EVALUATION OF ANTIFUNGAL ACTIVITY OF *CALOTROPIS PROCERA* LEAF EXTRACT: AN *IN VITRO* STUDY

Priyanka Sharma*[1] and Gajanand Modi[2]
Address: Priyanka Sharma, [1&2] IASE University, Department of Biotechnology, Sardarshahr-331403, Rajasthan

ABSTRACT
In developing countries of tropical regions skin diseases are more common due to mycotic infection. In many parts of the world the use of medicinal herbs in the treatment of skin diseases including mycotic infections is an age-old practice. The drugs which are being used against dermatophytosis exhibits several side effects and have limited efficacy. In this study, the in-vitro antifungal activity of *Calotropis procera* leaves extract in different solvents (petroleum ether, chloroform, ethyl acetate and ethyl alcohol and aqueous) were evaluated against two different genera of dermatophytes viz. *Trichophyton rubrum* and *Epidermophyton floccosum* by disc diffusion and broth dilution methods. The results revealed that ethyl acetate is the best extractive solvent for antifungal properties. The minimum inhibitory concentration (MIC) for ethyl acetate extract was also observed**.** The MIC values of this compound was 8 mg/ml. Present study concludes that leaves of *C. procera* demonstrated strong inhibitory effect on the test organisms and established a good support for the use of this plant 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: *Calotropis procera,* Antifungal activity, disc diffusion method, minimum inhibitory concentration.

INTRODUCTION

The plants which possess antimicrobial activities are being evaluated in the recent past. (**Clark *et al.*, 1993**). Due to increasing no. of immuno compromised individuals, fungal infections have increased in the last two decades, affecting millions of people worldwide (**Wong *et al*. 1994**). Among them, skin fungal infections are very difficult to eradicate (**Weitzman *et al*. 1995**). These infections produce verities of problems such as Athlete's foot and nail infections thus leading to debilitation of the patients and can spread to other areas of the body and to other individuals (**Ghannoum *et al*. 1999**). Many serious problems are being caused by Chemical fungicides which lead to environmental problems (**Anon, 2005**). In contrast to the synthetic pesticides which are dangerous to consumers and have hazardous impact on the environment, plant metabolites and plant-based pesticides appear to be one of the best alternatives (**Varma and Dubey, 1999**). Biologically active components present in the medicinal plants have always been great interest to scientists working in this field. To improve the treatment of superficial fungal infections 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 (**Domenico *et al*. 1999 and Barrett *et al*. 2002**).
Calotropis procera R.Br. is a large shrubby weed belongs to family Asclepiadaceae. Commonly known as milkweed or swallow-wort **(Singh *et al*., 1996**). The species has high socio-economic value and is native to tropical and subtropical Africa and Asia, common in the Middle East (**Parsons and Cuthbertson 2001; Lottermoser 2011**) and in Latin America (**Abbas *et al*. 1992**). It often grows in saline or slightly saline soils with low soil moisture, forming mono-specific stands (**El-Midany 2014**). The presence of alkaloids, cardiac glycosides, tannins, flavonoids, sterols and triterpenes on the aerial parts of the plants was shown by phytochemical studies (**Mossa *et al*.1991**). The leaves contain mainly the amyrin, amyrin acetate, ß-sitosterol, ursolic acid, cardenolides, calotropin and calotropagenin (**Sharma *et al*. 2011**). The plant is reported for analgesic activity, anti-inflammatory, and hepatoprotective effects (**Dewan *et al*., 2000; Alencar *et al*., 2004; Padhya *et al*., 2007**). Leaves also are used for fertilizer (**Akhtar *et al*. 1992**).

However, little known about the antimicrobial activities of *Calotropis procera* except for their activities against a small range of microorganisms (**Jain *et al*., 1996; Kareem *et al*., 2008**).

The dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissue (skin, hair and nails) of humans (**Maraki *et al*., 2007 and Mihali *et al*., 2012**). Infections which are caused by dermatophytes are known as dermatophytosis (**Dei and Vernes, 1986**). The genera *Microsporum, Trichophyton* and *Epidermophyton* causes Dermatophytosis. *Epidermophyton* is a human pathogen while *Trichophyton* are human and animal pathogens. The present research study is, therefore, designed to evaluate the antifungal potential of *Calotropis procera* leaves against the destructive plant pathogenic fungi *Trichophyton rubrum* and *Epidermophyton floccosum*.

MATERIALS AND METHODS

I. Collection of Plant material

Fresh and healthy leaves of *Calotropis procera* were obtained from hedges of agriculture fields, Mansa, Punjab. The sample specimen was identified based on the taxonomical characteristics. The healthy and disease-free leaves was separated and dried in shade to avoid decomposition of chemical constituents. The leaves were subsequently dried in a hot air oven at 40°C for 48 h, powdered to 100-120 mesh in a grinder and stored in clean and dry airtight container for further studies.

II. Preparation of Plant Extract

Serial exhaustive extraction which involves successive extraction with solvents of increasing polarity from a nonpolar (petroleum ether) to a more polar solvent (Ethanol and aqueous) to ensure that a wide polarity range of compound could be extracted will be used. The crushed and powdered leaves (200 g) were extracted over a period of forty-eight hours in soxhlet extraction unit successively with the solvents of increasing polarity (Petroleum ether, Chloroform, Ethyl acetate and Ethyl alcohol, aqueous) to cover a wide range of compounds. Solvents were removed in vacuum using rotary evaporation.

The above procedure was repeated with the same leaves and solvents as and when required. The evaporated extracts so obtained were preserved at 4°C in airtight bottles until further use.

III. Dermatophyte Isolates

For antifungal evaluation, both strains (MTCC 7859) *Trichophyton rubrum* and (MTCC 7880) *Epidermophyton floccosum* were collected from Institute of Microbial Technology, Chandigarh. fungal cultures were maintained on saborauds dextrose agar (SDA) medium. Cultures were reactivated before every test.

IV. Antifungal Susceptibility Testing

The in vitro assessment of antifungal susceptibility is done by two methods: Diffusion Assay and Broth Assay. 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. The minimum inhibitory concentration (MIC) was carried out by broth dilution method (Brantner and Grein 1994). The dermatophytes grown on SDA medium for a week were flooded with 0.85% saline. After settling of the larger particles, conidia were counted with a hemocytometer and diluted in saborauds dextrose broth to a final spore concentration of 1×10^6 spores/ml. For dermatophytic assay in broth, 5 ml of sterile saborauds dextrose broth medium taken in screw capped tubes were inoculated with 20 µl of fungal suspension and 1-10 mg/ml concentration of the extract. The tubes were incubated for a week at 30°C. The visible mycelial growth in the tubes expressed the degree of activity of the extract. Inhibition of bacterial growth was determined by measuring the absorbance at 600 nm in a colorimeter. Fungal growth inhibition was calculated by considering the control and sample zone of inhibition.

Ketoconazole was used as a standard antifungal agent. The tubes containing 5 ml of broth and 20 µl of fungal suspension served as bacterial control. The results are depicted in Table 1.

Statistical analysis

The antifungal activity evaluated by paper disc diffusion method was expressed as mean±SE of the diameter of the growth inhibition zones (mm).

RESULTS AND DISCUSSIONS

The antifungal activity of different solvent extracts of *C. procera* leaf extract against dermatophytes showed varied levels of inhibition. Among the solvent extracts tested, ethyl acetate extract had a broad spectrum of activity against both fungi tested and showed the highest zones of inhibition against *E. floccosum* (20.5 mm) as shown in table 1. The least zone of inhibition was observed with the aqueous extract which showed an inhibition of zone 8.8 mm against *T. rubrum.*

