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Full Length Research Paper

Susceptibility of a weed *Calotropis procera* (Ait.) against clinical isolates of dermatophytes

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Anti-dermatophytic activity of foliar extract of *Calotropis procera* in different solvents (petroleum ether, chloroform, ethyl acetate and ethyl alcohol) was determined using paper disc diffusion method against three target fungi *Microsporum canis* (MTCC 3270), *Microsporum fulvum* (MTCC 7675) and *Trichophyton mentagrophytes* (MTCC 7250). The results revealed that ethanol is the best extractive solvent for antifungal properties followed by chloroform, ethyl acetate and petroleum ether respectively. The best antifungal activity was recorded in the ethanol extract of *C. procera* leaves against *M. canis*. The minimum inhibitory concentration (MIC) for ethanol extract was also observed. 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 *C. procera* in traditional medicine. Ethanol extract of *C. procera* leaves is a potential antidermatophytic agent with a high anti-dermatophytic property.

Key words: Antidermatophytic activity, *Calotropis procera,* disc diffusion method, folk medicines, minimum inhibitory concentration.

INTRODUCTION

Nowadays, natural products most likely continue to exist and grow to become even more valuable as sources of new drug leads. This is because of a broader degree of chemical diversity and novelty of molecules found in natural products than that from another source. Fungal diseases have historically been a difficult clinical entity to effectively deal with. The available drugs to treat fungal infections have been limited. Furthermore, in this armamentarium there are problems with dose limiting nephratoxicity, the rapid development of resistance, drug interactions and fungi static mechanism of action. Thus, there is need for the development of more efficacious antifungal agents with fewer limitations and lesser side effects. Dematophytosis is a clinical condition caused by fungal infection of the skin in humans, pets and domestic animals. It is caused by fungi of several different species. The fungi that causes parasitic infection (dermatophytes) feed on keratin, the material found in the outer layer of the skin, hair and nails. These fungi thrive on skin that is warm and moist but may also survive directly on the outsides of hair shafts or in their interiors.

Dermatophytes love to grow at low temperature between 0 to 30°C. Higher temperature of the hot spot cause the fungus to move towards the periphery of the lesion where the temperature is comparatively low, thus producing concentric ring shaped lesions; the name “ring worm diseases” is derived from such lesions. The virulence of the infecting strain or species, the anatomic location of the infected site and local environmental factor determine the severity of infection. The plant used in the present investigation, *Calotropis procera* (Family: Asclepiadaceae**)** is a weed commonly known as Aak or Madar. Calotropis is used as a traditional medicinal plant (Agharkar, 1991) with unique properties (Dzoyem et al., 2006). Calotropis is also a reputed homeopathic drug (Ferrington, 1990). It is a widely used medicinal plant in the Indian sub-continent (Sehgal et al., 2005). It has longethnobotanical history in traditional medicine. The medicinal potential of *C. procera* has been known to the traditional system of medicine for a while now with its leaves being widely used. In folk medicine, the leaves of

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Table 1. Percentage yield of extracts from *C. procera.*

Part used	Percent yield of extracts (%)			
	PE	CHL	ETOAC	ETOH
Leaves	2.75±0.03	3.75±0.02	1.88±0.03	4.33±0.01

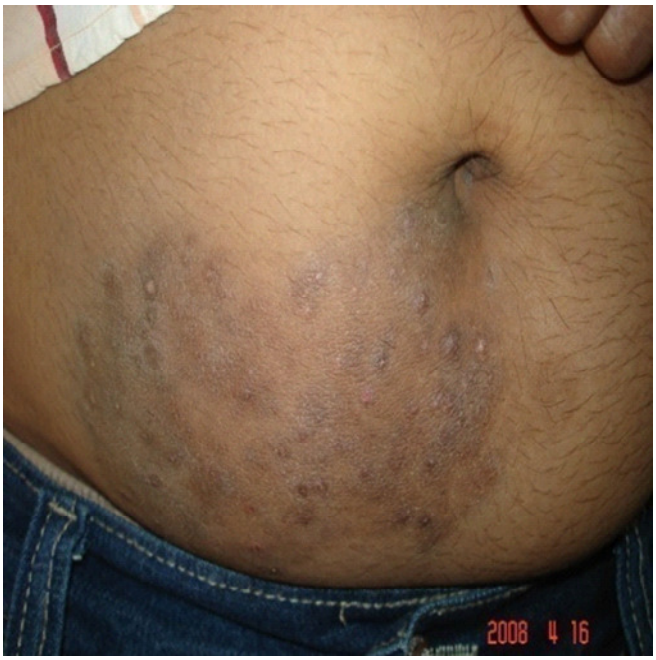

Figure 1. Photograph of patients showing *Tinea corporis* disease caused by dermatophytes.

C. procera are used to alleviate ear pain. Traditionally, Calotropis is used alone or with other medicines to treat common diseases such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting and diarrhea. There is a great demand for exploration of new antimicrobials from plant sources.

