

# Search for Effective Antimycotic Agents against *Microsporium Gypseum* from 61 Ethno Medicinal Plants of Hyderabad, Karnataka Region, Karnataka, India

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## Abstract

The 61 ethno medicinal plants of Hyderabad Karnataka region belonging to 33 different families used in skin diseases were screened for their antidermatophytic properties. Screening was carried out at 5 and 2.5 mg/ml concentrations of pet ether, chloroform, ethylacetate, methanol and aqueous extracts of each plant by agar well diffusion technique against *Microsporium gypseum*. Out of 61 plants, 05 (*Ceasalpinia bonducella*, *Coccinia indica*, *Corchorus olerarius*, *Lawsonia inermis* and *Tridax procumbens*) showed very effective antidermatophytic activity in ethyl acetate, chloroform and in aqueous extracts, effective activity observed in 11 plants (*Achyranthes aspera*, *Allium sativum*, *Celosia argentea*, *Citrus medica*, *Curcuma longa*, *Embllica officinalis*, *Gymnosporia montana*, *Lycopersicon esculentum*, *Milletia pinnata*, *Ricinus communis*, *Zingiber officinale*) in different extracts, whereas 38 plants showed moderate activity, 07 plants (*Euphorbia tirucalli*, *Lantana camara*, *Mentha viridis*, *Tinospora cordifolia* and *Tridax procumbens*) showed weak activity. The minimum inhibitory concentrations of 05 very effective plants were determined. The potential minimum inhibitory concentrations of 0.15 mg/ml conc. were detected from *C. indica*. This study provides scientific base for the isolation and purification of antidermatophytic compound(s) from ethno medicinal plants.

**Keywords:** Traditional medicinal plants; *Microsporium gypseum*; Antifungal screening

## Introduction

Medicinal plants have been used in traditional treatment of skin diseases in worldwide [1], India [2] and Karnataka [3]. Herbal medicines are the basis of treatment and cure for various diseases in traditional methods practiced, such as Ayurveda, Unani and Siddha [4]. The rich availability and easy access to these medicinal plant resources have made them almost inevitable in the healthcare practices especially for those residing in the forest, hilly, rural and remote areas. Mycotic infections are the most common cause of skin infection in tropical developing countries. The incidence of dermatophytosis raised dramatically in the past one decade. Humid weather, over population and poor hygiene are the ideal conditions for the growth of dermatophytes [5]. These dermatophytes invade skin, hair and nail and cause dermatophytosis. Though these dermatophytes respond to treatment with conventional antifungal agents, the disease had a tendency to reoccur in the same area or other ones [4]. The most frequent fungal pathogens include the dermatophytes *Microsporium sp.*, *Trichophyton sp.*, and *Epidermophyton sp.* [6]. In the past decade, the incidence of dermatophytosis has risen dramatically. The humid weather, over population and poor hygienic conditions are ideally suited for the growth of dermatophytes and these factors are more important in country like India. Among the infectious diseases, diseases caused by fungal infections account for a larger proportion of health problems in humans [7]. Therefore, the present report focused on the antidermatophytic activity of petroleum ether, chloroform, ethylacetate, methanol and aqueous extracts of 61 plant parts against common dermatophytic fungi *Microsporium gypseum*.

## Materials and Methods

### Plant materials

Plant materials were collected from various localities of Hyderabad Karnataka region and Identified with the help of Gulbarga district flora

[8] the voucher specimens deposited in herbarium centre, Department of Botany, Gulbarga University, Karnataka, India. The collected plant materials were initially rinsed with distilled water to remove soil and other contaminants and dried on paper towel in laboratory at 37 ± 2°C for week.

### Preparation of the plant extracts

The selected plant materials after shade drying were ground in a grinding machine in the laboratory. 25 g of shade dried powder was weighed and extracted successively with petroleum ether, chloroform, ethyl acetate, and methanol and aqueous in soxhlet extractor for 48 h. The extracts were concentrated under reduced pressure and preserved in refrigerator in airtight bottles for further use.

**Extract stock solution:** Dissolved 50 mg of crude in 10 ml DMF with glass beads, vortex to homogenize and a two-fold serial dilution was prepared. As a precaution not to miss trace amounts of antifungals for screening, a relatively high concentration of 2.5 to 5 mg/ml of each extract was prepared for bioassays.