The antifungal activity of leaf extract (petroleum ether, chloroform, methanol and water) of *C. procera* against *Candida albicans* and *A. niger* has been assessed by well plate diffusion method **(Suvarna and Patil, 2009**). The antimicrobial activity of apical twig of *C. procera* has been demonstrated **(Parabia *et al.*, 2008)**. Other authors had reported the antidermatophytic activity of leaves, latex and stem bark of *C. procera* (**Kareem *et al*.,2008; Kuta, 2006; 2008; Hassan *et al.,* 2006; Rai and Upadhyay, 1988a).**

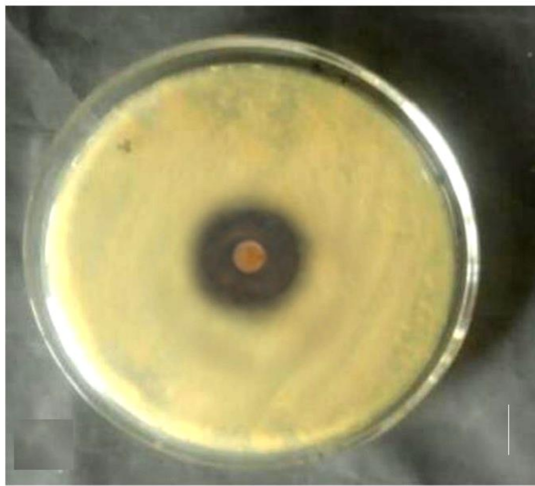
Table 2 reveals MIC values of ethyl acetate leaf extract of *Calotropis* sp. Minimum inhibitory concentration values for *T. rubrum* and *E. aerogenes* were found to be 8 mg/ml. At 600 nm, *Calotropis procera* ethyl acetate extract had the MIC values of 8 mg/ml against *T. rubrum* (95.63% growth inhibition) and *E. floccosum* (90.47% growth inhibition). Both dermatophytes, *T. rubrum* and *E. floccosum* were found to be more susceptible to the ethyl acetate leaf extract of all the three plants. The per cent growth inhibition of fungi is shown in Figure 1. **Sharma *et al.* (2002)** worked on screening of leaf extracts of *C. procera* for their fungicidal properties. **Kuta (2008)** again observed inhibition of aqueous extract of stem bark of *C. procera* against *Epidermophyton floccosum* and *Tricophyton gypseum* in a concentration range of 1 to 5 mg/ml. **Goyal and Mathur (2011**) also found that extracts of *C. procera* have antimicrobial potency. **Komathi *et al.* (2012)** confirmed the antifungal potency of ethanolic extract of leaves of *C. procera*. In an investigation performed by **Vadlapudi *et al.* (2012)** antimicrobial activity was reported by methanolic extract of aerial parts of *C. procera*. It gave moderate effect against tested bacteria and fungus. Similarly, **Sharma *et al*. (2018)** also reported that ethyl acetate extract of *C. procera* showed maximum antibacterial activity.

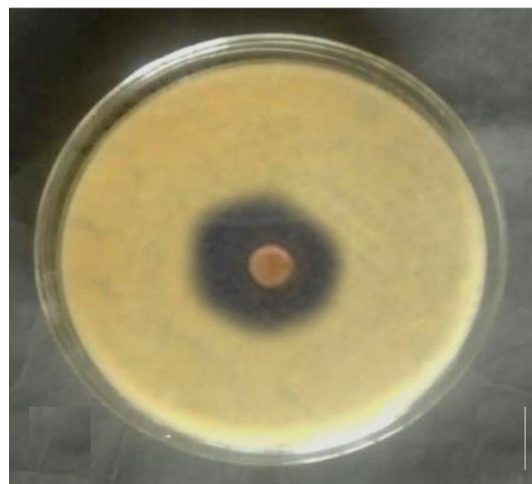
The results of present study revealed that, *Calotropis procera* leaf possesses antifungal activity. Future research should focus on the more elucidation of the chemical constituents and their mechanism of action to facilitate efficient uses of important plant resources as anti-microbial drugs.

