Full Length Research Paper

Dermatophytes and related keratinophilic fungi isolated from the soil in Gwalior region of India and in vitro evaluation of antifungal activity of the selected plant extracts against these fungi

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Soil is rich in pathogenic and non pathogenic keratinophilic fungi including dermatophytes. Most of these fungal species have already been reported as dermatophytes causing infections of skin, scalp, hair, etc. The present study was undertaken to assess the prevalence of dermatophytes and related keratinophilic fungi isolated and characterized from different public park soils in the Gwalior region of India and to evaluate the antifungal potential of selected herbal extracts against seven dermatophytes. Two keratinous fragments, human hair and nails were used for the growth of fungi by the hair-baiting technique. 160 fungal samples were collected and cultured on Sabouraud dextrose agar, containing chloramphenicol and incubated at 25 to 27°C. Isolates were identified on the basis of colony characterization and the morphology of the fungal strains. Eight different fungal species, namely, Trichophyton tonsurans, Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton equinum, Microsporum gypseum, Microsporum nanum, Microsporum audouinii and Aspergillus niger were isolated. Out of them, T. mentagrophytes is most common (22%) followed by T. rubrum (16%) and T. equinum (13%) in this region. Evaluation of antifungal activity was carried out with different plant extracts, namely, Punica granatum (Pomegranate), Curcuma longa (Turmeric), Hibiscus rosa sinensis (Gudhal), Vitis vinifera (Grapes), Citrus limon (Lemon), Nerium oleander (kaner), Aloe barbadensis (Aloe vera), Trigonella foenum graecum (Methidana) and Trachyspermum ammi (Ajwain) by the well diffusion method. In this assay, extracts from P. granatum and C. longa showed maximum antifungal activity. Therefore, these could be used as an alternative medicine against infections caused by dermatophytes.

Key words: Dermatophytes, keratinophilic fungi, antifungal activity, plant extracts.

INTRODUCTION

Dermatophytes are a group of morphologically and physiologically related fungi that have the capacity to invade keratinized tissue (skin, hair and nails) of humans and other animals (Dei Cas and Vermes, 1986; Emmons, 1934). These fungi are both keratinophilic and keratinolytic (Revell and Taplin, 1974). They have the ability to digest keratin in vitro in their saprophytic state and utilize it as a substrate. Some of these fungi may invade tissues in vivo and provoke tineas. Dermatophytes are spread by direct contact with infected people.

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(anthropophilic), animals (zoophilic) and soil (geophilic) and indirectly from fomites (Revell and Taplin, 1974; Hainer, 2003). Dermatophytosis is caused by fungi in the genera *Microsporum*, *Trichophyton* and *Epidermophyton*. These organisms, called dermatophytes, are the pathogenic members of the keratinophilic (keratin digesting), soil fungi. *Microsporum* and *Trichophyton* are human and animal pathogens. *Epidermophyton* is a human pathogen (Nweze, 2010; Deor and Mpriello, 2006; Aliello and Mays, 1998).

Many studies have shown that soils are important sources of dermatophytes and keratinophilic fungi. Dermatophytes in soil (especially Public Park) can be a reservoir for infection in human beings (Hedayati et al., 2004; Mercantin et al., 1980; Oyeka and Okoli, 2003). The geographic distribution of dermatophytes can vary according to climatic conditions and nature of soil (Ramesh and Hilda, 1998; Papini et al., 1998; Shadzi et al., 2002). To date, little epidemiological data on the fungal flora of the soil of Gwalior area of India has become available. This paper reports the prevalence of dermatophytes and other keratinophilic fungi and risk of dermatophytosis in soil.

Plants have been a prime source of highly effective conventional drugs for the treatment of many forms of dermatophytes (Fabry et al., 1996; Cowan, 1999). The actual compound isolated from the plant may not serve as the drug, but the compound leads to the development of potential novel agents. Thus this paper also reports antifungal activity of selected herbal extracts against isolated fungal species from park soil of Gwalior region.

**MATERIALS AND METHODS**

**Collection of samples**

160 samples were collected from different public parks in the Gwalior region of India by sterile brush and collected in sterilized plastic bags. The soil samples were processed by taking the sterile petri dish 90 mm diameter were half filled with soil and moistened with distilled water. It was then baited with autoclaved keratinous substance like human hair, horse hair, and human nail (Vanbreuseghem et al., 1952; Kurup and Schmitt, 1970). After incubation of one week, cultured was transferred to Sabouraud dextrose agar (Hi-media), containing chloramphenicol and incubated at 25 to 27°C for 48 to 72 h. Isolated colonies were then identified for the species on the basis of colony and morphological characterization.

**Plant extracts**

Plant material, namely, fresh leaves, flowers, stem and root of *Punica granatum* (Pomegranate), *Curcuma longa* (Turmeric), *Hibiscus rosa sinensis* (Gudhal), *Vitis vinifera* (Grapes), *Citrus limon* (Lemon), *Nerium oleander* (kaner), *Aloe barbadensis* (Aloe vera), *Trigonella foenum graecum* (Methidana), and *Trachyspermum ammi* (Ajwain) were collected, and made into a fine powder by grinding. Two grams of the powder were soaked in 10 ml of 80% methanol and ethanol in ratio of 2:5 (1 part powder and 5 parts each solvent). Aqueous extracts were prepared with distilled in the same ratio. The filtrate was then dried at 50°C. 100 mg of the dried extract was dissolved in 1 ml Dimethyl sulfoxide (DMSO) and finally working solution was prepared in a concentration of 5 μg/μl.

