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Reserach Article

ANTIMYCOTIC ACTIVITY OF FLOWER EXTRACT OF *CRATAEVA NURVALA* BUCH--HAM

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ABSTRACT

Dermal mycotic infections caused by superficial fungi are most prevalent disease of body surface. Dermatophytes comprising of three genera are responsible for these types of infections in human beings and other animals. The aim of present study was to evaluate the antimycotic activity of 50 % ethanolic extract of *Crataeva nurvala* (extracted by rotavapor process) using the technique of Broth Micro Dilution method, recommended by CLSI (NCCLS). The activities were analysed in units of MIC having 1.511 and 1.981 mg/ml for *Trichophyton mentagrophytes* and *Microsporum fulvum* respectively. The microbial activity of the *Crataeva nurvala* was due to the presence of various secondary metabolites. Further studies will to helpful to isolate the active compounds from those extracts with fungicidal potential.

INTRODUCTION

Skin is the important organ of body, covers the entire body surface and constitutes 15-20 percent of body weight. It performs so many functions including protection, homeostasis and sensation, and hence called 'jack of all trades'. A few microbes can penetrate unbroken skin and skin infection occur wherever the skin is broken, in ducts of sebaceous and sweat glands and hair follicles. Mycotic infection is the most common cause of skin infection in tropical developing countries. The incidence of dermatophytoses or cutaneous mycoses raised dramatically in the past one decade. Humid weather, over population and poor hygiene are the ideal conditions for the growth of dermatophytes (Vaijayantimala *et al*., 2001). Dermatophytes are a group of closely related keratinophilic fungi that can invade stratum corneum of the epidermis and keratinised humans and animals tissues such as skin, hair and nails causing dermatophytosis (Kumar *et al*., 2016).

The disease caused by fungi known as dermatophytes. Dermatophytes consist of three genera such as *Trichophyton*, *Microsporum* and *Epidermophyton*. They cause fungal infections of the epidermis and dermis that evoke an inflammatory reaction in the host, whereas superficial mycoses only involve the superficial stratum corneum, thereby not causing a host response. Dermatophytic infections are commonly referred to by the region of the body that they inhabit (Kumar *et al*., 2015).

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Most of the dermatophytoses in the United States involve *Trichophyton* species (tinea capitis, tinea barbae, tinea corporis, tinea cruris, tinea pedis, and tinea unguium), although some are also caused by organisms of the *Microsporum* and *Epidermophyton* species (Hossain and Ghannoum 2001).

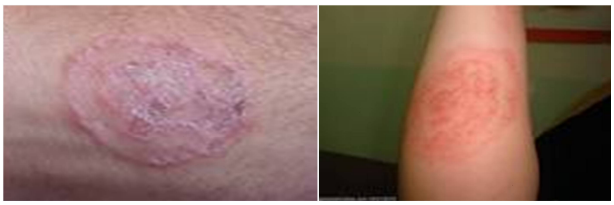

Fig 1 Tinea imbricata

Antimicrobial and antitoxin properties of some plants, herbs, and their components have been documented since the late nineteenth century. The natural plants involve garlic, ginger, neem, datura etc. They are not expensive and safe to human and the ecosystem than the chemical. In this context, the new antifungals of plant origin could be useful alternatives for the treatment of dermatophytoses, where a topical therapy is required. Keeping in view the reduced risk of side effects and lower cost, these natural compounds are preferred for human well being (Ghannoum *et al*., 2000). Therefore, in recent years, there has been growing interest in search of suitable medicinal plants for skin ailments. The numerous ethnic tribes in India have developed their own herbal remedies for all their diseases including the skin ailments.

MATERIALS AND METHODS

Selection of test pathogens

Two genera of pathogenic fungi i.e. *Trichophyton mentagrophytes* (MTCC-7687) and *Microsporum fulvum* (MTCC-7684) were selected in this study. They were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India.