### Microbial culture and growth conditions

Test microorganism *Microsporium gypseum* used in the present study was obtained from M.R. medical college, Gulbarga, Karnataka,

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India. The Culture of *M. gypseum* grown on Sabouraud dextrose broth (HiMedia) at 28°C for 48 h and it was maintained on agar slants at 4°C.

### Inoculum preparation

Stock inoculum of *M. gypseum* strain was prepared from 10-day cultures in Potato Dextrose Agar at 28°C to induce sporulation. Fungal colonies were covered with 5 mL of sterile saline solution (NaCl 0.85% w/v), the surface gently scraped with a sterile loop and the resultant mixture of fungal units was then transferred to a sterile tube. The turbidity of the final inoculum was standardized according to a McFarland scale 0.5 tubes and adjusted to a fungal population of 10<sup>6</sup> Colony Former Units (CFU). The confirmation of inoculum quantification was done by plating 0.01 mL of inoculum suspension in Sabouraud Dextrose Agar (SDA). The dishes were incubated at 28°C and examined daily for the presence of fungal colonies which were counted as soon as growth became visible [8,9].

### Agar-well diffusion method

The assay was conducted by agar well diffusion method [10]. About 15 to 20 ml of potato dextrose agar medium was poured in the sterilized petri dishes and allowed to solidify. Fungal lawn was prepared using 5 days old culture strains. The fungal strains were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (10<sup>8</sup> CFU/ml). 1 ml of fungal strain was spread over the medium using a sterilized glass spreader. Using flamed sterile borer, wells of 4 mm diameter were punctured in the culture medium and required concentrations of serially diluted extract (2.5, 5 mg/ml) was added to the 20 µl to each wells. The plates thus prepared were left for diffusion of extracts into media for one hour in the refrigerator and then incubated at 30°C. After incubation for 48 h, the plates were observed for zone of inhibition. Diameter zone of inhibition was measured and expressed in millimeters. Dimethyl formamide (DMF) was used as a negative control. The experiments were conducted in triplicates. The obtained results classified into three groups on the basis of zone of inhibition i.e., very effective (above 12 mm), effective (10-11 mm), moderate (7-9 mm), weak (No zone of inhibition).

### Minimum Inhibitory Concentration

One ml of sterile liquid Sabouraud medium was added to 08 sterile capped tubes, 1 ml of each solvent extracts suspension was added to tube 1 [11]. The contents were mixed and 1 ml was transferred to tube 2. This serial dilution was repeated through to tube six and 1 ml was discarded from tube 6. 50 µl of inoculum was added to tubes 1-8 and the contents were mixed. Medium control (no inoculum and no drug) and inoculum control (no drug) tubes were prepared. The final concentrations of each plant solvent extracts ranged from 0.5 mg/ml to 0.15 mg/ml. The tubes were incubated at 30°C for 96 h. The fungal growth in each tube was evaluated visually depending up on the turbidity in the tubes. MIC was defined as the drug concentration at which the turbidity of the medium was the same as the medium control.

### Statistical analysis

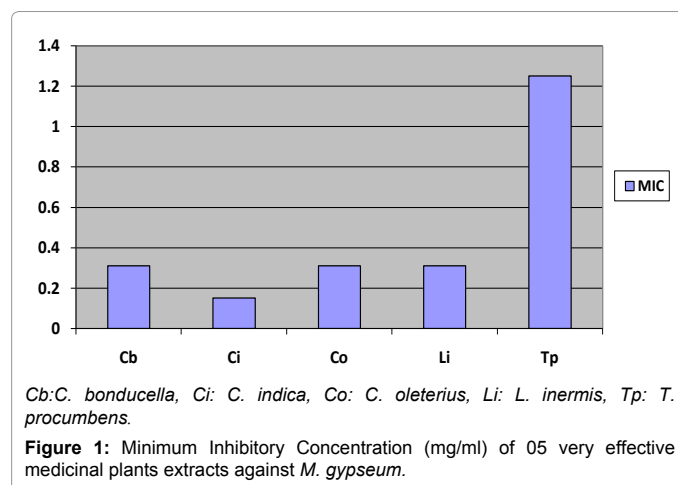
All the experiments were conducted in triplicate unless stated otherwise and statistical analysis of the data was performed by Analysis Of Variance (ANOVA), using STATISTICA 5.5 (Stat Soft Inc, Tulsa, Oklahoma, USA) software. A probability value of difference p~0.05 was considered to denote a statistically significance All data were presented as mean values ± Standard Deviation (SD).

