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Antifungal Activities of the Leaf Extract of *Clerodendrum infortunatum* Retz

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Abstract: The antifungal activity of the dealcoholized extract of the leaves of *Clerodendrum infortunatum* Retz. was determined on four different fungal organisms. The crude leaf extract significantly inhibited the growth of *A. niger*; *P. frequentance*; *P. notataum* and *B. cinera* when tested by turbidity and spore germination methods in concentration dependent fashion. The effects produced by the extract were compared with a standard antifungal agent.

Key words: *Clerodendrum infortunatum* Retz · Antifungal activity · *Aspergillus niger* · *Penicillium frequentance* · *P. notatum* and *Botrytis cinera*

INTRODUCTION

Clerodendrum infortunatum Retz. (Verbenaceae), commonly known as "Bhandira" in Sanskrit, "Bhant" in Hindi and "Bhari chuda" in Bengali is found throughout the India and is popularly known for its medicinal values [1-4]. Leaves and roots of this plant have been reported to be useful as antiperiodic, vermifuge, laxative and cholagogue [5-6]. The leaves are also used externally for treating tumors [7] and certain skin diseases. The fresh leaf juice can be as an injection given into rectum for ascardis. Sterol xanthophylls and carotene have been isolated from the leaves [8]. A configuration at C-24 of 24-alkylsterols was isolated from leaves and stem [9]

In view of its reputed use in treating skin diseases, the effect of dealcoholized extract of leaves of *C. infortunatum* Retz. was tested against four different fungal pathogens.

MATERIALS AND METHODS

Plant Material: The plant material (*C. infortunatum*) were collected from Manesar, U.P. The sample was authenticated by Taxonomist of National Botanical Research Institute, Lucknow (India).

Preparation of Leaf Extract: Powdered leaves (200 gms) was extracted with 1000 mL of 90% (v/v) ethanol. The extract was filtered and the filtrate was dried under vacuum on a water bath at 50°C to constant weight. A stock solution containing 300 µg/mL of this dealcoholized extract was made with sterile water containing propylene glycol. A solution of griseofulvin (1000 ppm) was prepared by dissolving in propylene glycol and diluted with water to be used as a standard. Chemical constitution of the leaves was estimated by AOAC method.

Media Used: Solid agar and liquid culture media "C" and "D" as specified in the India Pharmacopoeia was used for turbidity and spore germination methods.

Test Organisms: Test organisms were pure fungal cultures of *Aspergillus niger*; *Penicillium frequentance*; *P. notatum* and *Botrytis cinera*.

Method of Testing (Turbidity Method): This was performed by tube dilution technique. A series of Borosil make test tubes (15 x 120 mm), containing sterile culture medium and various concentrations of extract 100, 200 and 300 µg/mL, griseofulvin (1000 ppm) and one control (test tubes for each) was taken. All tubes were inoculated with fungal organisms to be tested and then incubated at 20-30°C (for 48 hrs). Turbidity produced was measured thereafter against a blank.

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Antifungal Assay: For assaying the antifungal activity of the extract by this spore suspension of 7 days old were prepared with the test compound. A drop of spore suspension was placed on a sterilized slide and incubated in humid chamber for 12 hrs and a number of spore germination were by the following formula:

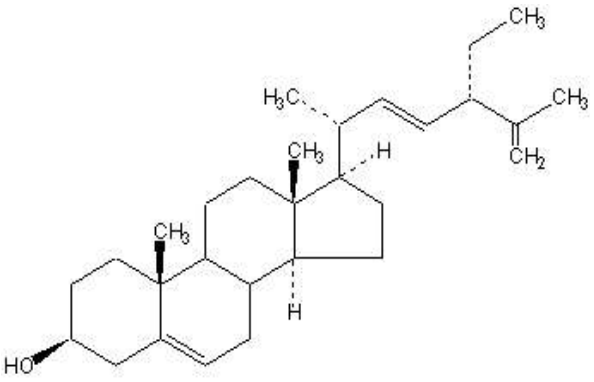
$$\% \text{ of spore germination incubation} = 100 - \frac{(\% \text{ spore germination in treated})}{\% \text{ spore germination in control}} \text{X } 100$$

RESULTS AND DISCUSSION

Analysis of the leaves showed the presence of ash (8.0), protein (21.2), crude fiber (14.8), reducing sugar (3.0) and total sugar (17.0) percent, respectively.

From the petroleum ether extract (3.85%) of the air dried powder of leaf, a bitter principle, clerodin, $C_{13}H_{18}O_3$, was isolated (Manzoor-i-Khuda, 1966; Barton *et al*. 1961). Leaves contained a fixed oil which consists of glycerides of lenolenic, oleic, stearic and lignoceric acid. The leaves also contained proteinase and peptidase. The results obtained were compared with the work already done by the previous workers [10].

The extract at concentrations of 100, 200 and 300 µg/mL showed the widest spectrum activity against all the organisms tested during spore germination (Table 1) and turbidiy tests. The growth inhibitory responses of the extract at concentration of 100, 200 and 300 µg/mL were obtained by the turbidity and spore germination method by comparing with that of griseofulvin (1000 ppm), a standard antifungal agent. The extract 300 µg/mL was 68.83%, 65.47%, 63.38% and 66.21% active with respect to griseofulvin when tested against *Aspergillus niger*; *Penicillium frequentance*; *P. notatum* and *Botrytis cinera* respectively as shown by the turbidity method. The extract showed significant inhibitory responses against all the organisms tested in the order of *Aspergillus niger* > *Penicillium frequentance* > *Botrytis cinera* > *P. notatum* in a concentration on dependent fashion both spore germination and turbidity tests.



Alkylsterol (22E,24S)-24ethylcholesta-5,22,25-trien-3β-ol) isolated from the leaves [11]

Table1: Antifungal Activity of *C. infortunatum* leaf extract by spore germination method

Treatment Extract (µg/ mL)	*A. niger*	*P. frequentance*	*P. notatum*	*B. cinera*
100	81.26±11.6	71.26±10.8	68.42± 8.6	70.43± 10.5
200	88.63± 9.6	76.09± 8.6	74.16± 10.8	75.86± 9.6
300	96.36± 12.3	90.68± 11.5	89.20± 10.6	89.62± 9.5
Griseofulvin (100 µg/ mL)	98.68± 9.8	88.58± 12.5	86.93± 10.8	88.56± 10.8
Control	0.0	0.0	0.0	0.0

[a]P < 0.001 comparing with control by student's t-test (n = 10)

Table 2: Inhibitory responses of *C. infortunatum* extract on various organisms (using turbidity method)

	Griseofulvin Extract					
Test Organism	Control	(1000 ppm)	100 µg/mL	200 µg/mL	300 µg/mL	300 µg/mL with respect to griseofulvin
A. niger	2.92±0.16	1.08±0.18	2.09±0.3	1.98±0.16	1.65±0.21	68.83±1.2
P. frequentance	2.95±0.9	1.23±0.14	2.44±0.32	2.34±0.26	1.89±1.80	65.47±2.8
P. notatum	2.88±0.14	1.37±0.17	2.54±0.25	2.42±0.17	2.12±0.16	63.68± 1.7
B. cinerea	2.86±0.23	1.45±0.13	2.68±0.23	2.52±0.15	2.42±0.12	66.21±1.6

p<0.001, p < 0.001, p <0.05 comparing with student's t-test (n = 10)

From the above observations it can be suggested that the dealcoholized extract of the leaves from *Clerodendrum infortunatum* served an effective antifungal agent when tested *in vitro*. This provides some evidence of the leaves being used in treating various skin diseases.

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