This paper discusses the microbiological properties of leaves of *C. procera.* Its antifungal properties were investigated against clinical isolates of *Microsporum canis, Microsporum fulvum* and *Trichophyton mentagrophyte* using disc diffusion sensitivity test. Although the anti microbial activity of different parts of *C. procera* extracts have been reported (Hassan et al., 2006; Kuta, 2008; Parabia et al., 2008; Kareem et al., 2008) but only few reports are available regarding the anti-dermatophytic activity of the leaf extract (Hassan et al., 2006; Rai and Upadhyay, 1988a; Kuta, 2006, 2008). To the best of my knowledge, there has not been found any report documented in the literature against the clinical isolates of dermatophytes. Therefore, the present research work was carried out. Consequently, this study was embarked upon to confirm or otherwise, the sensitivity of microbes to extracts of the plant and to investigate its potential for antifungal activity.

MATERIALS AND METHODS

Plant collection

The leaves of the selected plant used for the study were collected from the campus area of the Dayalbagh Educational Institute (Deemed University), Dayalbagh, Agra, India during July to August 2007 and were cleaned, shade dried at room temperature for 10 to 15 days. The dried plant material was powdered and stored in labeled air tight containers.

Authentification of plant

The plant has been characterized by the Taxonomy Division, Botanical Survey of India (BSI), Allahabad as *C. procera* and the assigned Accession No. is 79385 (BSA).

Extraction and processing

Dried and coarsely powdered plant material (200 g) was successively extracted with various solvents such as petroleum ether, chloroform, ethyl acetate and ethyl alcohol by using continuous hot extraction with Soxhlet extractor, for 48 h (Sheela and Kannan, 2003). The crude extracts obtained were filtered through Whatman filter paper no. 1 and the filtrates were evaporated at low temperature and reduced pressure to give a gummy solid residue. The dried extracts were stored in labeled sterile screw capped bottles in a refrigerator. The extracts were weighed and the percent yield was calculated (Table 1). The extracts were dissolved in respective solvents in sterile test tubes to obtain concentrations of 125, 250,500 and 1000 to 7000 ppm and were subjected to antifungal screening..

Collection of samples

The clinical samples were collected from patients, Dermatology Department, S.N. Medical College, Agra, from skin scrapings (Figures 1, 4 and 5), hair (Figure 2) and nails (Figure 3) of the patients (Whiting and Bisset, 1974). The skin scrapings were collected on sterilized butter paper with the help of sterilized new blade from the centre or edge of the lesion, after cleaning the site with 70% alcohol and carefully transferred from butter paper to the Petri dishes containing SDA (Sabouraud Dextrose Agar) medium. Hair samples were collected from base and shaft of hair in sterile Petri dish. Nails sample, after cleaning the nail site with 70% ethanol, the scrapings were collected.

Culturing, isolation and purification

All the samples were then inoculated into Petri dishes containing SDA medium directly and incubated at 28±2°C. In Petri dish, when fungal colonies appeared on SDA medium, it was transferred to other dishes for the purification. Sub culturing of respective colony was carried out to obtain a pure culture. Then the pure cultures were identified (Figures 6, 7 and 8) with the help of 'A Color Atlas of Pathogenic Fungi' (Frey et al., 1986) and sent to MTCC for confirmation.

Antifungal sensitivity test

The isolated fungal cultures used in the screening such as *M. canis* (MTCC 3270), *M. fulvum* (MTCC 7675) and *T. mentagrophytes* (MTCC 7250), were procured from S. N. Medical College, Agra.

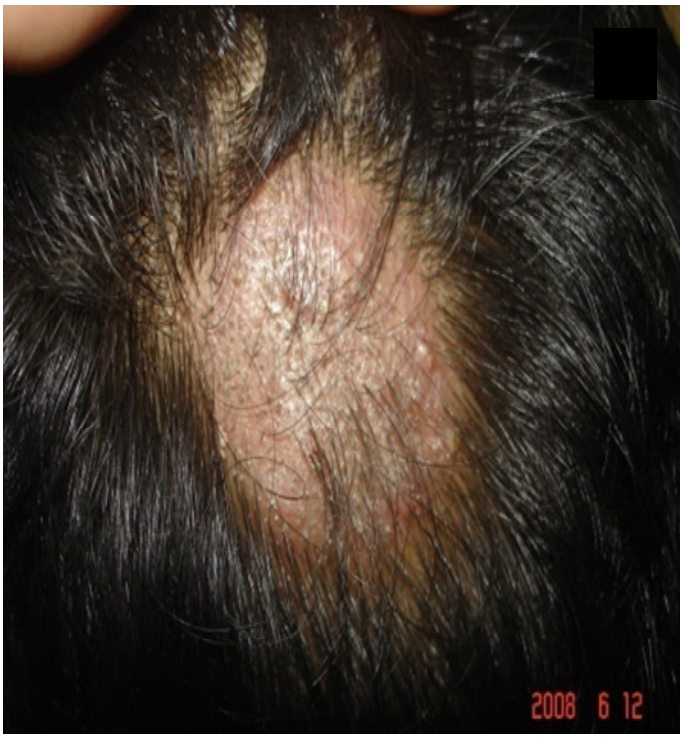

Figure 2. Photograph showing *Tinea capitis* disease caused by dermatophytes.