Table 1 Zone of inhibitory activity (in millimeter) of different solvent extracts of *Calotropis procera* leaves against *Trichophyton rubrum* and *Epidermophyton floccosum*

Micro-organisms	Ketonocazole	Aqueous Extract	Ethanol Extract	Chloroform Extract	Petroleum Ether Extract	Ethyl Acetate Extract
Trichophyton rubrum	14.5±0.96	8.8±0.58	14.5±1.05	13.5±0.34	17.2±0.75	19.7±0.84
Epidermophyton flocossum	13.4±0.57	9.2±0.67	16.2±0.97	15.4±0.51	14.7±0.29	20.5±0.68

Plates 1 Antifungal activity of ethyl acetate extracts of *Calotropis procera* against the test fungi (a) *Trichophyton rubrum* (b) *Epidermophyton floccosum*





Trichophyton rubrum *Epidermophyton floccosum*

Table 2 Minimum inhibitory concentration (MIC) of *Calotropis procera* ethyl acetate extract using broth dilution method

Concentration (mg/ml)	**Fungal strains**	
	Trichophyton rubrum	*Epidermophyton floccosum*
Control	2.52±0.1	2.73±0.08
1	2.05±0.06	2.25±0.07
2	1.9±0.08	2.07±0.08
3	1.65±0.08	1.91±0.12
4	1.38±0.1	1.6±0.17
5	1.1±0.05	1.22±0.05
6	0.67±0.1	0.98±0.09
7	0.3±0.05	0.6±0.07
8	0.11±0.03	0.26±0.06
9	0	0
10	0	0
11	0	0
12	0	0

1 mg/ml concentration of the extract use
Values are means of three independent replicates

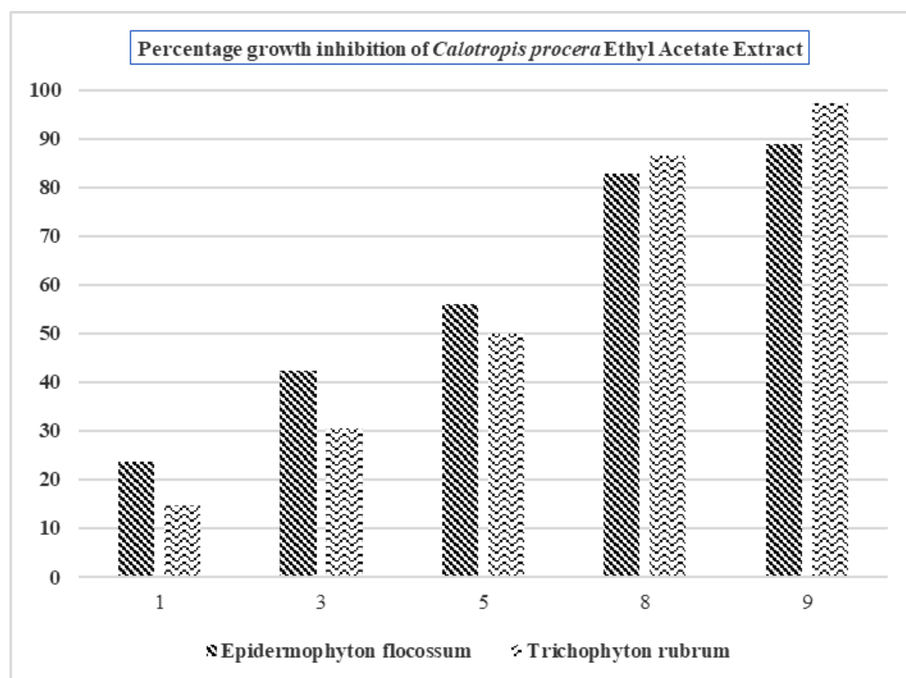


Figure 1 Percent growth inhibition of bacteria at different concentrations (1-10 mg/ml) of *Calotropis procera* ethyl acetate leaf extract

CONCLUSION

Based on results of this study, we can consider Ethyl acetate extract of *Calotropis procera* as a new source for developing novel antifungal agent against pathogenic fungi. 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 ensure the safety of the extract.

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