 1). However, the aqueous extract was not effective against *P.aeruginosa* and *S.pyogenes*. The result agrees with Takazawa *et al.*, (1982) that there is a need to employ broad range of extractive solvents in the extractions of possible phytochemicals from medicinal plants. The agar well diffusion methods however, gave larger zones of inhibition compared to paper disc method (Table 2). The zone of inhibition was 14.4mm for the ethanol extracts of *C.procera* latex against *E.coli* when agar diffusion method was used, as against 9.0mm for the paper disc method. According to Omenka and Osuaba (2000), agar well diffusion method allows better diffusion of the exteracts 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 components absorbed by the discs into the medium and may be responsible for the observed differences.

Table 1:
Antibacterial Properties of Calotropis procera Latex and Leaf Extracts using Paper Disc Method

Test Organisms	**Zone of inhibition (mm)**					
	Aqueous extract		Ethanol extract		Chloroform Extract	
	Leaf	Latex	Leaf	Latex	Leaf	Latex
E. coli	2.8c	4.5c	6.5c	9.0d	3.5c	6.5d
S. aureus	1.0a	3.5b	8.0d	10c	2.0a	5.0c
S. albus	1.6b	2.8a	3.5b	7.0bc	2.5b	3.3a
P. aeruginosa	I	I	2.0a	6.5b	2.0a	3.6ab
S. pyogenes	I	I	3.5b	5.5a	2.0a	3.7ab
S. pneumoniae	1.5b	3.0a	4.0bc	7.5b	2.5b	4.0b

Values followed by different letters along each vertical column are significantly different by Duncan's Multiple Range Test (P<0.005)
Key: - = No inhibition

Table 2:
Antibacterial Properties of Calotropis procera Latex and Leaf Extracts using Open Hole Diffusion

Test Organisms	Zone of inhibition(mm)					
	Aqueous Extract		Ethanol Extract		Chloroform Extract	
	Leaf	Latex	Leaf	Latex	Leaf	Latex
E. coli	2.5b	6.0c	8.5d	14.1d	5.0c	8.5d
S. aureus	3.0b	6.5c	7.0c	12.0c	4.5c	7.5c
S. albus	1.5a	4.5b	5.0b	9.0b	2.5a	5.5a
P. aeruginosa	1.0a	2.5a	3.5a	7.0a	2.0a	4.5a
S. pyogenes	I	2.0a	3.0a	7.5a	3.0b	4.3a
S. pneumoniae	_I____	4.0b	4.5b	9.0b	3.5b	5.5b

Values followed by different letters along each vertical column are significantly different by Duncan's Multiple Range Test (P<0.005); Key = No inhibition

Table 3:
Antifungal Properties of *Calotropis procera* Latex and Leaf Extracts

Test Organisms	Zone of inhibition(mm)					
	Aqueous Extract		Ethanol Extract		Chloroform Extract	
	Leaf	Latex	Leaf	Latex	Leaf	Latex
A. niger	1.5a	4.5a	3.5b	8.5c	2.5b	6.5d
A. flavus	1.0a	4.1a	3.0b	7.2b	3.0b	6.8b
C.albicans	I	I	4.6c	8.2c	4.1c	7.0b
M. boulardii	I	I	1.2a	2.5a	1.0a	2.0a

Values followed by different letters along each vertical column are significantly different by Duncan's Multiple Range Test (P<0.005); Key = No inhibition

Table 4: Minimum Inhibitory Concentration (mg/ml) of *Calotropis procera* Latex and Leaf Extracts

Test Organisms	Aqueous Extract		Ethanol Extract		Chloroform Extract	
	Leaf	Latex	Leaf	Latex	Leaf	Latex
E. coli	10.0a	7.5a	5.0a	2.5a	5.0a	2.5a
S. aureus	12.5b	7.5a	7.5b	5.0b	10.0c	5.0b
S. albus	20.0e	12.5c	12.5d	7.5c	15.0e	10.0c
P. aeruginosa	ND	20.0c	10.0c	5.0b	7.5b	5.0b
S. pyogenes	ND	20.0c	7.5b	5.0b	12.5d	10.0c
S. pneumoniae	17.5a	15.0d	10.0c	5.0b	15.0	12.5d
A. niger	15.0c	12.5c	10.0	7.5c	12.d	10.0c
A. flavus	15.0c	10.0b	7.5b	5.0b	20.0f	15.0
C.albicans	ND	ND	10.0c	5.0b	15.0e	10.0c
M. boulardii	ND	ND	17.5e	12.5d	20.0f	17.5e

