

**INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY,  
BIOLOGY AND CHEMISTRY****Research Article****Antifungal Activity of Calotropis Procera Towards  
Dermatophytes****S. Goyal\*, S. Kumar, P. Rawat and N. Dhaliwal**

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**ABSTRACT**

In developing countries of tropical regions skin diseases are more common due to mycotic infection. The use of medicinal herbs in the treatment of skin diseases including mycotic infections is an age-old practice in many parts of the world. The drugs which are being used against dermatophytosis exhibits several side effects and have limited efficacy. So that there is a distinct need for the discovery of new safer and more effective antifungal agents. Because herbal remedies used in traditional folk medicine may help to overcome the growing problem of resistance to antifungal drugs and their relative toxicity. In this study, the in-vitro antifungal activity of calotropis procera leaves extract were evaluated against three different genera of dermatophytes viz. *Microsporium*, *Trichophyton* and *Epidermophyton* by dilution agar method. *Calotropis procera* belongs to family *Asclepiaceae*. Medicinal properties of this plant are antimicrobial, anti-inflammatory, analgesic and useful in eczema and also for thrush and in mycotic enteritis. The ethanolic extracts from the leaves of this plant inhibited the growth of the dermatophytes tested at a concentration of 250  $\mu\text{gml}^{-1}$ . The MFC values of this compound was between 500-1000  $\mu\text{gml}^{-1}$ . Based on the results of this study, we can consider *calotropis procera* extract as a new source of developing local antifungal agents. However, further studies are needed to determine the efficacy of active component of this extract.

**Keywords:** Dermatophytes, *Calotropis procera*, Antifungal activity.**INTRODUCTION**

*Calotropis procera* belongs to family *Asclepiaceae*. The whole plant has a strong odour and found to be growing in all rural areas. Medicinal properties of this plant are antiseptic, anti-inflammatory, analgesic and useful in eczema and also for thrush and in mycotic enteritis (H. Misra et al. 1972). Due to increasing no. of immuno compromised individuals, fungal infections have increased in the last two decades, affecting million of people worldwide (Wong et al. 1994). Among them, skin fungal infections are very difficult to eradicate (Weitzman et al. 1995). They produce a variety of problems such as Athlete's foot and nail infections, leading to debilitation of the patients and can spread to other areas of the body and to other individuals (Ghannoum et al. 1999). Otherwise human mycoses are not always successfully treated since antifungal drugs show resistance or they have side effects and ineffective. Therefore, there is a need to find out more effective and less toxic, new antifungal agents by detection of antifungal compounds in medicinal plants and new antifungal agents are still needed to improve

the treatment of superficial fungal infections (Domenico et al. 1999 and Barrett et al. 2002).

**MATERIALS AND METHODS****I Plant Material**

*Calotropis procera* was collected locally from Amritsar district ASR, Punjab state India in Oct. 2007. Efforts were made to collect this plant for correct identification. It was identified with the help of Deptt. of Botanical Sciences, GNDU, Amritsar. The healthy and disease free leaves was separated and dried in shade so as to avoid decomposition of chemical constituents. These were powdered in grinder and stored in clean and dry airtight container for further studies.

**II Preparation of Plant Extract**

*Calotropis procera* leaves hydroalcoholic extracts prepared with 10 g (DW) of air dried powder to 100 ml organic solvent, ethanol 80% (drug/solvent ratio 1:10 w/v) in a conical flask, plugged with cotton and then kept on a rotary shaker at 180-220 rpm for 3 × 24 h by maceration at room temperature. Following filtration of the suspension through a Buckner fennel and whatman

filter paper # 1, the crude ethanol extract was evaporated at 50°C to near dried. Then 1 gm dried extract dissolved in 1 ml dimethylsulphoxide (DMSO). Final concentration of each extract adjusted to 1000 mg/ml, then divided to volume 1 ml in sterile vials and stored at -20°C for bioassay.

### III Dermatoplyte Isolates

For antifungal evaluation, all the strains were collected from Institute of Microbial Technology, Chandigarh. The samples were transferred to Sabourand Culture medium containing cyclohexamide and chloramphenicol agar (SCC) slants and subcultured every 15 days to prevent pleomorphic transformations.

### IV Preparation of Fungal Inoculum

A standardized inoculum was prepared by counting the microconidia microscopically. Sterile normal saline (0.85%) solution containing 0.05% Tween 80 was added to the slant tube culture gently with a glass rod to dislodge the conidia from the hyphal mat. The suspension was transferred to a sterile centrifuges tube and the volume was adjusted to 5 ml with sterile normal saline. The final suspension of conidia was adjusted to  $10^4 - 10^5$  cells/spores with colony forming units (CFU/ml) on a hemocytometer cell counting chamber (Shin & Lim 2004).

