

Inhibitory effect of extracts of latex of *Calotropis procera* against *Candida albicans*: A preliminary study

Nosocomial fungal infections due to *Candida* species are an important cause of morbidity and mortality especially in immunocompromised patients. The use of available treatment options for invasive mycoses is limited due to limited spectrum of activity, drug resistance, toxicity and drug–drug interactions.^[1,2] In view of this, there is a need to develop more effective and less toxic agents for the treatment of common, as well as drug resistant fungal infections.

The latex of the plant, *Calotropis procera* has been reported to possess *in vitro* larvicidal and anthelmintic activities. Different parts of the plant also exhibit antimicrobial and cytostatic properties.^[3] In the present study the activities of different extracts prepared from the latex of *Calotropis procera* have been evaluated against *Candida albicans*.

The latex collected from the aerial parts of the plant was dried (DL), soxhlated successively with petroleum ether (PE), methanol and water and dried under vacuum and dissolved in sterile water (methanol and aqueous extract) and 0.05% Tween-20 (PE extract). The standard antifungal drugs with low, moderate and high efficacy against *C. albicans* namely, griseofulvin, clotrimazole and nystatin were dissolved in 1% di-methylsulfoxide. The anti-candidial activity of these extracts

was tested by the Cup-plate method using Potato Dextrose Agar as growth medium.^[4] A previously liquefied medium was inoculated with *Candida albicans* suspension (100:1) at 35–40 °C and 25 ml of this inoculated medium was dispensed into 8.5 cm Petri plates. After complete solidification, holes were made aseptically with a 6 mm sterile cork borer and 0.1 ml of the test solution as well as standard drugs were poured in it. The plates were incubated at 25–27 °C for 72 h and the zone of inhibition was measured. The activity of the extracts was compared with respective solvents and standard antifungal drugs such as griseofulvin, clotrimazole and nystatin. The activity index was expressed as the ratio of zone of inhibition obtained with test drug and clotrimazole, a widely used anticandidal drug. The minimum inhibitory concentration (MIC) of the extracts was determined following incubation for 48 h.^[5]

The values are given as mean \pm SEM and $P < 0.05$ was considered statistically significant. The data were analysed by one-way ANOVA followed by *post hoc* test (LSD) using SPSS (Version 10).

Both petroleum ether and methanol extracts produced significant increase in the zone of inhibition compared to griseofulvin that was found to be ineffective. The activity in-

Table 1

Inhibitory effect of fractions of latex of *Calotropis procera* and standard anti-fungal drugs against *Candida albicans*

Treatment		Zone of inhibition (mm)			MIC mg/ml
		1 mg/ml	10 mg/ml	20 mg/ml	
DL of <i>Calotropis procera</i>					
	Petroleum ether extract	8.63 ± 0.69 (0.72)	12.23 ± 0.43** (0.77)	18.18 ± 1.62* (0.76)	128
	Methanol extract	9.15 ± 0.52** (0.77)	11.91 ± 0.80** (0.75)	17.51 ± 0.69** (0.73)	128
	Aqueous extract	3.16 ± 0.46 (0.26)	4.55 ± 1.10 (0.28)	7.05 ± 1.60 (0.29)	1600
Standard drugs					
	Griseofulvin	4.70 ± 0.30 (0.39)	4.93 ± 0.61 (0.31)	7.93 ± 0.52 (0.33)	800
	Clotrimazole	11.86 ± 0.94**	15.78 ± 1.40**	23.86 ± 2.70	2
	Nystatin	13.86 ± 0.52** (1.16)	19.46 ± 0.86** (1.23)	30.86 ± 1.37** (1.29)	1
One-way	F	59.52	78.12	193.80	
ANOVA	P	<0.001	<0.001	<0.001	

MIC: Minimum inhibitory concentration. df=5,12; Values given in parentheses are activity index with respect to clotrimazole. * $P < 0.01$ Vs griseofulvin; ** $P < 0.001$ Vs griseofulvin.

dex of these extracts ranged from 0.72 to 0.77 against 0.31 to 0.39 in case of griseofulvin. The inhibitory effect of these extracts was comparable to clotrimazole. The zone of inhibition at 20 mg/ml concentration was 18.18 ± 1.62 , 17.51 ± 0.69 and 23.86 ± 2.70 mm with petroleum ether extract, methanol extract and clotrimazole, respectively. The aqueous extract was ineffective in this regard. Nystatin was found to be more effective against *Candida albicans* with an activity index ranging from 1.16 to 1.29 and a zone of inhibition of 30.86 ± 1.37 mm at 20 mg/ml concentration. The respective solvent controls did not show any anticandidal activity. Further, the MIC of petroleum ether and methanol extract was found to be 128 $\mu\text{g/ml}$ and that of aqueous extract was 1600 $\mu\text{g/ml}$. The MIC of nystatin, clotrimazole and griseofulvin was 1, 2 and 800 $\mu\text{g/ml}$, respectively. (Table 1)

Our study is the first of its kind that demonstrated the anticandidal activity of the extracts of DL of *Calotropis procera*. Such a property could be related to the presence of enzymes and stable cysteine proteases in the latex.^[6] Further, the anticandidal activity of latex suggests that it might be effective against other fungal strains as well.

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