Occurrence of tinea infection with comparative study of commercial antifungal and traditional herbs in district Swat, Khyber Pakhtunkhwa, Pakistan

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Abstract

The current study was aimed to pinpoint the prevalence of tinea infection causing pathogens, comparative study of commercial antifungal drugs and traditional herbs against two fungal species Trichophyton rubrum and Aspergillus fumigatus isolated from dermatophytosis patients in Swat. A total of 190 samples were collected from skin, nails and hairs of infected peoples in different hospitals and private medical facilities. The samples were cultured on potato dextrose medium, labelled carefully and incubated. Moreover, the growths were observed under microscope and species were identified on the basis of morphological characteristics. A total of 12 different fungal species were isolated. Among all T. rubrum specie was recorded with high percentage 25%, followed by Candida (19.4%) and Penicillium spp (16.6%). The minimum rate was recorded for Aureobasidium pullans, Epidermphyton floccosum, Trichophyton basicola, T. verrucosum, T. tonsurans and T. tonsultans with 2.78% each. A total of six anti-fungal were examined in which fluconazloe and clotrimazole showed best results against T. rubrum and A. fumigatus. A total of eight traditional herbs were studied against T. rubrum and A. fumigatus. Ethyl acetate extract showed best results against both species followed by methanol extract. n Hexane extract was found less effective. The study concluded that fluconazole, clotrimazole and Ethyl acetate extract of medicinal plants were more effective against T. rubrum and A. fumigatus.

Introduction

Dermatophytosis especially Tinea infections are caused by more than 30 species of dermatophytes [1]. Tinea infection are usually restricted to superficial skin, but it has potential of systemic infection particularly in immune compromised patients [2]. According to WHO estimation about 25% of the world population are affected by dermatophytes [3]. Clinical mycosis of tinea infection is mainly related with Tricophyton, Microsporium and Epidermphyton. There are different types of tinea infection depending on infection sites and types of host. Trichophyton rubrum is the main causative agent isolates from skin infection [9–11]. To control these pathogens, various commercial antifungal drugs are used. However, due to their elevated toxicity and problems of residues its uses is restricted nowadays [15].

In the resent past the interest in the use of natural plants extracts as a therapeutic become increasing. Several plants, herbs and their components have been recognized since the late 19th century that has antimicrobial and antitoxin properties. The plants extract are secure to human and ecosystem then the chemical drugs. It can be used without any trouble by community who used plants extract for thousands of years to improve taste and odor of foods plus economic value [16–18]. The use of plants extract against tinea infection is well documented [19]. However, limited studies have been conducted on the comparative studies of commercial antifungal drugs and traditional herbs.

To fulfil this gap, the present study was designed to find the frequency distribution of Tinea infection causing fungal pathogens. Furthermore, to find out the in-vitro activity of commercial antifungal and traditional herbs against these pathogens.
Materials and methods

Characterization of fungal pathogens

The ethical committee of Centre for Biotechnology and Microbiology, University of Swat, Pakistan approved the study. A total of 190 samples were collected from skin, nails and hairs of infected peoples visited/admitted in different hospitals and private medical facilities located at district Swat, Pakistan. A proper consent form was signed from each individuals before sampling. The samples were inoculated directly on Potato Dextrose Agar (PDA) medium under the biosafety cabinet [20]. The plates were examined and identified under the microscopic observation for various taxonomic features i.e., colony color, colony shape, types of mycelia, types of fungal spores and fruiting body. The observed traits were compared to the key monographs on dermatophytes. The identified fungal species were again grow on media separately and pure culture were obtained and maintained for further study.

Antifungal activity of commercial drugs

Standard powders of six (06) antifungal drugs (Table 1) were dissolved in their specific DMSO (Dimethyl sulfoxide) solvent. Small pieces of seven (07) days old culture were taken from cultured media and inoculated on PDA plates. The solution of drugs were applied by well diffusion methods. A wells were made in agar plates by punching with a sterile crock borer of 4 mm and 100µl of each drugs solution were poured in to the well. The plates were allowed to standby for about 30mins. The plates were then incubated at 28°C and the inhibition zones were measured after 24 to 168 hours.

