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In-Vitro Antimicrobial Activity of Latex and Leaf Extract from a Common Weed *Calotropis procera* Ait.

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Abstract

The antimicrobial effect of aqueous and methanol extracts of leaf and latex of *Calotropis procera* Ait. was evaluated against ten microorgnisms comprising of five bacteria (*Bacillus subtilis, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa)* and five fungi *(Alternaria pori, Aspergillus flavus. Aspergillus niger, Aspergillus oryzae, Penicillium chrysogenum)* using paper disc and well diffusion method. The *in vitro* study revealed that methanol extract was more effective than aqueous extract. Agar well diffusion methods gave larger zones of inhibition compared to paper disc method and the inhibitory effect was more pronounced in the latex extract than the leaf. *Bacillus subtilis* was reported to be the most succeptible microorganism.

Key words : *Calotropis procera* Ait., Antibacterial, Antifungal, Aqueous, Methanolic.

Antimicrobial substances are the substance that inhibits the growth and existence of microorganisms (1). These microorganisms could be pathogenic or non-pathogenic hence, antimicrobial substances are used in the treatment of various ailments. Quite a large number of antimicrobial substances exist and they are gotten from diverse sources such as microbes, plants, animals and chemicals (2). Plants have been an integral part of human civilization and their derived substance have recently become of great interest and importance owing to their versatile applications (3). *Calotropis procera* Ait. is member of plant family Asclepiadaceae, a shrub of about 6 m high and is widely distributed in the tropics. The plant is erect, tall, large, much branched and perennial. All parts of the plant exude white latex when cut or broken. In India, the secretion from the root bark is traditionally used for the treatment of skin diseases and intestinal worms (4). In the traditional Indian medicinal system, it has been used for pain, asthama, leprosy, tumors, piles. It is also either used alone or with other herbs to treat common diseases such as fever, rheumatism, indigestion, cold, eczema and diarrohea. However, antibacterial and antifungal activities of Calotropis procera Ait. have not been properly documented. The present study explores the antimicrobial activities of Calotropis procera Ait. using known microbial pathogens as test organisms.

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Methods

Collection and Processing of Plant Materials

Fresh plants of *Calotropis procera* were collected randomly from the outskirts of Noida and Greater Noida city. Leaves from fresh plants were taken and washed under running tap water, and rinsed with distilled water and air dried. The dried leaves were blended into powder. The latex was aseptically collected and centrifuged using a bench centrifuge at 1,500 rpm for 5 minutes. The samples were stored in air tight bottles until further use.

Extraction Procedures

Leaf and lates extraction of *Calotropis procera* Ait. was done with water and methanol ; 10 g each of

leaf powder and latex were dissolved in 100 ml of each solvent. The suspended solutions were leaf to stand for five days. The extract was filtered using Whatman's Filter paper No. 1. The filtrate was collected and stored at 4 C in sterile tubes.

Preparation of Test Organisms

Ten microorganisms used in this study as test organisms comprising of five bacteria *(Bacillus subtilis, Enterobacter aerogenes, Escherichia coli. Klebsiella pneumoniae, Pseudomonas aeruginosa)* and five fungi *(Alternaria pori, Aspergillus flavus. Aspergillus niger, Aspergillus oryzae, Penicillium chrysogenum)* were obtained from Department of Microbiology, IARI, New Delhi. The typed cultures of bacteria and fungi were sub-cultured on nutrient agar (NA) and potato dextrose agar (PDA) slants respectively and stored at 4c until required for study.

Antimicrobial Activity Testing

The antimicrobial assay was performed by two methods viz. disc diffusion method (5) and agar well diffusion method (6). Bacterial suspension was prepared and 1 ml of suspension was pippeted onto the surface of solidified agar plate, and gently rotated by hand so as to cover the entire surface with the microbial suspension. The plates were allowed to dry for at least 15 minutes. Sterile dise of 7 mm diameter was saturated with 100 µl of the test compound (aqueous/alcohol), allowed to dry and was introduced on the upper layer of the seeded agar plate by means of sterile forceps. The control discs were also incorporated on the fourth quarter. The plates were incubated overnight at 37 C in inverted position. The fungal isolates were similarly cultured on PDA plates and incubated at 30 C for 72 hours.

