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Antibacterial analysis of crude extracts from the leaves and latex of *Calotropis procera*

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ABSTRACT

The antibacterial effect of ethanol, acetone, aqueous and chloroform extracts of leaf and latex of *Calotropis procera* on bacteria namely, *Bacillus subtilis* (ATCC 6633) and *Pseudomonas putida* (MTCC 1194) were determined using agar well diffusion and paper disc methods. The results revealed that the acetone was the best extractive solvent for antibacterial active compounds of leaf and latex of C. procera followed by chloroform, ethanol and aqueous extracts .The acetone extracts of *C. procera* latex gave the widest zone of inhibition (25mm) against *Bacillus subtilis* (ATCC 6633) as tested using agar well diffusion while 22mm was recorded for the same organism in the paper disc method. The growth of both the bacterial isolates was inhibited by the extracts namely acetone, ethanol and chloroform extracts while the aqueous extract was the least effective. The best antibacterial activity was recorded in acetone extract of *C. procera* latex against *Bacillus subtilis*. This study revealed that the *C procera* latex demonstrated strong inhibitory effect on the test organisms than *C. procera* leaf. These results established a good support for the use of *C. procera* in traditional medicine

KEY WORDS: PREVALENCE, CHANNA STRIATUS, HELMINTH, PARASITES

INTRODUCTION

Calotropis procera is an important medicinal plant used for the cure of number of diseases among humans. It belongs to the family Asclepiadaceae. It is a wild shrub, which grows up to a height of 1 to 3 meters and it is commonly known, as 'Akra' and is also known by various names viz. swallow wort, dead sea apple, sodom apple or milk weed.

The genus *Calotropis* is distributed in tropical and subtropical regions of Asia and Africa (The wealth of India, 1959). There are two species, *C. procera* and *C. gigantean* found in India. The plant is erect, tall, large, much branched and perennial with milky latex throughout. In India, the secretions from the root bark are traditionally used for the treatment of skin diseases, enlargement of abdominal viscera and intestinal worms (Parrotta, 2001). The paste of root bark is locally applied in elephantiasis and is used in the treatment of diarrhea and dysentery. In diarrhea, it changes the faecal matter into a semisolid mass (Jain *et al*., 1985).

The milky latex of *C. procerais* is locally applied for the treatment of cutaneous diseases viz. ringworm, syphilitic sores and leprosy. It is either used alone or with other herbs to treat common diseases viz. fever, rheumatism, indigestion, cold, eczema and diarrohea. Besides, preparations from latex with honey are used as anti-rabies and also in the treatment of toothache and cough (Kew, 1985).

Kumar and Basu (1994) have also reported use of latex of *C. procerais* in leprosy, eczema, inflammation, cutaneous infections, syphilis, malarial and low hectic fevers, and as abortifacient. Latex of the plant is filled in spaces between

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nails and finger tips of patient twice daily for a few days to cure conjunctivitis and used as antiseptic (Kumar *et al.*, 2007).

Leaf extracts, chopped leaves and latex of *C. procera* have shown great promise as a nematocide *in vitro* and *in vivo* (Khirstova and Tissot, 1995). The potential of *C. procera* leaves in water treatment and its ability to reduce total viable counts have also been reported (Shittu *et al.*, 2004).

The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered to be good candidates for developing new antimicrobial drugs. Biologically active compounds present in the medicinal plants have always been of great interest to scientists working in this field. In recent years this interest to evaluate plants possessing antibacterial activity for various diseases has increased enormously.

Researchers have shown that all different parts of the plants which include stem, root, flower, bark, leaves possess antimicrobial property. Microorganisms have potential to cause human diseases. In the traditional Indian medicinal system, it has been used for pain, asthma, bronchitis, and dyspepsia, leprosy, ulcers, tumors, piles, spleen, liver and abdomen. In this report, we studied antibacterial activity of *C. procera* leaf and latex using different solvent extracts against known microbial pathogens as test organisms.

MATERIAL AND METHODS

TEST ORGANISMS

Microorganisms used in this study as test microorganisms are *Bacillus subtilis* ATCC 6633 and *Pseudomonas putida* (MTCC 1194) were procured from the School of Life Science of this University .The typed cultures of bacteria were sub-cultured on nutrient agar slants and stored at 0 to 4°C until required for study.

