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Evaluation of antidermatophytic property of lichen: *Flavoparmelia caperata* (L) Hale

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ABSTRACT

Dermatophytosis or cutaneous mycoses caused by the filamentous, keratinophilic fungus viz. *Trichophyton*, *Microsporum* and *Epidermophyton*. Dermatophytosis is amongst the most prevalent disease around the globe. In India, there are significant increase in the number of cases of resistant and recurrent dermatophytic infection. In present study, the antidermatophytic activity of a lichen *Flavoparmelia caperata* (L) Hale (Family: Parmeliaceae) was investigated against three dermatophytosis causing fungus viz., *M. canis*, *T. mentagrophytes* and *T. rubrum*. Lichen thallus was collected from the Chakrata district, Uttarakhand, India. The susceptibility of aforementioned pathogens were evaluated via Clinical Laboratory Standard Institute (CLSI) recommended broth microdilution method and the results were calculated in terms of Minimum Inhibitory Concentrations (MICs). The antidermatophytic activity of *F. caperata* acetone extract was compared to the synthetic drug Fluconazole. *F. caperata* extract inhibited the growth of *M. canis*, *T. mentagrophytes* and *T. rubrum* at 0.556±0.03, 1.014±0.06 and 0.525±0.03mg/ml respectively and the total activity was reported as 0.305, 0.167 and 0.323 ml/g respectively. Although, usnic acid is the one of the characteristic compound produced by the *F. caperata* having hepatotoxic effect when administered orally so, the topical application might be the safe option for the treatment of dermatophytosis, but before that in vivo potency and efficacy must be studied.

KEYWORDS: Dermatophytosis; *Flavoparmelia caperata*; Lichen; Minimum Inhibitory Concentration (MIC).

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INTRODUCTION

Human dermatophytes are the keratinophilic fungi which causes cutaneous mycoses or dermatophytosis and are categorized into *Trichophyton*, *Microsporum* and *Epidermophyton*[1]. Till date around thirty species of human dermatophytes are known to us[2]. Cutaneous mycoses is a prevalent disease and surveys conducted in sixteen countries of Europe exhibited that 35-40% of individuals were suffering from tinea pedis while another survey conducted among the childrens' of US exhibited that around 22-55% childrens' were suffering from hair scalp dermatophytic infection[3,4]. Rarely dermatophyte drug resistance had been reported for chemically synthesized first line drugs viz. fluconazole, griseofulvin and terbinafine[1,5-8]. In India, recurrence of dermatophytosis and increasing reports of drug resistant is a major concern[9]. In current scientific exploration the biological activity of lichens in terms of antidermatophytic activity was evaluated. Lichens are the consortium of mycobiont and photobiont in which major partner is mycobiont and produces many secondary compounds of biological importance. There are numerous activity reported from the lichen viz. antiviral, antioxidant, photoprotection, allelopathy, antifungal, antibacterial[10].

EXPERIMENTAL SECTION

Collection of lichen thallus and preparation of extract

Lichen thallus was collected from Koti, Chakrata district, Uttarakhand, India. Thallus was identified with the help of relevant key[11] as *Flavoparmelia caperata* (L.) Hale and was further determined by Dr. D.K. Upreti, CSIR-NBRI, Lucknow. The voucher specimen was submitted to Central Regional Centre, Botanical Survey of India, Allahabad, Accession No.: 8764. Two gram of thallus was weighed and subjected to cold extraction in acetone as solvent. Crude extract was obtained via vacuum drying using rotary evaporator[12]. Percent yield was calculated using formula:

Percent yield (%) = (Dry weight of extract/Dry weight of sample) × 100

And the stock solution of extract was prepared in dimethyl sulphoxide (DMSO) as 50mg/ml[13]. Further, a synthetic drug Fluconazole, purchased from TCI Chemicals (India) Pvt. Ltd. was also purchased and a stock solution was made in equivalent concentration as that of extract.

Procurement of dermatophytes

Human dermatophytes viz., *Microsporum canis* (MTCC No. 3270), *Trichophyton mentagrophytes* (MTCC No. 7687) and *T. rubrum* (MTCC No. 296) were procured as live cultures from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India and the inoculum prepared according to 0.5 McFarland standard corresponding to 0.5 x 10^6 CFU/mL[14].

Antidermatophytic assay

Antifungal susceptibility test was performed on the recommended guidelines of Clinical Laboratory Standard Institute (CLSI) via broth microdilution method[15]. The broth used for the aforementioned test was RPMI-1640 medium supplemented with HEPES modification (Sigma Aldrich) and MOPS (3-morphollinopropane-1-sulfonic acid) buffer. Detailed steps involved were listed in Pathak et al.[16]. The tested concentrations for extract and drug are in between 1.25 to 0.009mg/ml. The results were expressed in terms of Minimum Inhibitory Concentrations (MICs) and was calculated based on optical density assay using SpectraMax Plus[384], Molecular Devices Corporation, USA at 530nm after the incubation of 96hours. The effect of coloured extract was nullified via subtracting the treated columns with the corresponding drug control ones[12].