For assessing the antibacterial activity of the prepared extracts by agar well diffusion method, 0.6 ml of standardized bacterial stock suspension was thoroughly mixed with 60 ml of sterile nutrient agar ; 20 ml of the inoculated nutrient agar were distributed into sterile petri dishes. The plates were allowed to dry for at least 15 minutes. A sterile cork borer No. 4 was used to make wells of 10 mm diameter in each plate for extracts. The bottoms of the wells were sealed with one drop of the sterile nutrient agar, to prevent diffusion of the extracts under the agar. In 3 of 4 wells 100 µl of extract (aqueous/alcohol) was poured, the 4th well marked as control, filled with 100 µl sterile water/alcohol. Then the plates were incubated overnight at 37 C. The activity was evidenced by the presence of zone of inhibition surrounding the well. Each test was repeated three times and the antibacterial activity was expressed as the mean of diameter of the inhibition zones (mm) produced by the plant extract when compared to the controls. The same method as for bacteria was adopted for fungal species ; instead of nutrient agar PDA was used.

Table 1. *In vitro* antimicrobial activity of aqueous and alcoholic extracts of *Calotropis procera* Ait. on test microorganisms (disc diffusion method).

Test organisms	Calotropis procera			
	Aqueous extract		Methanolic extract	
	Leaf	Latex	Leaf	Latex
	Zone of inhibition (mm)			
Bacterial Species				
Bacillus subtilis	10	12	12	14
Enterobacter aerogenes	8	9	10	11
Escherichia coli	7	9	9	10
Klebsiella pneumoniae	10	11	11	12
Pseudomonas aeruginosa	4	6	5	7
Fungal Species				
Alternaria pori	3	5	4	6
Aspergillus flavus	3	3	5	6
Aspergillus niger	2	3	5	6
Aspergillus oryzae	2	3	5	6
Penicillium chrysogenum	4	6	6	7

Results and Discussion

The finding of this study reported in Tables 1 and 2 presents the antimicrobial activity of the aqueous and alcoholic extracts of leaf and latex of *Calotropis procera* Ait. The results indicate that both type of extracts from the plant showed inhibition of growth against tested microorganisms to various degrees.

The results of antibacterial activities (Tables 1 and 2) showed that aqueous and alcoholic extracts of both leaf and latex obtained from *Calotropis procera* Ait showed activities against bacteria with the widest zone of inhibition of against *Bacillus subtilis* of

Table 2. *In vitro* antimicrobial activity of aqueous and alcoholic extracts of *Calotropis procera* Ait. on test microorganisms. (Well diffusion method).

Test organisms	*Calotropis procera* Aqueous extract Leaf	Latex	Methanolic extract Leaf	Latex
	Zone of inhibition (mm)			
Bacterial Species				
Bacillus subtilis	13	14	15	16
Enterobacter aerogenes	10	12	12	14
Escherichia coli	8	10	10	12
Klebsiella pneumoniae	12	13	14	15
Pseudomonas aeruginosa	6	8	8	10
Fungal Species				
Alternaria pori	5	6	7	8
Aspergillus flavus	4	6	6	8
Aspergillus niger	4	5	6	7
Aspergillus oryzae	4	5	6	7
Penicillium chrysogenum	6	8	8	9

16 mm by the methanolic extract of the latex in well diffusion process followed by *Klebsiella pneumoniae* (15 mm) and minimum zone of inhibition against *Pseudomonas aeruginosa* (4 mm) by aqueous extract in disc diffusion method. According to the antibacterial assay done for screening purpose, the gram-positive bacteria *Bacillus subtilis* was reported to be more susceptible in comparison to gram- negative bacterial species. These observations are likely to be the results of the differences in cell wall structures between gram-positive and gram-negative bacteria, with gram-negative outer membrane acting as a barrier to many environmental substances including antibioties (7).

On comparing the antifungal activity of leaf and latex extracts obtained from both the processes (Tables 1 and 2) showed that methanolic extract from latex by well diffusion method showed highest zone of inhibition of 09 mm against *Penicillium chrysogenum* followed by *Alternaria pori* (8 mm) and almost negligible inheritance was shown by aqueous leaf extract by disc diffusion method against *Aspergillus* spp.

The results show that methanolic extracts exhibited a considerably broader antimicrobial activity compared to aqueous extract in both the cases (Tables 1 and 2). This may be due to better solubility of the active compounds in organic solvents (8). The agar well diffusion methods gave larger zones of inhibition compared to disc diffusion method (Tables 1 and 2). According to Omenka and Osuoha (9) agar well diffusion method allows better diffusion of the extract 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.

Although both leaf and latex extract showed considerable degree of inhibition against test microorganisms but, the inhibitory effect was more pronounced in the latex extract than the leaf. This could be due to the presence of calactin, mudarin and calotropin which are active constituents of *Calotropis procera* latex (1, 10).

Demonstration of antimicrobial activity of *Calotropis procera* Ait. against these microorganism is an indication that there is possibility of discovering promising alternative antibiotic substance in this plant for the development of newer antimicrobial agents and carry out further pharmacological evaluation.

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