Values followed by different letters along each vertical column are significantly different by Duncan's Multiple Range Test (P<0.005); **Key:** ND = Not Detectable

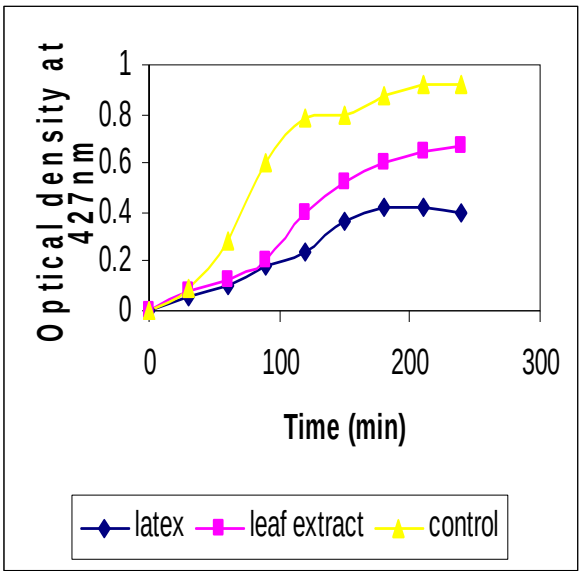

Figure 1.
Kinetics of antimicrobial activities of ethanolic extracts of *Calotropis procera* against *Escherichia coli*

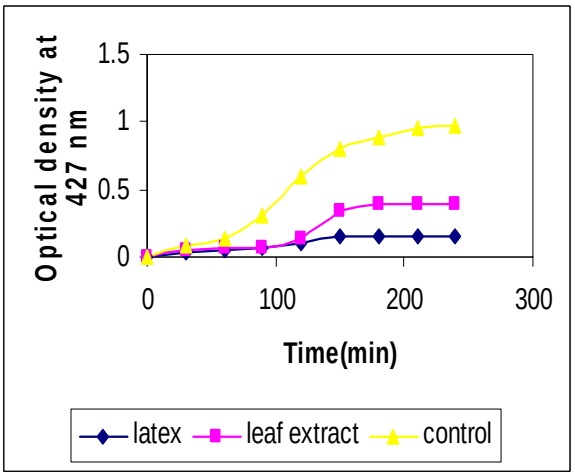

Figure 2.
Kinetics of antimicrobial activities of ethanolic extracts of *Calotropis procera* against *Candida albicans*

The results of antifungal activities (Table 3) showed that the ethanolic and chloroform extracts of both leaf and latex *C.procera* showed activities against the four test fungi with the widest zone of inhibition of 8.5mm against *A. niger* by the ethanolic extracts of the latex. However, *Candida albicans* and *Microsporum boulardii* were not inhibited by aqueous extracts of both leaf and latex of the plant. The Minimum inhibitory concentration (MIC) values of the extracts showed that the highest activity was recorded agains *E.coli* (MIC 2.5mg/ml) in ethanol extracts of *C.procera* latex and the lowest activity was observed against *Pseudomonas aeruginosa* and *Streptococcus pyogenes* (20mg/ml) in aqueous extract of the latex. However, aqueous extract of leaf of *C.procera* had no activity against two bacteria, *P.aeruginosa*, and *S.pyogenes* and two fungi *C.albicans* and *M.boulardii* (Table 4).

The study on the effect of plant extracts on the growth dynamics of *E.coli* and *Candida albicans* when compared with the normal growth curve showed that the ethanolic extracts of leaf and latex exhibited different characteristics on the two isolates (Figures 1 & 2). The inhibitory effect of *C.procera* was more pronounced in the latex than the leaf. It was observed that the leaf extract could be said to be bacteriostatic while the latex extract exhibited bacteriocidal effects. The bacteriocidal activity of *C.procera* latex could be due to the presence of calactin, mudarin and protein called calotropain which are active constituents of *C.procera* latex (Parotta, 2001); Kerharo and Adam, 1974). Moreover, the results agree with the use of latex and leaf *C.procera* in waste water treatment because of its bacteriocidal effect on *E.coli* and other pathogens. The potentials of *C.procera* in the reduction of total viable count have been recently reported (Shittu *et al*, 2004). 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 promisng antimicrobial agent.

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