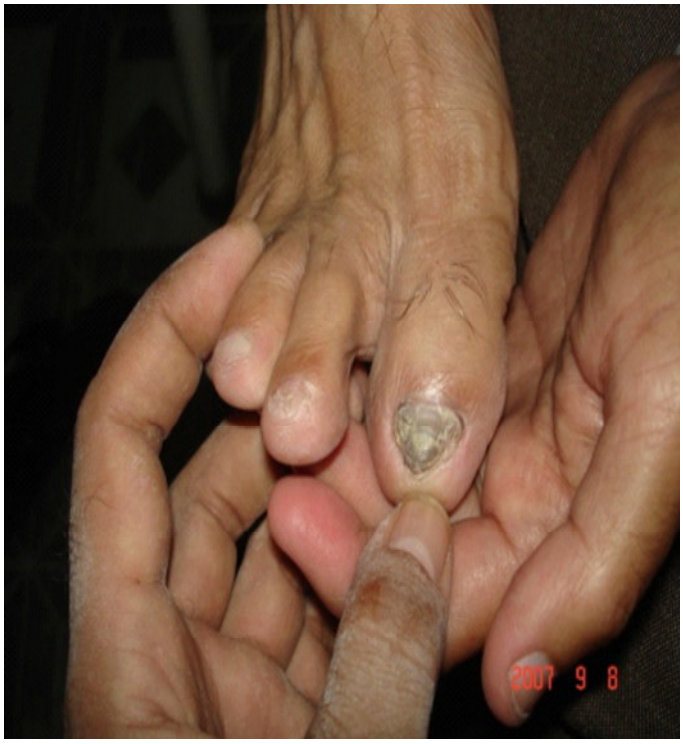

Figure 3. Photograph showing onychomycosis disease caused by dermatophytes.

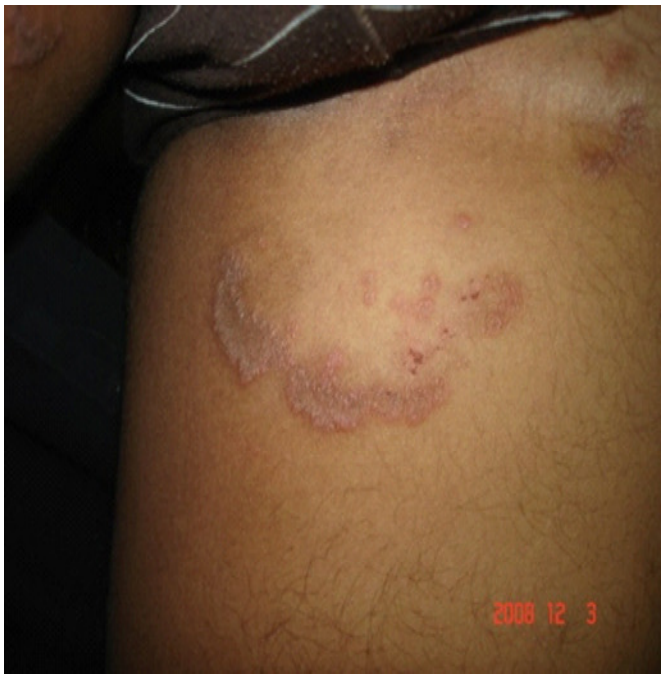

Figure 4. Photograph showing *Tinea cruris* disease caused by dermatophytes.

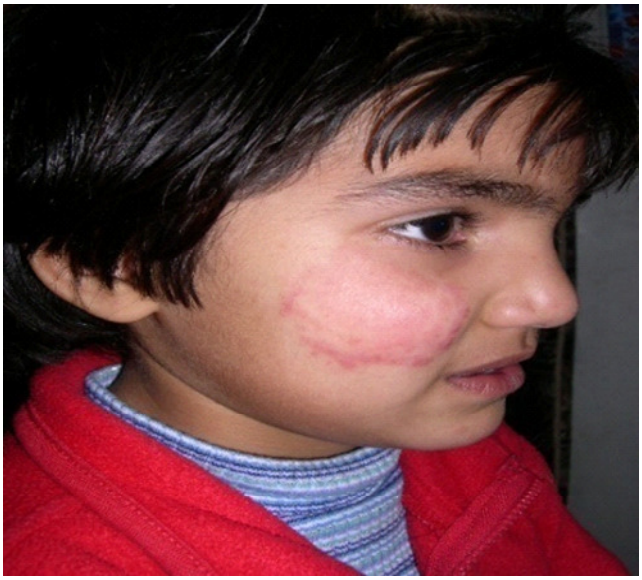

Figure 5. Photograph showing *Tinea facei* disease caused by dermatophytes.

