Research Journal of Medical Sciences 6 (1): 13-17, 2012
ISSN: 1815-9346

An *in vitro* Antimicrobial Activity of *Calotropis procera* (Ait). R.Br. Extracts on Certain Groups of Pathogenic Microorganisms

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Abstract: Leaves, stems, fruits, roots and latex of *Calotropis procera* extracts were evaluated for their antimicrobial activity against four types of bacteria namely *Staphylococcus aureus*, *Bacillus subtilis*, *Eschreiashia coli* and *Pseudomonas aeruginosa*. Two species of fungi i.e., *Aspergillus niger* and *Candida albicans* were also bioassayed for their response when the extracts were used. All of the plant extracts irrespective of their types, inhibited the growth of all microbes to varying degrees. Aqueous extract showed strong and superior antibacterial activity against all bacterial strains especially with regard to gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) as compared to methanol or chloroform. Less or no activity was observed against *Aspergillus niger* and *Candida albicans* in some of the extracts used. The Minimum Inhibitory Concentration (MIC) value of the different extracts was 10 mg mL^{-1} in chloroform extract against *Aspergillus flavus*, *Candida albicans* and *Bacillus subtilis* and it was 25 mg mL^{-1} in methanol extract against *Candida albicans* and aqueous extract for *E. coli*. It was clearly noticed that the latex of the plant had a broad spectrum activity against all of the bacteria and fungi in all of the tested extracts. These findings support the traditional use of the plant in the treatment of different infections in the area.

Key words: *Calotropis procera*, antimicrobial activity, bacteria, fungi, strains, Sudan

INTRODUCTION

Bacteria and fungi are extremely pathogenic causing serious human infections. The discovery of antibiotics to combat these pathogens marked a resolution in the 20th century (Saadabi, 2007). Unfortunately because of the inappropriate use of antibiotics in human and veterinary medicine, certain strains of bacteria and fungi developed the ability to produce substances which block the action of antibiotics or change their target or ability to penetrate cells (Saadabi *et al*., 2006). Therefore, disease causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem. Tuberculosis, gonorrhea, malaria and childhood ear infections are just a few of the diseases that have become hard to treat with antibiotics. However, a large part of the problem is due to the increasing use and misuse of existing antibiotics in human and veterinary medicine and in agriculture (Saadabi, 2006). To substitute synthetic antibiotics, many of today's modern and effective drugs have their origin in traditional folk medicine (Aliya *et al*.,1991). Plants have been used to treat human, animals and plant diseases from time immemorial. Also herbal medicines have been known to man for centuries (Almagboul *et al*., 1985). Therapeutic efficacy of many indigenous plants for many disorders has been described by practitioners of traditional medicine (Almagboul *et al*., 1985). The milk weed *Calotropis procera* (Ait). R.Br. is one such plants known since with healing attributes and is now the subject of intense scientific study (Khanzada *et al*., 2007). The plant is belongs to the family Asclepiadaceae and in Sudan locally known as Usher (Sodom's apple). It is used globally as an antirheumatic, antidysenteric, anthelmintic as an expectorant. Also for the treatment of bronchial asthma and skin infections (Margaris and Vokou, 1985). In African countries, the latex of *C. procera* is utilized as fungicide, an anti-syphilitic, an anti-inflammatory for the treatment of dropsy and rheumatism to remove *Taenia* sp. and the treatment of toothache (Kalita and Saikina, 2001). However, the dried leaves are smoked in pipe as cure for cough (Aftab and Rizvi, 1990; Csurhes and Edwards, 1998). *C. procera* is known to contain cardio active glycoside calotropine which has shown an antitumer effect in human epidermoid carcinoma cells of the rhinopharynx (Khirstova and Tissot, 1995).