COLLECTION AND PROCESSING OF PLANT SAMPLES

Leaves of *Calotropis procera* were collected from the plants grown on the University campus and were sun dried for 4-6 days and blended into powder using an electric blender. The samples were stored in air tight containers for further analysis. The latex was aseptically collected from the plants and centrifuged using a bench centrifuge at 1500 x g for 5 minutes. The supernatant was discarded and the pellet was evaporated to dryness using water bath at 100°C.

EXTRACTION OF PLANT EXTRACTS

Extraction of leaf and latex of *C. procera* was done with water, 60% ethanol, chloroform and acetone. 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 0 to 4°C

ANTIBACTERIAL ACTIVITY TEST

The antibacterial activities of aqueous, acetone, chloroform and ethanolic extracts were determined using 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°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°C for 24 hours.

AGAR WELL DIFFUSION

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, filled with 100 µl of the extract. The plates were incubated at 37°C for 24 hours. The zones of inhibition were measured and recorded.

RESULTS AND DISCUSSIONS

Both the leaves and latex extracts of *Calotropis procera* exhibited bacteriocidal activity. The results are shown in Table I. The results indicated that acetone extract was the best solvent out of the solvents tested for extracting antibacterial substance(s) from this plant. The widest zone of inhibition (25mm) was demonstrated by the acetone extract of *C. procera* latex while the value dropped to 16 mm for chloroform,14mm for ethanol and 4mm for water extract respectively when tested against *Bacillus substilis* (Table II).

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

Test Organism	Inhibition Zone Diameter in mm							
	Aqueous Extract		Ethanol Extract		Chloroform Extract		Acetone Extract	
	Leaf	Latex	Leaf	Latex	Leaf	Latex	Leaf	Latex
Bacillus substilis (ATCC 6633)	1	2	2	6	10	11	16	22
Pseudomonas Putida (MTCC 1194)	1	3	2	7	8	12	15	16

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

Test Organism	Inhibition Zone Diameter in mm							
	Aqueous Extract		Ethanol Extract		Chloroform Extract		Acetone Extract	
	Leaf	Latex	Leaf	Latex	Leaf	Latex	Leaf	Latex
Bacillus substilis (ATCC 6633)	2	4	4	8	10	12	18	25
Pseudomonas Putida (MTCC 1194)	2	3	4	10	11	13	16	18

The aqueous extract was least effective against *Bacillus substilis* and *Pseudomonas putida*. The present results are in agreement with the results reported by Takazawa *et al*., (1982) where they showed need to employ broad range of extractive solvents in the extractions of possible phytochemicals from medicinal plants. Agar well diffusion method showed larger zones of inhibition compared to paper disc method (Table II).

On using agar diffusion method, zone of inhibition was 25 mm with the acetone extract of *C. procera* latex against *Bacillus subtilis* where as zone of inhibition was 22 mm on using paper disc method.

These results are in agreement with the results of Omenka and Osuaba (2000) where they showed better diffusion of the extracts into the medium with agar well diffusion enhancing contact with the organisms. Paper discs may act as a barrier between the extract and the organisms preventing total diffusion of active components absorbed by the discs into the medium and may be responsible for the observed differences.

Bacillus subtilis is a gram- positive bacterium and is responsible for bronchial disease infection and food poisoning in human beings. Acetone latex extract from *Calotropis procera* showed a widest zone of inhibition of 22 mm and 25 mm for Bacillus substilis using disc and open hole diffusion methods, respectively. The results indicated that acetone latex extract from *Calotropis procera* are effective in treating the bacterial infection. *Pseudomonas putida*, a non-fermenting Gram-negative bacillus, is an opportunistic human pathogen responsible for bacteraemia and sepsis in neonatal, neutropenic and cancer patients, as well as in urinary tract infections (UTIs). Sometimes, *P. putida* is also a cause of nosocomial infections in compromised hosts. Acetone latex extract from *Calotropis procera* showed a widest zone of inhibition of 16 mm and 18 mm for Pseudomonas putida using disc and open hole diffusion methods, respectively. Therefore, acetone latex extract from *Calotropis procera* may be considered to be a good agent in treating bacterial infections.

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