Statistical analysis

An independent sample *T*-test was performed between the non-treated dermatophytes and fluconazole-treated dermatophytes; and between the non-treated dermatophytes and extract-treated dermatophytes for the equality of means via SPSSv20 software.

Total activity

It is a measure of degree of dilution of extract obtained from one gram of plant material and remain effective, inhibiting the growth of pathogens[17,18]. It depends upon the percent yield and MIC. Total activity = amount of extract obtained from 1g/MIC.

RESULTS AND DISCUSSION

Amount of crude extract obtained from the two gram of *F.* caperata thallus was 0.17g and the percent yield calculated was 8.5%. The antifungal efficacy of *F. caperata* extract was evaluated and found that it was most effective against *T. rubrum* (MIC = 0.525±0.03mg/ml) and least effective against *T. mentagrophytes* (MIC= 1.014±0.06 mg/ml) whereas the MIC obtained for *M. canis* was 0.556±0.03mg/ml. When compared to synthetic drug fluconazole similar pattern in the susceptibility

was observed amongst the human dermatophytes. Fluconazole was found highly effective against *T. rubrum* (0.135±0.02mg/ml) whereas least effective against *T. mentagrophytes* (MIC=0.353±0.01mg/ml). The MIC value for *M. canis* was 0.223±0.02 mg/ml. The efficacy was listed in terms of IC_{50} and MIC were listed in Table 1 and represented graphically in Fig 1.

Table 1: Antifungal activity of Fluconazole and *F. caperata* against tested human dermatophytes.

	Fluconazole		*F. caperata*	
	IC_{50} (mg/ml)	MIC (mg/ml)	IC_{50} (mg/ml)	MIC (mg/ml)
M. canis	0.123±0.02	0.223±0.02	0.036±0.03	0.556±0.03
T. mentagrophytes	0.041±0.01	0.353±0.01	0.137±0.06	1.014±0.06
T. rubrum	0.107±0.02	0.135±0.02	0.170±0.03	0.525±0.03

± Standard Error

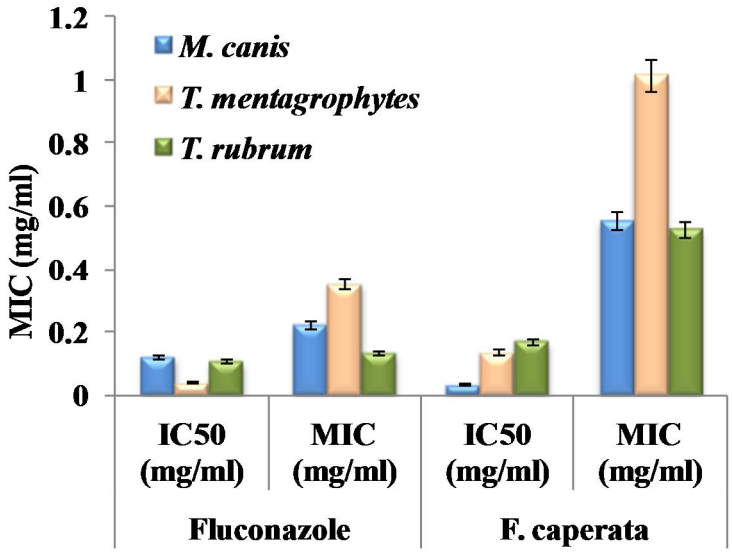

Fig 1: Minimum Inhibitory Concentration (MIC) values of Fluconazole and *F. caperata* against tested dermatophytes.

The p-value obtained for all the results are below 0.05 which exhibited that there is a significant variance in the means of treated and non treated pathogens. The total activity obtained for *F. caperata* were 0.305, 0.167 and 0.323ml/g for *M. canis*, *T. mentagrophytes* and *T.rubrum* respectively. *F. caperata* is a monospecific genera of lichen found in India[11]. It is a foliose, coticolous lichen and produces usnic acid, caperatic acid, protocetraric acid, atranorin and unknown substances[19]. Amongst all the compounds produced by the *F. caperata*, usnic acids are widely known for their biologically activities viz. antibacterial, antifungal, antifeedant, antiprotozoal, anti-inflammatory, antipyretic,

analgesic and many more[20]. In current study, *F. caperata* exhibited the promising antifungal activity against all three most prevalent species of human dermatophytes. As usnic acids are produced by the *F. caperata* and the toxicity of usnic acid have been reported by Durazo et al.[21], usnic acid causes liver failure when administered orally. So, the topical treatment of *F. caperata* extract might be a good option for the treatment of human dermatophytosis. *F. caperata* extract or in a combination with other drug can be used for the treatment of resistant or recurrent dermatophytosis but before that in vivo efficacy and potency has to be evaluated for the *F. caperata* extract or such combinations.

CONCLUSION

F. caperata exhibited promising antifungal activity against *M. canis*, *T. mentagrophytes*, and *T. rubrum* and have the potential to develop into new biological resource of commercial purpose for the treatment of dermatophytosis.

Ethical Issue: There is no ethical issue in present study.

Conflict of Interest: No conflict of Interest

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