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In Sudan, *C. procera* is traditionally and widely used in folk medicine as a rich source of biologically active compounds capable of promoting diverse benefits such as control of dermal fungal infections, antimicrobial activities and pain relief among other useful properties (Almagboul *et al*., 1985; El-Badwi, 1997). *C. procera* is widespread and grown abundantly in Gezira and Rahad areas in the Nile banks as soft wooded tree-like shrub usually around 2.0 m high. The leaves of the plant are opposite, broadly ovate or obovate, acute at the apex, truncate to cordate at the base with short and glabrous or absent petioles. Stems are of soft wood with a light brown spongy bark. Inflorescences are axillary and clustered. Fruit consists of a spongy inflated glabrous pericarp, sheathing two diverged follicles. Seeds are ovate flat, glabrous, brown, crowned with silky hairs. As a medicinal plant because of its attributed antibacterial, antifungal and antihemorrhagic effects (Mitscher *et al*., 1972) it has also been used as a folk remedy against inflammatory cases of eyes. According to phytochemical analysis of *C. procera*, the plant contain enzymes and many stable cysteine prteases in the latex and many fatty acids in the whole plant (Dubey and Jagannadham, 2003; Kalita and Saikia, 2004; Khanzada *et al*., 2008). Antimicrobial properties of *C. procera* were investigated by several workers the world over (Margaris and Vokou, 1985). The paucity of pharmacological and chemical data of Sudanese usher plant prompted an investigation into its antimicrobial activity. Therefore, the present study was planned to find out the antimicrobial activities of *C. procera* and its efficacy against different fungal and bacterial strains.

MATERIALS AND METHODS

Plant material: The study was carried out at the department of Microbiology and Microbial Technology, Al-Neelain University, during March to July 2009. *Calotropis procera* (Ait). R.Br. Leaves, stems, fruits, roots and latex samples used in this study were collected from Gezira area central Sudan, near the Nile banks. It was identified and authenticated by the Department of Biology and Environmental studies, a voucher specimens were deposited at the departmental herbarium. Fresh leaves, stems, fruits and roots were dried in shade then were ground to powder. The latex was aseptically collected and centrifuged using a bench centrifuge at 1,500 rev min^{-1} for 5 min. The supernatant was discarded and the pellet was evaporated to dryness using water bath at 100°C.

Preparation of extracts: About 10 g of the coarsely powdered plant material (Leaves, stems, fruits and roots) were successively Soxhlet extracted with Chloroform ($CHCl_3$) and Methanol (MeOH) for 24 h. The extracts were evaporated under vacuum and the residues were separately dissolved or suspended in the same extracting solvent (10 mL) and kept in refrigerator till use. Water extracts were prepared by adding distilled water to 10 g of coarsely powdered plant material in a conical flask and left to soak overnight. The residue was then filtered and the final volume was adjusted to 10 mL with distilled water and the solution used immediately.

Fungal strains: Four fungal strains were obtained from departmental culture stock which were originated from clinical cases. These fungi were *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Candida albicans* (Table 1). Each organism was cultured on Sabouraud's dextrose agar medium incubated at 25°C for 7 days, to obtain inoculums for testing.

Bacterial strains: Four types of bacteria namely *Staphyllococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* were used. The bacteria was cultured on nutrient broth (Oxoid) at 37°C for 24 h.

Determination of antifungal bioassay: Sterile, filter paper discs of 6 mm diameter were impregnated with about 0.1 mL per disc of extract which have been dissolved in Dimethyl Sulphoxide (DMS) and placed in duplicates onto

Table 1: Antifungal activity of *Calotropis procera* (*In vitro* tests)

	Inhibition of fungal growth											
	Ch				Me				H_2O			
Part used	A.fl	A.fu	A.n	C.a	A.f	A.fu	A.n	C.a	A.fl	A.fu	A.n	C.a
Leaf	+++	-	-	+++	-	++	++	-	-	++	++	-
Stem	++	+	+	++	++	++	++	+++	-	++	+	++
Fruit	+	+	-	-	++	++	+++	+++	-	-	+	+
Root	+++	+	+	+++	-	+	+	-	-	+	+	-
Latex	+++	++	-	+++	-	++	-	-	-	+	+	-

*Data are presented as follows: - = No inhibition of fungal growth, + = Slight inhibition, ++ = Moderate inhibition, +++ = Strong inhibition as an average of two separate experiments with five replicates in each treatment; A.fl = *Aspergillus flavus*, A.fu = *Aspergillus fumigatus*, A.n = *Aspergillus niger*; C.a = *Candida albicans*; Ch = Chloroform; Me = Methonol; H_2O = Water