Assessment of anti-fungal activity of plant extract against dermatophytic fungal species

The antifungal activity of extract of plant *C. procera* against the target dermatophytic fungi was studied in terms of:

Paper disc diffusion method: The SDA media was poured into

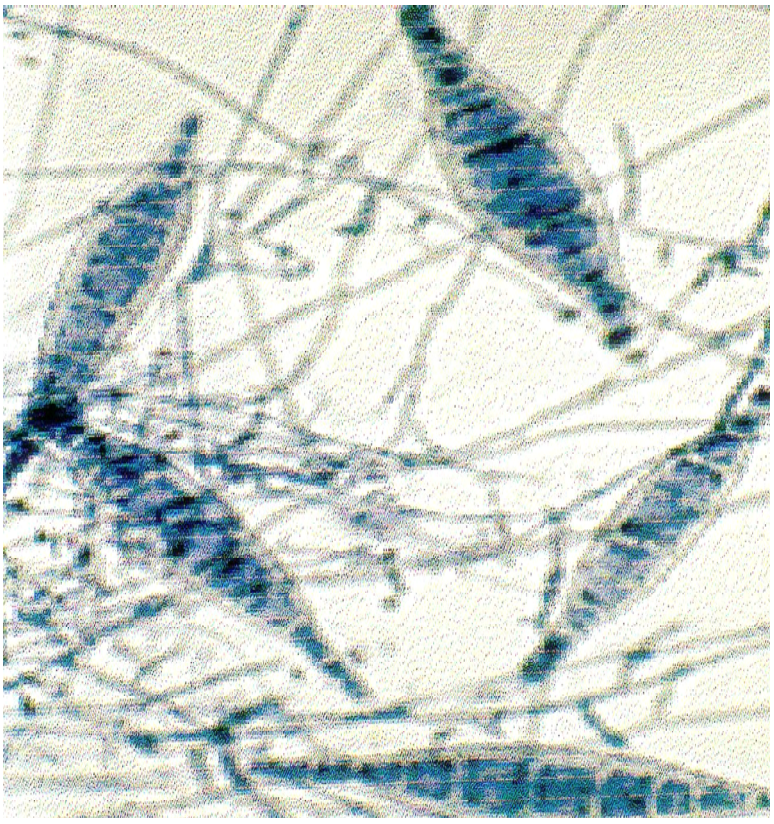

Figure 6. Microphotograph of *M. canis.*

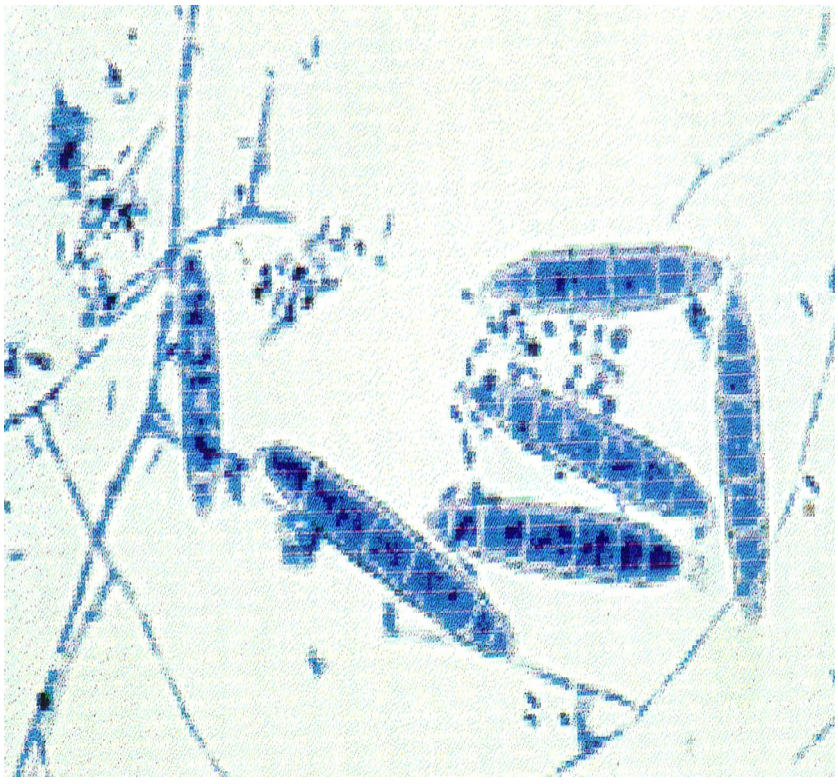

Figure 7. Microphotograph of *M. fulvum*

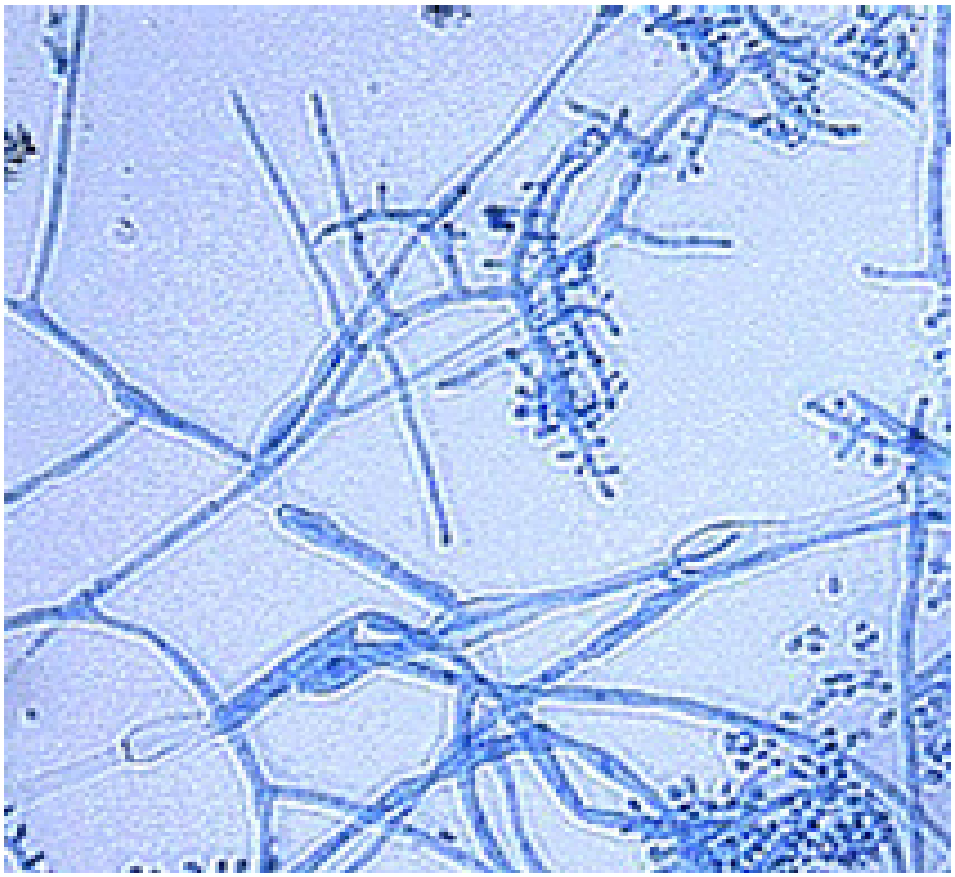

Figure 8. Microphotograph of *T. mentagrophytes.*

sterile Petri dishes (diameter 9.0 cm) and allowed to set. The Paper Disc Diffusion method was employed for the antifungal susceptibility testing (Pelczar et al., 1993). Whatman filter paper discs (No. 1, diameter 10 mm) saturated with different extracts containing varying concentrations (125, 250, 500, 1000 to 7000 ppm) were placed on culture medium seeded with the test organism 1×10^5 spores/ml. Disc fed with corresponding solvent alone served as control. These agar plates were incubated at 27±2°C for 3 to 5 days. After incubation, the zone of inhibition around the disc was measured in mm diameter and the mean value of triplicate was recorded. Griseofulvin (6000 ppm concentration) was the standard antifungal used. Thereafter, the minimum inhibitory concentration (MIC), the lowest concentration of a sample that inhibits the growth of a microorganism was determined.