## Results and Discussion

The 61 ethno medicinal plants of Hyderabad Karnataka region belonging to 33 different families used in skin diseases were screened for their antidermatophytic properties against *Microsporum gypseum*. The screening was carried out at 5 and 2.5 mg/ml concentrations of pet ether, chloroform, ethylacetate, methanol and aqueous extracts of each plant by agar well diffusion technique the obtained results were given in (Table 1). Out of 61 plants, 05 (*Ceasalpinia bonducella*, *Coccinia indica*, *Corchorus olerius*, *Lawsonia inermis* and *Tridax procumbens*) showed very effective antidermatophytic activity in ethyl acetate, chloroform and in aqueous extracts. The effective activity observed in 11 plants (*Achyranthes aspera*, *Allium sativum*, *Celosia argentea*, *Citrus medica*, *Curcuma longa*, *Embllica officinalis*, *Gymnosporia montana*, *Lycopersicon esculentum*, *Milletia pinnata*, *Ricinus communis*, *Zingiber officinale*) in different extracts, whereas 38 plants showed moderate activity, 07 plants (*Euphorbia tirucalli*, *Lantana camara*, *Mentha viridis*, *Tinospora cordifolia* and *Tridax procumbens*) showed weak activity. The negative control (DMF) was not showed activity, while the standard drug, Ketoconazole significantly inhibited ( $28.66 \pm 1.15$  to  $12.33 \pm 1.52$  mm) the growth of the test dermatophyte.

The minimum inhibitory concentrations of very effective 05 plants were determined. The 0.15 mg/ml of minimum inhibitory concentration was recorded with *C. indica* followed by 0.31 mg/ml conc. observed with three plants extracts i.e., *C. bonducella*, *C. olerius*, *L. inermis*. Whereas the weak 1.25 mg/ml minimum inhibitory concentration was observed with *T. procumbens* (Figure 1).

The response of *M. gypseum* to treatment with various plants extracts varied from solvent extract to extract. The ethyl acetate seed extract of *C. bonducella* was showed effective activity against test dermatophyte. This was supported by the previous work of Kavitha Sagar and Vidyasagar GM. Where they used ethyl acetate leaves extracts of *C. bonducella* [12]. Farukh and Iqbal reported antimicrobial activity using seed extract of *C. bonducella* [13]. In the present report *Lawsonia inermis* leaves showed very effective activity in chloroform extract it was correlated with past reports of Bhatnagar et al. [14], Misra and Dixit [15]. Whereas in another report the effective activity showed in ethanolic leaves extract against *T. rubrum*, *T. mentagrophytes* [16]. The ethanolic extract of the whole plant of *Lawsonia inermis* showed antifungal activity against *Trichophyton mentagrophytes* and *Microsporum canis* [17]. In the present study, the ethylacetate seed extract of *Corchorus olerius* showed an effective activity. Though no report is available on the antidermatophytic activity of *Corchorus*





*oleterius*. In this study at lower concentration of *Allium sativum* aqueous extract has not showed activity whereas the report of Sowjanya NC, and Manohara C Chary revealed the aqueous extracts of *Allium sativum* and *Ocimum sanctum* at 10% concentration were more pronounced antifungal properties against *Microsporum gypseum* [18].

In the present report the best MIC was obtained on *M. gypseum* with remarkable activity for ethylacetate leaf extract of *C. indica*. This is supported by the work of Narasimha et al., [19] where they reported effective antibacterial activity of floral petal extracts of *C. Indica*.

## Conclusion

Of the antimycotic activity of 61 ethno medicinal plants (05 very effective + 11 effective) showed maximum activity against *M. gypseum*. This may be attributed to the various phytochemical constituents present in the crude extracts. The purified components may have even more potency with respect to inhibition of dermatophytes. The work carried was a basic approach to find out the effective antimycotic agents from 61 medicinal plants. Further works on the types of phytoconstituents and purification of individual group (s) of bioactive compounds (s) can reveal the exact potential of the plants to inhibit skin pathogenic microbes.

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