Table 1

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Antifungal</th>
<th>Group</th>
<th>Concentration/well</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fluconazole</td>
<td>Triazole</td>
<td>60 µl</td>
</tr>
<tr>
<td>2</td>
<td>Ketoconazole</td>
<td>Imidazole</td>
<td>40 µl</td>
</tr>
<tr>
<td>3</td>
<td>Nystatin</td>
<td>Polyene anti-fungal</td>
<td>30 µl</td>
</tr>
<tr>
<td>4</td>
<td>Clotrimazole</td>
<td>Imidazole</td>
<td>40 µl</td>
</tr>
<tr>
<td>5</td>
<td>Candazole</td>
<td>Imidazole</td>
<td>30 µl</td>
</tr>
<tr>
<td>6</td>
<td>Econazole</td>
<td>Imidazole</td>
<td>60 µl</td>
</tr>
</tbody>
</table>

Antifungal activity of traditional herbs

The plants used traditionally for skin diseases from the local areas of district Swat, Pakistan were collected (Table 2). After collection these plants were identified by Professor Dr. Ghulam Dastagir, Department of Botany, University of Peshawar, Pakistan. The selected parts of these plants were separated and thoroughly washed with tap water to remove extra mud and dust. The washed parts were then air dried for about 9 to 10 days. These plants materials were then finally powdered after complete
dryness. The powdered plant material was mixed with methanol, ethyl acetate and n-Hexane solvents in extraction flask and then placed in shaking incubator for about seven days. The solvent of the filtrate was then evaporated by rotary evaporator at 37 °C. Detail methodology of the extraction can be viewed in our recent publication [36]. Well diffusion methods were applied to check antifungal sensitivity assay of the herbal extracts. Same size of 4mm well was made in ager plates by sterilized cork borer. From each extract solutions were prepared in DMSO (Dimethyl sulfoxide) such that 0.5 mg of each extract was prepared in 3ml of DMSO is mixed in a solution. The zone of inhibition (ZI) were measured by the way that growth towards extract and growth opposite to extract, because fungi have radial growth and by comparing with positive and negative control.

Table 2
Local and botanical names of plants and their parts used against tinea infection

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Local names</th>
<th>Botanical names</th>
<th>Parts used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Papra</td>
<td>Fumaria indica</td>
<td>Whole plant</td>
</tr>
<tr>
<td>2</td>
<td>Sumbal</td>
<td>Adiantum incisum</td>
<td>Leaves</td>
</tr>
<tr>
<td>3</td>
<td>Kwarai</td>
<td>Berberis lyceum</td>
<td>Roots bark</td>
</tr>
<tr>
<td>4</td>
<td>Neem</td>
<td>Azadiracht indica</td>
<td>Leaves</td>
</tr>
<tr>
<td>5</td>
<td>Turmeric</td>
<td>Curcuma longa</td>
<td>Rhizome</td>
</tr>
<tr>
<td>6</td>
<td>Butey</td>
<td>Ajugabracteosa</td>
<td>Whole plant</td>
</tr>
<tr>
<td>7</td>
<td>Aijai</td>
<td>Debregeasia saeneb</td>
<td>Leaves</td>
</tr>
<tr>
<td>8</td>
<td>Azghake</td>
<td>Fagonia indica</td>
<td>Whole plant</td>
</tr>
</tbody>
</table>

**Results**

Among the total 190 collected samples, in 72 samples fungal growth were observed. Total 12 fungal isolates growth were obtained in these positive samples. The most prevalent fungal pathogen was found *T. rubrum* n = 18 (25%). The occurrence of other fungal pathogens were presented in Table 3. For the assessment of antifungal activity of commercial drugs and traditional plants two fungal isolates were selected that includes; *T. rubrum* and *A. fumigatus.*
Table 3
Tinea infection causing fungi isolated from different samples collected in Swat

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Pathogens</th>
<th>Frequency</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>T. rubrum</em></td>
<td>18</td>
<td>25.00</td>
</tr>
<tr>
<td>2</td>
<td>Candida spp.</td>
<td>14</td>
<td>19.44</td>
</tr>
<tr>
<td>3</td>
<td>Penicillium spp.</td>
<td>12</td>
<td>16.67</td>
</tr>
<tr>
<td>4</td>
<td>Aspergillus fumigatus</td>
<td>8</td>
<td>11.11</td>
</tr>
<tr>
<td>5</td>
<td>Alternaria spp.</td>
<td>4</td>
<td>5.56</td>
</tr>
<tr>
<td>6</td>
<td>Microsporum canis</td>
<td>4</td>
<td>5.56</td>
</tr>
<tr>
<td>7</td>
<td>Aureobasidium pullans</td>
<td>2</td>
<td>2.78</td>
</tr>
<tr>
<td>8</td>
<td>Epidermphyton floccosum</td>
<td>2</td>
<td>2.78</td>
</tr>
<tr>
<td>9</td>
<td>Trichophyton basicola</td>
<td>2</td>
<td>2.78</td>
</tr>
<tr>
<td>10</td>
<td><em>T. verrucosum</em></td>
<td>2</td>
<td>2.78</td>
</tr>
<tr>
<td>11</td>
<td><em>T. tonsurans</em></td>
<td>2</td>
<td>2.78</td>
</tr>
<tr>
<td>12</td>
<td><em>T. tonsultans</em></td>
<td>2</td>
<td>2.78</td>
</tr>
<tr>
<td></td>
<td>Total samples</td>
<td>72</td>
<td>100</td>
</tr>
</tbody>
</table>