**Antifungal activity of extract**

The antifungal activity of these plant extracts against dermatophytes was performed according to CLSI guideline. Briefly, Muller Hinton Agar (MHA) plates were prepared and 3 to 4 wells of 7 mm were punched on a plate with cork borer (Canton et al., 2009). A suspension of isolated dermatophytes in 0.005% Tween-80 in distilled water were spread on the plate with the help of cotton swabs and allowed them to incubate for half an hour. Then, 20 μl of the plant extract was transferred into each well including one well with 100 μg of fluconazole as a control. The plates were allowed to incubate for 48 h and the diameter of zone of inhibition was measured in mm.

**RESULTS**

In this study, total of 160 samples were collected from soil in different parks of Gwalior region of India, and were tested against different keratin-containing samples like horse hair, human hair, nails, etc. A positive diagnosis of keratinophilic fungi was obtained with some dermatophytic species like *Trichophyton tonsurans*, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Trichophyton equinum*, *Microsporum nanum*, *Microsporum gypseum*, *Microsporum audouinii* and few non dermatophytes like *Aspergillus niger*. Out of them, *T. mentagrophytes* is the most common (22%) followed by *T. rubrum* (16%) in the region (Figure 1).

The antifungal activity of extracts from different plants like *P. granatum*, *H. rosa sinensis*, *A. barbadensis*, *N. oleander*, *T. foenum graecum*, *C. longa*, *T. ammi*, *C. limon* were tested, and the extracts showed varying level of activity. A zone of inhibition ≥10 mm diameter was taken as a positive result. The results of antimicrobial susceptibility pattern obtained are presented in Table 1.

The mean diameter of the zone of inhibition for different fungi varied with different plant extracts. *P. granatum* and *C. longa* showed maximum antifungal activity against *Microsporum* species and *Trichophyton* species (Table 1 and Figure 2), while *H. rosa sinensis* and *T. foenum graecum* were not effective against these dermatophytes and the other plant extracts show antifungal activity with little less magnitude against different test fungi.

**DISCUSSION**

Plants have shown the limitless ability to synthesize antimicrobial substances most of which are secondary metabolites. In many cases, they serve as plant defense mechanisms against predation by microorganisms, insects and herbivores (Martin and Ernst, 2003). In the soil from public parks, there is a prevalence of keratinophilic fungi which may be dermatophytes or non
dermatophytes. The dermatophytes may infect human and animals, thus they must be treated with antifungal drugs.

The antifungal activities of various methanolic extracts of different plants were found effective against many keratinophilic dermatophytes fungi. Plant extracts from medicinal plants are an excellent candidate for the development of remedies for many infectious diseases, including mycosis due to the increasing development of antifungal resistance as well as the appearance of undesirable effect of some antifungal agent.

Compound identified from extracts of plants such as *P. granatum*, *H. rosa sinensis*, *A. barbadensis*, *N. oleander*, *T. foenum graecum*, *C. longa*, *T. ammi*, etc., may be useful as antifungal agents. Turmeric has the ability to prevent skin infection as it contains toxic flavonoids including 1.25% Karajan and 0.8% porogamal. Sunflower have two antifungal benzo-pyron derivative, 6-acetyl 2-2 dimethyl, 1-2 benzo-pyron, and 6-acetyl 7-hydroxi, 2-2 dimethyl benzo-pyron. Yellow kaner is also used to treat fungal skin diseases.

The extracts of several plants have been tested against keratinophilic fungi found to have antifungal activity to some extent against certain dermatophyte fungi.
Table 1. Antifungal activity of different plant extract.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Plant extracts</th>
<th>T. mentagrophytes</th>
<th>T. rubrum</th>
<th>T. tonsurans</th>
<th>T. equinum</th>
<th>M. nanum</th>
<th>M. audouini</th>
<th>M. gypseum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P. granatum</td>
<td>15 mm</td>
<td>R</td>
<td>15 mm</td>
<td>R</td>
<td>17 mm</td>
<td>R</td>
<td>20 mm</td>
</tr>
<tr>
<td>2</td>
<td>C. limon</td>
<td>R</td>
<td>12 mm</td>
<td>R</td>
<td>14 mm</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>3</td>
<td>A. barbadensis</td>
<td>11 mm</td>
<td>R</td>
<td>13 mm</td>
<td>R</td>
<td>R</td>
<td>12 mm</td>
<td>R</td>
</tr>
<tr>
<td>4</td>
<td>H. rosa sinensis</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>5</td>
<td>V. vinifera</td>
<td>R</td>
<td>7 mm</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>6</td>
<td>C. longa</td>
<td>12 mm</td>
<td>15 mm</td>
<td>R</td>
<td>8.2 mm</td>
<td>R</td>
<td>13.6 mm</td>
<td>12 mm</td>
</tr>
<tr>
<td>7</td>
<td>T. spermum ammi</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>10 mm</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>8</td>
<td>T. foenum graecum</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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</tr>
<tr>
<td>9</td>
<td>N. oleander</td>
<td>R</td>
<td>8.5 mm</td>
<td>12.5 mm</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

Conclusion

Conclusively, T. mentagrophytes, T. rubrum and T. equinum are prevalent in the soil of public park in the Gwalior region of India. Extracts from P. granatum and C. longa have potential antifungal activity and may be useful against various infections caused by dermatophytes.

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REFERENCES