Table 2: Antibacterial activity of *Calotropis procera* (*In vitro* tests)

	Inhibition of bacterial growth											
	Ch				Me				H_2O			
Part used	B.s	S.a	E.c	P.a	B.s	S.a	E.c	P.a	B.s	S.a	E.c	P.a
Leaf	16	18	14	14	17	16	16	15	14	14	12	12
Stem	15	13	12	12	16	-	15	14	13	14	11	13
Fruit	13	14	13	12	12	16	14	16	11	13	12	14
Root	14	15	17	15	17	17	15	17	13	12	13	10
Latex	20	16	15	20	15	17	15	19	14	14	12	14

*B.s, *Bacillus subtilis*; S.a, *Staphyllococcus aureus*; E.c, *Escherichia coli*; P.a, *Pseudomonas aeruginosa*; concentration of extracts 0.1 mL/cup (100 mg mL^{-1}), inhibition zones are the mean of three replicates; - = Not tested; Ch = Chloroform; Me = Methonol; H_2O = Water

Table 3: Antibacterial effects of some standard antibiotics (Gram positive master multi disk) against different species of bacteria*

Antibiotics	Sensitive	*Staphylococcus aureus*	*Bacillus subtilis*
Ciprofloxacin	19	23	25
Clindamycin	19	21	20
Erythromycin	20	19	20
Gentamycin	13	22	19
Ofloxacin	14	22	17
Tobramycin	13	18	19

* = Mean measurement of inhibition zone (mm)

Table 4: Antibacterial effects of some standard antibiotics (Gram negative master multi dick) against *E. coli* and *Pseudomonas aeruginosa**

Antibiotics	Sensitive	*Escherichia coli*	*Pseudomonas aeruginosa*
Amikacin	15	25	22
Ciprofloxacin	19	15	17
Gentamycin	12	22	10
Lomefloxacin	20	17	22
Netilmicin	13	15	7
Ofloxacin	14	17	12

* = Mean measurement of inhibition zone (mm)

the Sabouraud's dextrose agar plates, seeded with 0.2 mL of fungal suspension. The plates were then incubated at 25°C for 10-14 days. The zone of inhibition around each disc was presented as no inhibition of fungal growth, slight inhibition, moderate inhibition and strong inhibition. Clotrimaxole and nystatin (25 μg/disc) were used as a reference standard drugs for comparison (Table 1).

Antibacterial bioassay: Leaf, stem, root, fruit extract (Water, methanol and chloroform) or latex was tested separately against the four types of bacteria using the Cup-Plate Agar Diffusion method (Groove and Randall, 1955) with same concentration as in antifungal bioassay and the inhibition zones were measured. The means of the diameters of the inhibition zones are shown in Table 2. Ciprofloxacin, Clindamycin, Erythromycin, Gentamycin, Ofloxacin, Amikacin, Lomefloxacin, Netilmicin and Tobramycin (25 μg/disc) were used as a reference standard drugs for comparison (Table 3-5).

Minimum Inhibitory Concentration (MIC): Different concentrations of the latex extract of *C. procera* were prepared to obtain 5.0, 7.5 and 10.0 mg mL^{-1}. Three drops of overnight cultures of the test organisms were

Table 5: Antifungal effects of some standard antibiotics against different species of fungi *

		Inhibition zone (mm)			
Antibiotics	Sensitive	*Aspergillus flavus*	*Aspergillus fumigatus*	*Aspergillus niger*	*Candida albicans*
Nystatin	16	22	19	24	20
Clotrimaxole	13	17	12	10	19

* = Mean measurement of inhibition zone (mm)

Table 6: Minimum Inhibitory Concentration (MIC) (mg mL^{-1}) of *Calotropis procera* Latex*

