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(Research Article)

Antimicrobial Activity of Flower Extracts of *Calotropis Gigantea*

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ABSTRACT

Antimicrobial potential of Calotropis gigantea against a wide range of microorganisms was studied. The ethyl acetate extract of this plant showed significant showed significant antibacterial and antifungal effect against most of the pathogenic organic organisms: Bacillus subtilis [MTCC (121)], Staphylococcus aureus [MTCC (96)], Pseudomonas aeruginosa [MTCC (429)], Escherichia coli [MTCC (443)], and two fungi Candida albicans [MTCC (183)], Tinea capitis [MTCC (7739)]. Phytochemical screening of the extracts showed the presence of a number of bioactive constituents such as cardiac glycosides, tannins, saponins, terpenes etc.

Key Words: *Calotropis gigantea*, Phytochemical screening, Bioactive, Antimicrobial potential, Asclepiadaceae, Milkweed

INTRODUCTION

The manufacture and clinical evaluation of herbal remedies and/or their isolates have made it increasingly feasible to transform traditional medicine into a modern industrial enterprise capable of making significant contribution to both healthcare deliveries and economic growth of developing countries[1]. Today, traditional medical practice has been recognized by the World health Organization (WHO) as a building block of primary healthcare[2]. But it emphasizes the fact that safety should be the overriding criterion in the selection of herbal remedies for use in healthcare[3]. There is no longer any doubt regarding the value and potential of traditional remedies[1].

Calotropis gigantea Linn. (Asclepiadaceae) is a glabrous or hoary, laticiferous shrubs or small trees, commonly known as THE SWALLOW-WORT or MILKWEED[4](Figure-1). The flowers of the plant contains the cardiac glycosides, calotopin, uscharin, calotoxin, calactin and uscharidin; gigantin. The flower also contains the protease calotropin DI and DII and calotropin FI and FII[5]. The flowers contains some poisonous constitute due to which it has somewhat caustic effect on the mucous membrane and tender skin, and may secondary dermatitis[6]. The aim of this present study is to evaluate the antimicrobial potential of *Calotropis gigantea* Linn against a wide range of microorganisms. Preliminary Phytochemical screening was also conducted in order to identify the chemical profile of active substances.

MATERIALS AND METHODS

Plant Materials

The flowers of *Calotropis gigantea* Linn used in this study were obtained from the local area of Uttarakhand and were identified based on its physical characteristics. The flowers were dried and crushed to small pieces using pestle and mortar and powered in an electric grinder.

Test Microorganisms
Clinical isolates of *Bacillus subtilis* [MTCC (121)], *Staphylococcus aureus* [MTCC (96)], *Pseudomonas aeruginosa* [MTCC (429)], *Escherichia coli* [MTCC (443)], *Candida albicans* [MTCC (183)] and *Tinea capitis* [MTCC (7739)] were obtained from the Department of Pharmaceutical Sciences, M.D.U. Rohtak, Haryana.

Preliminary Phytochemical Screening
The powder of the flowers of *Calotropis gigantea* Linn was subjected to successive extraction with different solvents in increasing order of polarity of solvents[7]. The dry extracts were subjected to various chemical tests in order to detect the presence of different phytoconstituents[8].

Preparation of Ethyl Acetate Extracts
The shade dried flowers were crushed into small pieces and powdered. The powder was loaded into soxhlet extractor and was subjected to extraction for about 25–30 h with ethyl acetate. After extraction the solvent was distilled off and the extract was concentrated under reduced pressure on a water bath at a temperature below 50°C to a syrupy consistency. Then it was dried in the dessicator. The yield was about 6%.

Sterilization of Materials
The Petri dishes and pipettes packed into metal canisters were appropriately sterilized in the hot air oven at 170^{o}C for 1 h at each occasion. Solution of the extract and culture media were autoclaved at 121^{o}C for 15 min.

Maintenance and Standardization of Test Organisms
The microorganisms were maintained by weekly sub culturing on sabouraud agar slant. Before each experiment, the organism was activated by successive sub culturing and incubation. Standardization of the test microorganism was done according to previously reported method[9, 10].