Among the commercial drugs, fluconazole showed highest ZI = 29mm antifungal activity against *A. fumigatus* while clotrimazole showed highest ZI = 23mm antifungal activity against *T. rubrum* (Fig. 1). In comparison with these results the traditional plant *F. indica* ethyl acetate extract showed highest ZI = 16mm against *T. rubrum* (Fig. 2). Whereas *F. indica* ethyl acetate extract showed highest ZI = 20mm against *A. fumigates* (Fig. 3). Moreover, among all plant extract the ethyl acetate extract was found more effective.

**Discussion**

Skin, the outer most layer of human body is exposes to different types of pathogenic fungi including Tinea infection. They are very important keratinize degrading skin diseases. These infections are distributed all around the world [4]. Various types of keratin degrading fungi are well reported to be in association with Tinea infections. According to a study, *Microsporum* species, *Trichophyton* species and *Epidermophyton* species are the major groups of Tinea infections [4, 24]. The present study also indicated that 12 different fungal species *T. rubrum*, *Candida spp.*, *Penicillium spp.*, *Aspergillus spp.*, *Alternaria spp.*, *Microsporum canis*, *Aureobasidium pullans*, *Epidermphyton floccosum*, *Trichophyton basicola*, *T. verrucosum*, *T. tonsurans* and *T. tonsultans* were isolated from different infected parts of the
patient's body. The study conducted by Cervelatti et al., 2004 showed that 90% of Tinea infections were due to *Trichophyton rubrum* which is in agreement with our results [25].

Treatment of tinea infection is rapidly through anti-fungal drugs. Topical anti-fungal therapy shows more effects. Sometime pathogen become resistant to antifungal. Therefore, it is necessary to study the activity of drugs followed standardized *in vitro* test procedure. It is well documented that Fluconazole was more affective against *T. rubrum* [29]. In the present study, fluconazole is recorded more affective against *T. rubrum*. Our study is in line with findings of Esteban and friends [30].

Antifungal are major therapy, however due to toxicity and problem of antifungal residue agents, people got interested in the use of herbs against pathogenic fungi. Present study was conducted to compare the anti-fungal drugs and traditional herbs extract against two important selected dermatophytic fungi, i.e. *T. rubrum* and *A. fumigatus*. In our present study ethyl acetate extract has better activity followed by methanol extract. Whereas, less activity has been showed by n-hexane extract. *F. indica* showed better result against *T. rubrum* in ethyl acetate that is followed by *Curcuma longa*. Similar results had been obtained by [34, 35] in methanol extract *F. indica* showed maximum zone of inhibition of 12mm followed by *C. longa* (10mm). The lowest zone of inhibition 01mm was observed against *D. saeneb*.

**Conclusion**

The present study concluded that some anti-fungal such as fluconazole and clotrimazole are more effective against *T. rubrum* and *Asphergillus fumigatus*. Usually anti-fungals are of high cost and have some side effects. From the results of our study it is revealed that *Fumaria indica, Curcuma longa* are more effective in ethyl acetate extract against *T. rubrum* and *Aspergillus fumigatus*. So, it is concluded from the study that we can use different solvent for extraction due to different polarity and solubility and ethyl acetate is best solvent followed by methanol for plants extraction. In our study, we exposed that medicinal plants are as effective as commercial anti-fungal drugs. Interest of peoples in herbs increased due to potential, minimum side effect and environmental friendly, cheap and is easily available in society.

**Declarations**

**Conflict of Interest**

The authors have no conflict of interest

**Author Contribution**

T.U.D and M wrote the main manuscript text and prepared the figures and tables, F.A.S and S.L performed the experimental work, M.N.U and W.K guide in experimental work, manuscript writing and also reviewed the manuscript. All authors reviewed the manuscript and equally contributed.
Acknowledgement

The authors declare that there are no acknowledgements.

References


**Figures**

![Figure 1](image)

**Figure 1**

Susceptibility of antifungal drugs against *T. rubrum* and *A. fumigatus* after 96 hours
Figure 2

Antifungal activity of traditional plants against *T. rubrum* after 96 hour

Figure 3

Antifungal activity of traditional plants against *A. fumigates* after 96 hour