### V Antifungal Susceptibility Testing

The fungistatic activities of different extracts was evaluated via the Agar Dilution Method (Brass et

al. 1979). 1000 mg of the crude extract was dissolved in 1 ml of sterile DMSO. It was served as stock solution. For the assay, stock solutions of extracts were two-fold diluted with sterile normal saline (0.85%) solution to produce serial decreasing dilutions ranging from 3.9 – 1000 µg/ml. 5ml of sabouraud culture medium containing cyclohexamide and chloramphenicol agar (SCC) was added into Petri-dishes (55 mm) and then cooled to 45°C. The non-solidified SCC media was added with 100 µl of the serial dilutions extracts and 50 µl the inoculum removed after seven days from old culture of fungi and mixed thoroughly in it. Inhibition of fungal growth was observed after seven days of incubation at 28<sup>o</sup> – 30<sup>o</sup>C for dermatoplyte strains in a moist, dark, and at a time according to the control of fungus growth upto 15 days for dermatoplyte strains. The antifungal agents Kete conazole and Griseofulvin were used as positive controls. Drug free solution (only with appropriate amount of DMSO) was also used as a blank control for verification of fungal growth. The minimal inhibition concentration (MIC) value was defined as the lowest extract concentration and MFC minimal fungicidal concentration showing no visible fungal growth after incubation time. MIC 50 and MIC 90 values are the lowest extract concentration at which 50% and 90% of the clinical isolates inhibited (Marco et al. 1998). The results are depicted in Table 1 and 2.

**Table 1: In vitro evaluation of antifungal activity of ethanolic extracts of Calotropis procera leaf against dermatophytes**

Plant Specie	Extract	MIC Value (µg/ml)				
		T.M. <sup>1</sup>	T.R. <sup>2</sup>	M.G. <sup>3</sup>	M.C. <sup>4</sup>	E.F. <sup>5</sup>
Calotropis procera leaf	EtoH	250	250	250	250	250
Griseofulvin		<200	>200	40	160	60
Keteconazole		<200	>200	<200	50	160

\*Values are the mean of three replicates.

<sup>1</sup>Trichophyton mentagrophytes T.M

<sup>2</sup>T.rubrum (TR)

<sup>4</sup>Microsporium canis, (MC)

<sup>3</sup>M.gypreum (MG),

<sup>5</sup>Epidermophyton floccousm (EF)

**Table 2: Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of ethanolic extract of calotropis procera leaf against dermatophytes**

Organism (No. of isolates)	MIC and MFC Value (µg/ml)			
	Range	50%	90%	MFC
<sup>1</sup> T.M. (3)	250 – 1000	500	1000	500
<sup>2</sup> T.R. (2)	250 – 1000	500	1000	1000
<sup>3</sup> M.G. (4)	250 – 1000	500	1000	500
<sup>4</sup> M.C. (2)	250 – 1000	500	1000	500
<sup>5</sup> E.F. (3)	250 – 1000	500	1000	1000

\*Values are the mean of three replicates.

## RESULTS AND DISCUSSION

To carry out the antifungal evaluation with agar dilution assays, extracts in concentrations of upto 1000 µg/ml were incorporated into the growth media according of materials and methods. Extracts with MIC values < 1000 µg/ml were considered active. The ethanolic extract of *calotropis procera* was active against dermatophytes causative fungus of many superficial infections. We tested it against several strains of *E.floccusum* that produces arthroconidia, which survive for a longer time than other dermatophytes, therefore constituting an environmental source of contagion, sometimes leading to recurrent outbreaks of dermatophytosis in individuals and in institution (Domenico et al. 1999) and *T.rubsum* and *T.mentagrophytes*, which are the main cause of athlete's is foot and onichomycoses in human beings. Athlete's foot is the most prevalent superficial infection in the developed world (Evans, 1997) and onichomycoses affects 3-13% of the population worldwide and upto 30% of groups at high risk such, as elderly and diabetic people (Levy, 1997) and (Gupta et al. 1998). The ethanolic extract of *calotropis procera* leaves, inhibited all the species of dermatophyte genus tested, with MIC values between 250 and 1000 µg/ml (MIC 90 and MIC 50 values = 1000 and 500 µg/ml, respectively). The MFC values of there compounds were between 500 – 1000 µg/ml.

## CONCLUSION

Based on results of this study, we can consider ethomalic extract of *calotropis procera* as a new source for developing local antifungal agents. However, further studies are needed to determine the efficacy of active chemical constituent of this plant extract. Toxicological studies on the extract must also be performed to unsure the safety of the extract. The discovery of a potent herbal remedy that is safe will be a big advancement in fungal infection therapies. It is vital for systemic fungal infections that are usually in immunocompromised patients as toxicities induced by commercial antifungal drugs are often observed in these patients due to high dosage and prolonged therapy.

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