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Antidermatophytic Effects Of Crude Leaf Extracts Of *Alseodaphne Andersonii*

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ABSTRACT

Leaf extracts of *Alseodaphne andersonii* extracted with petroleum ether, chloroform, acetone and methanol were screened for antidermatophytic activity against various pathogenic fungi namely *Epidermophyton floccosum* var *nigricans*, *Microsporum canis*, *Microsporum gypseum*, *Trichophyton rubrum*, *Sporothrix schenckii* and *Aspergillus fumigatus*. Acetone extract showed moderate antidermatophytic activity against tested organisms as reflected by the zone of inhibition, while methanolic extract showed a significant control of growth of *Sporothrix schenckii*, *Trichophyton rubrum* and *Epidermophyton floccosum* var *nigricans*. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of methanolic extract were determined against *Sporothrix schenckii* and *Trichophyton rubrum* only. On the basis of above results we can conclude that leaf extracts of *Alseodaphne andersonii* is potentially active against dermatophytes.

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KEYWORDS

Dermatophytosis;
Methanolic extract;
Mycosis;
Skin disease.

INTRODUCTION

Alseodaphne, a genus of medium sized tree of the wet evergreen tropical forests belonging to family lauraceae, distributed from Yunan to Malaysia and also occurs in India, some of which yield valuable

timber. *Alseodaphne andersonii* is a tree of moderate size and widely distributed within tropical belt^[1]. Although there are reports of this plant being a source of furniture and isolation of some compounds from this species has also been reported, but there has been no evaluation in reference to its medicinal use.

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It is infact totally unexplored species and systemic phytochemical investigation can lead to isolation of biologically active compounds. Lee *et. al.*, (1994) had isolated lactones from *A. andersonii*. Lactones are reported to be antimicrobial in nature^[2]. So with this fact in mind the present study has been designed to explore antidermatophytic principles of *Alseodaphne andersonii*. Present communication involves the investigation on the efficacy of the plant extracts against various organisms responsible for skin infections. It is hoped that this study might lead to the discovery of new compounds that could be used to formulate new and more potent antimycotic drugs that might over come the problem of resistance to the currently available antimycotic agents.

EXPERIMENTAL

Collection of Plant material

The fresh leaves of plant were collected from the botanical garden of Forest Research Institute (F.R.I.) Dehradun, India. *Alseodaphne andersonii* leaves were identified and authenticated by Dr.H.C.Pandey, Taxonomist, Botanical Survey of India. Voucher specimens (AA-29) were prepared and stored in the department of Pharmaceutical sciences, S.B.S (P.G) Institute of Biomedical Sciences & Research, Balawala, Dehradun (U.A.), India.

Preparation of extracts

The 500g air-dried leaves were powdered and extracted in Soxhlet assembly with solvents of increasing polarity (petroleum ether, chloroform, acetone, methanol). The extracts were concentrated under reduced pressure in rotary evaporator and yielded 6.25%, 3.0%, 3.5% and 4.0%(w/w) respectively. The material was stored in clean glass bottles for further qualitative analysis and carrying antidermatophytic activity.

Test organisms

The test organisms used for the screening of antidermatophytic activity were *Epidermophyton floccosum* var. *nigricans* MTCC 613, *Microsporium canis* MTCC 2820, *Microsporium gypseum* MTCC 2819, *Trichophyton rubrum* MTCC 296, *Sporothrix schenckii*

MTCC 1359 and *Aspergillus fumigatus* MTCC 2483. These microorganisms were obtained from Institute of microbial technology (IMTECH), Chandigarh, India. The organisms were maintained on Sabouraud agar slant at 4°C and activated prior to use.

Preliminary phytochemical analysis

The plant material was screened for the presence of some biologically active substances including glycosides, proteins, alkaloids and steroids using the methods described by Odebiyi^[3].