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) of *C. procera* leaf extract was determined by using paper discs in different concentrations as described by Shanmuga et al. (2002). The discs fed with different dilution range of 152, 250 and 500 ppm were placed on the surface medium containing fungus culture. The zone of inhibition was measured as the diameter and was recorded as the MIC.

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

The results showed that the ethanolic extract of *C. procera* leaves yield was 4.33% (Table 1). The antifungal potency of different leaf extracts of *C. procera* against three different dermal fungal species that is *M. canis, M. fulvum* and *T. mentagrophytes* was evaluated by the presence or absence of inhibition zones and zone diameters (mm) (Table 2). From the results, it is evident that the ethanoilc extract showed a maximum inhibitory zone 12.5, 12.5 and 9.13 mm, respectively for *M. canis, M. fulvum* and *T. mentagrophytes* (Figures 9b, 10b and 11b) while the values dropped 4.5, 11.3 and 10.6 mm for ethyl acetate and 4.6, 11.9 and 3.6 mm for chloroform leaf extracts respectively when tested against the same organisms at 6000 ppm concentration.

It was noticed that at higher concentration (7000 ppm) inhibition zone values were dropped down as 1.5, 1.0 and 7.1 mm for *M. canis* , 3.9, 7.3 and 0 mm for *M. fulvum* and 0 mm for *T. mentagrophytes* in chloroform, ethyl acetate and ethanol leaf extracts respectively. This is because when any organism is exposed to the same treatment over and over, a variety of biochemical processes occur within this organism. These processes

Table 2. Antifungal activity of *Calotropis procera* leaf extract against fungal species tested by disc diffusion and MIC.