Antimicrobial Activity
The agar diffusion method[11] was used to evaluate the antimicrobial activity. Bacteria were cultured overnight at 37^{0}C in Mueller Hinton Broth (MHB) and fungus at 28^{0}C for 72 h in Potato Dextrose Broth (PDB) and used as inoculum. A final inoculum, using 100 µl of suspension containing 10^8 CFV/ml of bacteria and 10^4 spore/ml of fungus spread on Mueller Hinton Agar (MHA) and Potato Dextrose Agar (PDA) medium, respectively. The disc (6mm in diameter) was impregnated with 10 µl of 100 mg/ml (1mg/disc) extracts placed on seeded agar. Gentamicin (10µg/disc), Streptomycin (10µg/disc), and Tetracycline (10µg/disc) were used as positive controls for bacteria and Fluconazole (10µg/disc) and Ketoconazole (10µg/disc) for fungi. The test plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 72 h for fungi depending on the incubation time required for a visible growth. MIC values were also studied for microorganisms, which were determined as sensitive to the extract in disc diffusion assay. Sterile filter paper discs (6mm in diameter) containing 2.5–1000 µg/disc of plant extracts were placed on the surface of a medium. MIC was defined as the lowest concentration of extract that inhibited visible growth on agar.

Statistical Analysis
Pharmacological data were subjected to statistical analysis using SPSS 17.0 for windows. The values are represented as mean ± S.E.M. for microorganisms. Paired t-test was used for reporting the p-value and significance with respect to the control group.

RESULTS AND DISCUSSION
The disc diffusion method was used to determine zones of inhibition of *Calotropis gigantea* Linn extracts (organic and aqueous). The plant showed significant antibacterial and antifungal activity against almost all the organisms (Table-1) and especially good activity was found against *Pseudomonas aeruginosa*. However, the water extracts of this plant showed little antimicrobial activity against the test organisms used, *Pseudomonas aeruginosa, E. coli, Staphylococcus aureus, Tinea capitis, Candida albicans* and *Bacillus subtilis*. Increased inhibition was found at higher levels of extract concentration. MICs of these extracts are summarized in Table-2. Some of the extracts like the ethyl acetate extract of *Calotropis gigantea* Linn gave very low MIC values, and inhibited the growth of *Pseudomonas aeruginosa, E. coli* and *Tinea capitis* with a concentration of 2.5 µg/disc. Preliminary Phytochemical screening of *Calotropis gigantea* Linn showed the presence of a number of bioactive constituents such as cardiac glycosides, tannins, saponins, terpenes etc. The antimicrobial activity could be due to the presence of terpenes[12].

CONCLUSION

Thus the plant extract might be useful as antimicrobial agent. The potent antimicrobial activities of the extracts are as shown from the area of zone inhibition on Muller Hinton agar medium.

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Table 1: Antimicrobial activity of *Calotropis gigantea* Linn extracts (1µg/disc) inhibition zone in diameter (mm) standard antibiotic

Sr. No.	Micro-organisms	Ethyl acetate extract	Ethanol extract	Water extract
	Bacterial strains	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)
1	*Bacillus subtilis*	16.05±1.86	13.05±1.26	15.76±1.08
2	*E.coli*	21.05±0.64	16.87±1.04	14.43±1.02
3	*Staphylococcus aureus*	18.05±0.76	11.05±1.09	17.05±1.31
4	*Pseudomonas aeruginosa*	25.67±0.75	22.5±0.0	18.53±1.75
	Fungal strains			
1	*Candida albicans*	17.05±1.88	14.33±1.51	15.05±1.58
2	*Tinea capitis*	18.21±1.07	12.77±1.06	17.25±1.16

Values are mean± S.E.M. of triplicates experiments

The values of negative control were subtracted from the values of sample and the corrected values are given.

Table 2: The MIC values (µg/disc) of *Calotropis gigantea* Linn extracts against the microorganisms

Sr. No.	Microorganisms	Ethyl acetate extract	Ethanol extract	Water extract
Bacterial strains				
1	*Bacillus subtilis*	100	100	100
2	*E.coli*	2.5	100	100
3	*Staphylococcus aureus*	10	200	10
4	*Pseudomonas aeruginosa*	2.5	100	5
Fungal strains				
1	*Candida albicans*	10	100	100
2	*Tinea capitis*	2.5	100	100

Figure-1: Photograph of flower of *Calotropis gigantea* Linn

Figure-2: Photograph of a plate showing zone of inhibition for a single concentration (in triplicate set) on Muller Hinton agar medium

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