Antidermatophytic activity

The disc diffusion method used to determine the *in vitro* antidermatophytic activities of the extracts^[4]. The cultures were sub cultured in modified Emision Sabouraud and Czepecdox agar and incubated at 27°C-35°C for 72-120 hr. From these plates the spore suspension was prepared containing 10⁵ spore/ml. 100µl of suspension was plated on agar medium. Sterile 6 mm diameter filter paper disc were prepared and allowed to soak and absorb the extract of known concentration for 24 hrs before draining off the excess and drying in the oven at 60°C^[5]. These disc were placed on the agar plates against the control (solvents). Plates were incubated at 25-37°C for 72-120 hrs and observed for the zone of inhibition. Disc diameter (6 mm) was deducted while recording the zone size .The test was conducted in triplicate.

Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) of methanolic extract (0.0060-50 mg/ml) of leaves was evaluated by dilution method against *Sporothrix schenckii* and *Trichophyton rubrum* only. The MIC of methanolic extract was compared with a standard fluconazole. Tubes were incubated at suitable temperature for 72 - 120 h. The tubes were observed for appearance of any growth. The MIC was interpreted as the lowest concentration of the extract that did not permit any visible growth (no turbidity) when compared with that of the control tubes.

Minimum fungicidal concentration

Minimum fungicidal concentration (MFC) was determined by sub culturing methods^[6]. Subcultures made from sample obtained from those test tubes

TABLE 1: Antidermatophytic activity of leaf extracts of *Alseodaphne andersonii*.

Organism	ZONE OF INHIBITION (mm) *			
	Petroleum ether extract	Chloroform extract	Acetone extract	Methanolic extract
<i>Sporothrix schenckii</i>	10	11	25	34
<i>Microsporum gypseum</i>	10	13	23	27
<i>Microsporum canis</i>	9	9	23	25
<i>Trichophyton rubrum</i>	8	12	25	30
<i>Epidermophyton floccosum</i> var. <i>nigricans</i>	12	12	24	29
<i>Aspergillus fumigatus</i>	10	11	21	25

* Zone size excludes the disc diameter (6 mm)

which showed no visible turbidity or growth in MIC assays, were made on freshly prepared Sabouraud's agar plates. After 72h incubation, the MFC was regarded as the lowest concentration of the extract that did not permit any growth on the agar plate surface used.

RESULTS AND DISCUSSION

The leaf extracts (petroleum ether, chloroform, acetone and methanol) were subjected to qualitative analysis for the presence of various phytoconstituents. Acetone and methanolic extracts showed the presence of alkaloids, glycosides, proteins and amino acids while petroleum ether and chloroform extracts showed the presence of steroids. Among all the extract, acetone extract exhibited moderate activity while methanolic extract revealed prominent effect on fungi like *Sporothrix schenckii* with zone of (25, 34 mm), *Microsporum gypseum* (23, 27mm), *Microsporum canis* (23, 25mm), *Trichophyton rubrum* (25, 30 mm), *Epidermophyton floccosum* var. *nigricans* (24, 29 mm) and *Aspergillus fumigatus* (21,25 mm) respectively (TABLE 1).

The MIC and MFC of methanolic extract of

TABLE 2: Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of methanolic extract of leaves of *Alseodaphne andersonii*.

Organism	Methanolic extract	
	M.I.C. (mg/ml)	M.F.C. (mg/ml)
<i>Trichophyton rubrum</i>	0.786	12.288
<i>Sporothrix schenckii</i>	0.384	6.144

Results were compared with standard fluconazole.

leaves against *Sporothrix schenckii* was found to be 0.384 and 6.144 mg/ml and for *Trichophyton rubrum* 0.768 and 12.288 mg/ml respectively (TABLE-2).

CONCLUSION

On the basis of results it can be concluded that leaf extracts of *Alseodaphne andersonii* showed remarkable antidermatophytic activity against a number of pathogenic fungi responsible for severe skin infections. So further research is required for purification of the active principles and elucidation of their nature and structures. This study might lead to the discovery of new compounds that could be used to formulate new and more potent antidermatophytic drugs that might overcome the problem of resistance to the currently available antimycotic agents.

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