Conc. range (ppm) / Microbe	Zone diameter (mm) in different Solvent leaf extracts of *C. procera*			
	Petroleum ether (mean±SE)	Chloroform (mean±SE)	Ethyl acetate (mean±SE)	Ethyl alcohol (mean±SE)
M. canis				
125	-	1.0±0.0	-	2.25±0.02
250	-	1.0±0.0	-	3.25±0.02
500	-	1.0±0.0	0.5±0	4.13±0.001
1000	-	1.75±0.02	0.75±0.02	4.75±0.02
2000	-	2.25±0.02	1.0±0	5.0±0.04
3000	-	3.5±0.04	1.0±0.0	5.25±0.04
4000	-	3.9±0.03	1.5±0.0	5.38±0.001
5000	-	4.6±0.001	2.25±0.02	6.9±0.001
6000	-	2.75±0.02	4.5±0.04	12.5±0.04
7000	-	1.5±0.0	1.0±0.0	7.1±0.08
Gresiofulvin	-	-	-	-
Control	-	-	-	1.0±0.0
M. fulvum				
125	-	2.6±0.001	1.5±0.0	2.3±0.02
250	-	3.1±0.04	2.0±0.05	2.9±0.001
500	-	3.3±0.02	3.0±0.0	3.5±0.0
1000	-	3.4±0.08	4.1±0.001	4.4±0.001
2000	-	4.5±0.05	6.5±0.02	5.6±0.001
3000	-	5.0±0.11	7.0±0.0	5.8±0.02
4000	-	6.4±0.19	10.3±0.02	6.5±0.04
5000	-	11.4±0.06	10.5±0.04	8.6±0.02
6000	-	11.9±0.06	11.3±0.09	12.5±0.04
7000	-	3.9±0.06	7.3±0.02	-
Gresiofulvin	10±0.4	10±0.04	10±0.04	10±0.3
Control	-	-	-	-
T. mentagrophytes				
125	-	1.13±0.001	-	1.0±0.0
250	-	1.63±0.001	-	2.5±0.02
500	-	1.63±0.03	-	3.3±0.02
1000	-	1.81±0.01	-	4.75±0.02
2000	-	2.0±0.0	1.63±0.001	6.5±0.02
3000	-	2.13±0.03	2.25±0.02	7.4±0.03
4000	-	2.75±0.02	2.75±0.02	7.75±0.02
5000	-	2.9±0.001	10.4±0.03	8.0±0.05
6000	-	3.6±0.001	10.6±0.03	9.13±0.03
7000	-	0.75±0.02	3.0±0.11	4.5±0.04
Gresiofulvin	-	-	-	-
Control	-	-	-	1.0±0.0

All values indicate antimicrobial activity in mm, (-) value indicates no activity

may keep the dose out of the cell, alter the target of the drug and the response may start to decrease at higher concentrations. The decrease in zone values may be due to the saturation. However, the petroleum ether extract was not effective against tested fungi. The antifungal potency of *C. procera* leaf extract on *M. canis* and *M. fulvum* showed a larger diameter of clearance than that of *T. mentagrophytes*.

Moreover, the zone of inhibitions achieved by *C. procera* leaf extract is comparable to that of the standard

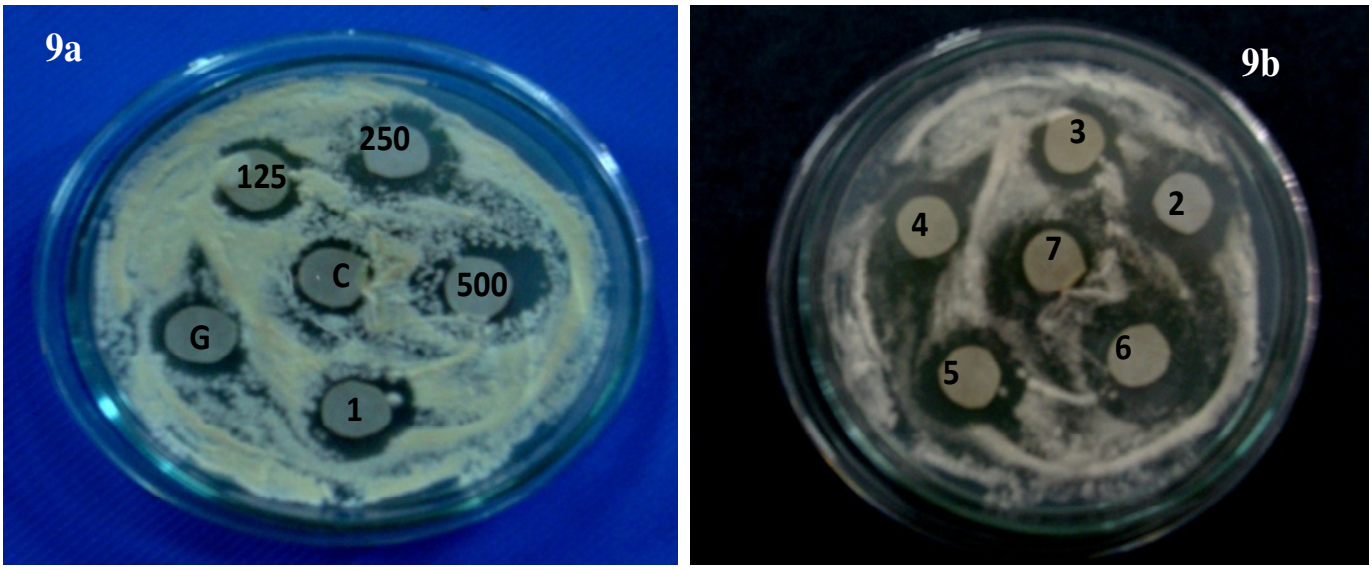

Figure 9. a) Showing MIC (125 ppm) b) Inhibition zone of *M. canis* by disc diffusion method, C is indicative of Control, G- Griseofulvin, 1-7: 1000 to 7000 ppm concentration of extract.