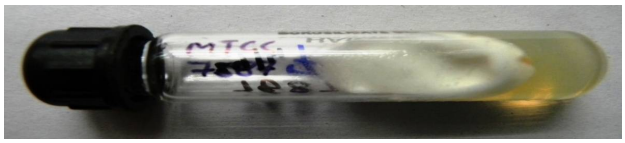

Fig 2a Pure Culture of *Trichophyton mentagrophytes* (MTCC-7687)

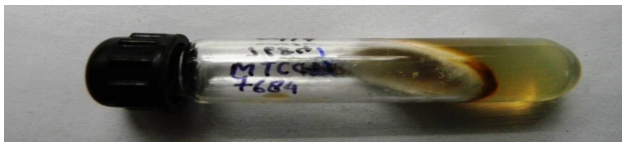

Fig 2b Pure culture of *Microsporum fulvum* (MTCC-7684)

Revival of cultures

Dermatophytes strains were revived on Sabouraud Dextrose Agar (SDA) petridishes supplemented with 0.5% streptomycin and incubated at 30°C for 7 days. The isolates were re- cultured again on same medium and incubated for another 5 to 7 days at 33°C.

Fig 3 petridish containing colony of (A) ***Trichophyton mentagrophytes,*** **(B)** ***Microsporum fulvum***

Preparation of herbal flower extracts

Collected flowers were washed with water and placed it on blotting paper for drying and left it in shade 72 hours for complete drying. After shade drying plant was chopped in to small pieces. 10 gram of chopped plant was soaked in 50 ml of 50% ethanol in a conical flask. The mouth of conical flask was wrapped using silver foil and left for 24 hours. The extract was filtered with Whatman filter paper No.1. The extract solution were evaporated by using rotary evaporator and stored in the deep freezer.

Experimental set up

Plant extracts: *Crataeva nurvala* (Capparidaceae) flower extract.

Common name: Varun, Varuna
Collected from: Department of Botany, University of Allahabad
These extracts were assayed against tested fungus viz.

- *Trichophyton mentagrophytes*
- *Microsporum fulvum*

Antifungal susceptibility assay using Broth Micro Dilution method

Broth micro dilution method was performed according to CLSI guidelines in flat bottom 96-well micro-titer plates. Here, RPMI 1640 medium (with L-glutamine but without sodium bicarbonate) was used as assay medium for viability of pathogens. 7- day old cultures of each pathogen were collected with an inoculating needle and suspended in sterile saline containing 0.1% Tween 80. After heavy particles were allowed to settle, cells (with spores) were transferred in 5 ml of RPMI1640. The turbidity of the full growing fungal suspension was compared and matched with the turbidity of 0.5 McFarland units. The McFarland 0.5 Standard corresponds approximately to a homogeneous suspension of $1.5x\ 10^6$ cells per ml. Serial dilutions were performed from rows 4 to 11, to obtain the final drug concentrations which ranged from 2.5 mg/ml to 0.020 mg/ml. Fungal inoculum of 100µl was added to each well except row number 2 and 3, making the final volume of 200µl (fig.4).

Quantification assay for growth of pathogens

After inoculation, the micro- titer plates were kept in a wet chamber and incubated for growth of pathogens at 33°C inside a B.O.D. Incubator. The Optical Density ($OD_{530\ nm}$) was recorded spectrophotometrically at every 24 hr, 48 hr and 72 hr (3 times). After the first 24 hr of incubation inside the plate reader, incubation of the Micro titre plates was continued under the same conditions in same incubator. The changes in OD over concentration of herbal extracts were used to generate growth inhibition curves at each drug concentration and for the drug-free growth control.

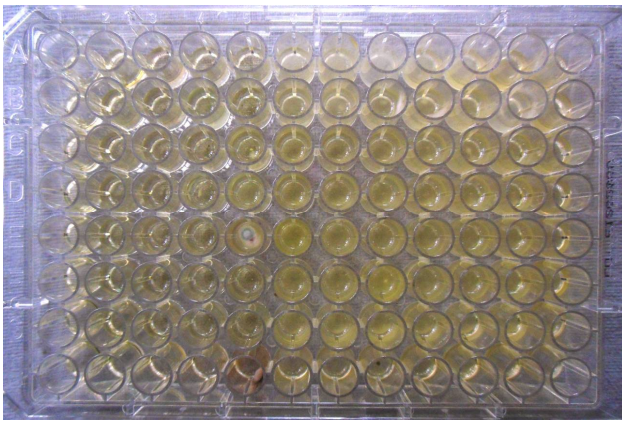

Fig 4 96 well microtitre plate used for Broth microdilution protocol

Determination of Minimum Inhibitory Concentrations (MICs)

The MICs were determined visually and spectrophotometrically after the above mentioned incubation periods for each species according to the CLSI guidelines. For herbal concentration, the MIC was determined as the lowest concentration showing absence of visual growth or 80% growth inhibition compared with the growth in the drug-free well. 50% growth inhibition compared to the growth in the drug-free well was determined as IC_{50}

RESULTS AND DISCUSSION

The broth micro dilution antifungal assay the plant sample was tested again *Trichophyton mentagrophytes* and *Microsporum fulvum.* The 50% ethanolic extract was found active against both pathogen *T. mentagrophytes* and *M. fulvum* with MIC range of 1.511 and 1.981 mg/ml respectively. The MIC difference clearly indicates the difference in activity of extract resulted due to more

susceptibility towards *T. mentagrophytes* than *M. fulvum* (table of IC-50 and MIC).