	Extract		
Test organism	Methanol	Chloroform	Aqueous
Aspergillus niger	12.5^a	7.5^a	15.0^a
Aspergillus flavus	12.5^a	10.0^b	17.0^a
Aspergillus fumigatus	17.5^b	12.5^b	22.0^b
Candida albicans	25.0^c	10.0^b	22.5^b
Bacillus subtilis	20.0^c	10.0^b	20.0^b
Pseudomonas aeruginosa	17.5^b	12.0^b	20.0^b
Staphyllococcus aureus	15.0^b	17.0^c	15.0^a
Escherichia coli	22.0^c	17.0^c	25.0^c

*Values followed by different letters in vertical columns are significantly different using Duncan's Multiple Range test ($p \leq 0.005$). Concentrations used are: 5.0, 7.5 and 10.0 mg mL^{-1}

inoculated into the dilutions and incubated at 25 and 37°C for 24 h and 4 days (Kareem *et al*., 2008). The lowest concentration of the extracts that inhibited the growth of the test organisms was recorded as the minimum inhibitory concentration (Table 6).

RESULTS AND DISCUSSION

As a general rule, plant extracts are considered active against both fungi and bacteria when the zone of inhibition is >6 mm or in the category of moderate growth inhibition or more (Groove and Randall, 1955). Extracts of different types obtained from *Calotropis procera* drastically suppressed ($p = 0.05$) the growth of the tested organisms (Table 1 and 2). The aqueous extract was superior in activity when compared to that of methanol and chloroform. The maximum inhibition zones were found in the latex and leaf extract of the plant against both fungi and bacteria (Table 1 and 2). In case of fungi, chloroform extract and methanol extract were effective against

Aspergillus flavus and *Candida albicans* in most of the different types of the extract with less activity in roots and latex. Methanol extract of the stems and fruits was found effective against all of the fungal species tested. On the other hand, latex was found very suppressive against all of the four tested bacterial species (Table 2). The maximum inhibition zone determined was 20 mm in case of *Bacillus subtilis* and *Pseudomonas aeruginosa* in chloroform extract followed by methanol and aqueous extract (Table 2). The least inhibition zones were obtained in water extract (10 mm and 11) in *Pseudomonas aeruginosa* and *Escherichia coli* in the roots and stems of the plant. For fungal species, latex was slightly active against *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans* in methanol and aqueous extract (Table 1). However, there is no detectable suppression in the growth of *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* in roots and latex.

The Minimum Inhibitory Concentration (MIC) values of the different extracts of *Calotropis procera* showed that the highest activity was recorded against *Aspergillus flavus*, *Candida albicans* and *Bacillus subtilis* (10 mg mL^{-1}) in chloroform extract of the latex (Table 6). The lowest activity was obtained against *Candida albicans* (25 mg mL^{-1}) in methanol extract and aqueous extract (25 mg mL^{-1}) against *E. coli*. It was clearly noticed that the latex of the plant had a broad spectrum activity against all of the bacteria and fungi in all of the tested extracts.

Results obtained from *in vitro* antimicrobial activity showed that the different types of *Calotropis procera* extracts have substantial inhibitory effects to varying degrees against the eight tested bacterial and fungal strains. Still the latex and leaves were superior in suppressing the bacterial and fungal growth followed by the roots and stems irrespective of the type of the extract used (Table 1 and 2).

CONCLUSION

When the obtained results were compared to antibiotics findings; it could be concluded that extracts of the different types obtained from *Calotropis procera* was less effective than the standard antibiotics used. These results are in close agreement with other findings obtained by other researchers (Adams *et al*., 1984; Aliya *et al*., 1991; Altaf, 2006; Ahmad and Beg, 2001; Campbell, 1983; Carr *et al*., 1986; Emon and Seiber, 1984; Shittu *et al*., 2004). It is not possible to make a direct correlation between the observed activity of the tested plant extracts *in vitro* and the actual effects when used *in vivo* for the diseases observed by the indigenous people and traditional healers. Therefore, it is important that the plant species which have demonstrated growth-inhibiting activity in these assays be further investigated to evaluate the significance of these extracts, clinical role and the medical system of indigenous people. Additional research is also necessary to isolate and characterize their active compounds for pharmacological testing.

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