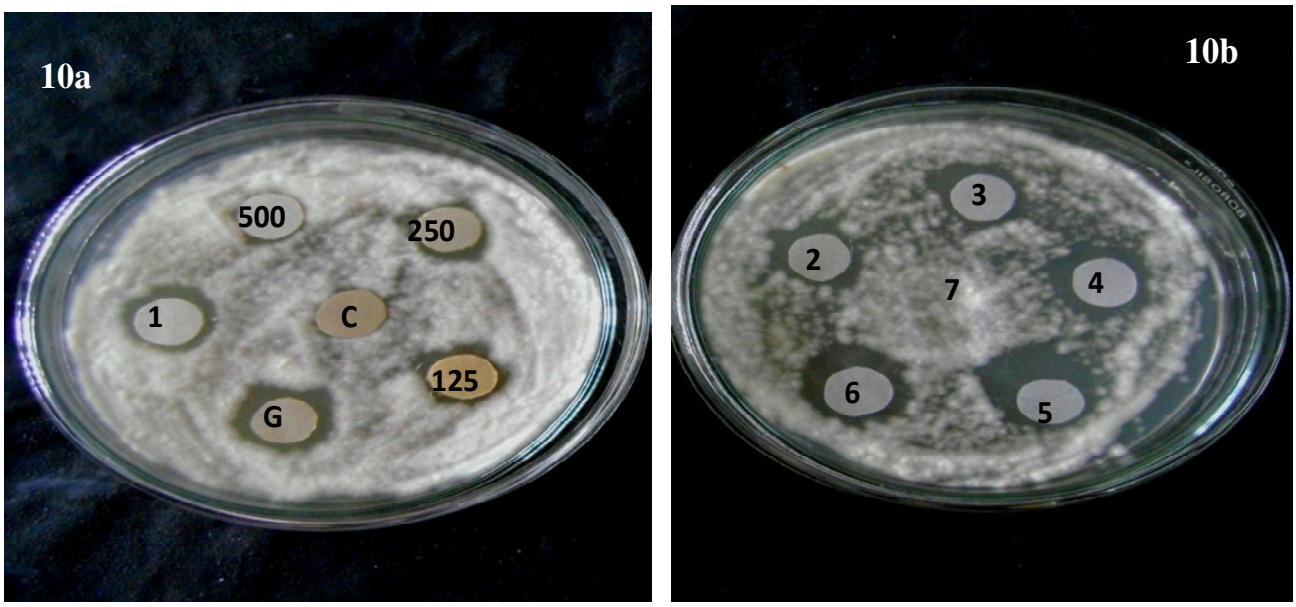

Figure 10. a) Showing MIC (125 ppm) b) Inhibition zone of *M. fulvum* by disc diffusion method, C is indicative of Control, G-Griseofulvin, 1-7: 1000 to 7000 ppm concentration of extract.

drug Griseofulvin. The MIC, which is the concentration giving to the last inhibitory activity and below which there is no further inhibition. It was taken as the concentration given to the lowest possible zone of inhibition. The MIC of antifungal component on *M. canis, M. fulvum* and *T. mentagrophytes* was recorded 125 ppm for chloroform and ethanol leaf (Figures 9a, 10a and 11a) extracts (Table 4). The mean growth of inhibition zone (mm) with standard error is presented in Table 2. Analysis of variance showed highly significant variation at 5, 1 and 0.1% levels for *T. mentagrophytes* in ethyl alcohol leaf extract. Values are highly significant at 5, 1 and 0.1% levels for *M. canis* and *M. fulvum* and significant at 5 and 1% levels for *T. mentagrophytes* in ethyl acetate leaf extract. For *T. mentagrophytes* values are significant at 5 and 1% levels in chloroform leaf extract. Calculated values of ANOVA for different fungi in different solvents are also shown (Table 3).