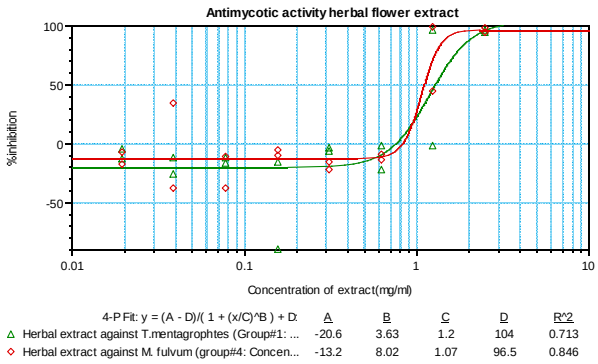

Fig 5 Graph showing of the antimycotic activity of flower extract

Table of IC_{50} and MICs (mg/ml) of flower extract against fungal pathogens (after 48 hr)

Dermatophytes	50% flower extract	
	IC50	MIC
T.mentagrophytes	1.180	1.511
M. fulvum	1.212	1.981

Though by increasing extract conc.in graph, the percentage inhibition was increased, yet in case of *T. mentagrophytes* it has been observed that with increase in concentration extract growth inhibition was also increased more than *M. fulvum* (fig.5).

The above mention results shows the effectiveness of flower extracts against *Trichophyton mentagrophytes* and *Microsporum fulvum* by broth micro dilution method. A wide range of anti fungal activity was found. Preliminary experiments were carried out in in-vitro using the RPMI-1640 medium by essential extract against *Trichophyton mentagrophytes* and *Microsporum fulvum* to measure the activity of extract on the basis of minimum inhibition concentration .The herbal extract (stock conc. 50mg/ml) exhibited a strong mold growth inhibition against Trichophyton *mentagrophytes* and *Microsporum fulvum.*

The flower extract was more against active *Trichophyton mentagrophytes* than *Microsporum fulvum,* in other words we can observe that *Microsporum fulvum* was less susceptible to extract. The need for a standard, reproducible method for microbial growth inhibition including bacteri has been also evaluated by several authors (Kumar *et al*., 2016). In views of this, many methods have been developed specifically for determining the antimicrobial activity of essential oils.

Extracts of leaves and stems of *Catharanthus roseus* showed good efficacy against *T. mentagrophytes.* Stem of *C. roseus* showed highest antifungal activity of water extract, whereas water extract of leaves reported less antifungal activity. Pandey *et.al*.,(2014) reported broad spectrum antifungal of lichen as a potential source of therapeutic agent. Others reported that the essential oil of *Curcuma domestica* was found strongest toxicant against the test fungus against *Epidermophyton floccosum* and *Microsporum gypseum* and *Trichophyton rubrum*; however, it was fungicidal (Lachoria *et al*., 2000). The presence of phenolic compounds in the experimented flower extract might be responsible for the antimycotic activity. Flavonoids are one of the most important classes of phenolic compounds which is associated with multiple features of chemical structures, responsible for antimicrobials and scavenging activity (Saeed *et al*., 2012, Qidwai *et al*., 2017). Additionally, investigations using more experimental paradigms particularly with isolated bioactive compounds may be required for further confirmation of antimycotic potential of other plant parts of *C. nurvala* for treatment of other skin diseases.

CONCLUSION

A broth micro dilution antimicrobial susceptibility testing was the most accurate method and considered a standard for determining the antimicrobial susceptibility of pathogens. In present study it was found that *Crataeva nurvala* flower extract was more effective against *T. mentagrophytes* but its activity is less effective on *M. fulvum* at 50mg per ml of stock concentration. The preliminary *in vitro* investigations reveals that the extract of herbal drugs, having an edge over some synthetic antifungal, can be used successfully in the form of broad spectrum herbal anti- dermatophytic agents. The commercial viability of the same can be determined after detailed *in vivo* as well as successful multi central clinical trials.

Acknowledgements

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