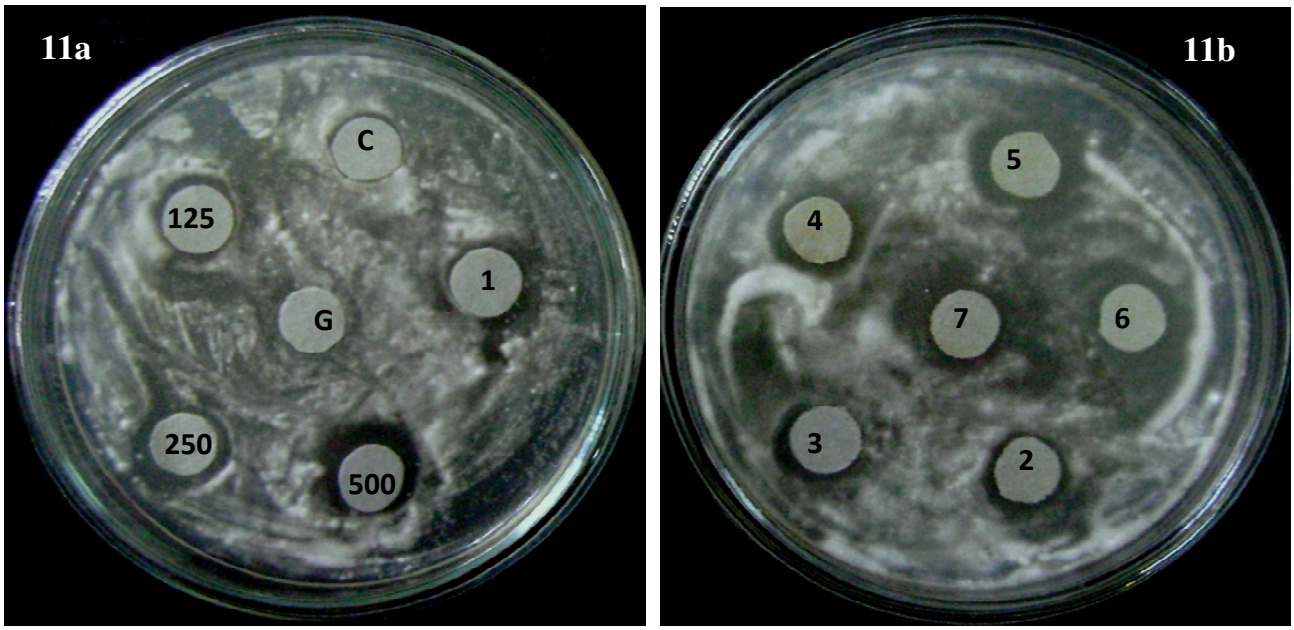

Figure 11. a) Showing MIC (125 ppm) b) Inhibition zone of *T. mentagrophytes* by disc diffusion method, C is indicative of Control, G- Griseofulvin, 1-7: 1000 to 7000 ppm concentration of extract.

Table 3. Calculated values ANOVA between zone diameter (mm) of fungal spp. and concentrations.

Fungal species	F Value of microbes in different solvent extracts			
	PE	CHL	ETOAC	ETOH
M. canis	-	0.09	498.3***	0.507
M. fulvum	-	0.19	11.79**	0.648
T. mentagrophytes	-	7.96**	6.55**	209.62***

***, significant at 5, 1 and 0.1% levels, **, significant at 5 and 1% levels.

Table 4. Minimum Inhibitory concentration for test microbes in ppm.

Test organism	MIC for test organism (ppm)
M. canis	125
M. fulvum	125
T. mentagrophytes	125

DISCUSSION

However, some reports are available on the antimicrobial activity of *C. procera*. Recently, the antimicrobial activity of stem, leaves and flowers of *C. procera* was checked in hexane, chloroform and methanol extract against *Alternaria alternate, Aspergillus flavus, Aspergillus niger, Bipolaris bicolor, Curvularia lunata, Pencillium expansum, Pseudomonas marginales, Rhizoctonia solani, Ustilago maydis* by agar well diffusion method (Vadlapudi and Naidu, 2009). The antifungal activity of leaf extract (petroleum ether, chloroform, methanol and water) of *C. procera* (100 mg/ml concentration) against *Candida albicans* and *A. niger* has been assessed by well plate diffusion method (Suvarna and Patil, 2009). The antimicrobial activity of apical twig and 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). Kareem et al. (2008) reported the antimicrobial effect of ethanol, aqueous and chloroform extracts of leaf and latex on six bacteria and three fungi namely *A. flavus, A. niger* and *Microsporum boulardii* and one yeast *C. albicans*. The results of this study indicate that ethanol was the best extractive solvent

followed by chloroform and water extracts. The MIC for the ethanol extract was between 5 to 20 mg/ml for fungi. Similarly, the strong inhibitory effect of aqueous extracts of *C. procera* stem, root and leaves has been demonstrated on the test microorganisms *A. niger, Microsporum gypseum* and *Tricophyton rubrum* (Hassan et al., 2006). Kuta (2006) observed the inhibitory effect of methanolic crude extract against *M. canis* and *T. rubrum* at 5.0 mg/ml concentration by well diffusion method. Kuta (2008) again observed inhibition of aqueous extract of stem bark of *C. procera* against *Epidermophyton flocosum* and *Tricophyton gypseum* in a concentration range of 1 to 5 mg/ml. Rai and Uadhyay (1988a) screened 19 medicinal plants against *T. mentagrophytes* and reported 50 to 75% inhibition in the growth of *T. mentagrophytes* with the treatment of leaf and stem extracts of *C. procera*.

These observations, therefore, support the use of *C. procera* in herbal cure remedies. The demonstration of antifungal activity of *C. procera* leaf extract against fungal species may be an indicative of the presence of broad spectrum antibiotic compounds. The mechanism of action of the constituents of *C. procera* could be by inhibition of fungal cell wall, protein amino acid, sphingolipid biosynthesis and electron transport chain. However, it is important to note the crude extract of *C. procera* leaf, need to be further purified through bioactivity guided fractionation to isolate and identify the compound responsible for antifungal activity.

Conclusion

The remarkable fungicidal effects of *C. procera* leaf extract suggest that leaves may be a useful source for the development of novel antifungal agent against pathogenic fungi.

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