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Original research article

OCCURRENCE OF TINEA INFECTION WITH COMPARATIVE STUDY OF COMMERCIAL ANTIFUNGAL AND TRADITIONAL HERBS IN DISTRICT SWAT, KHYBER PAKHTUNKHWA, PAKISTAN

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Abstract: *Dermatophytosis, mainly Tinea infections, is caused by more than 30 species of dermatophytes. Tinea infections are usually restricted to superficial skin, but it has the potential for systemic infection, particularly in immune-compromised patients. The use of plant extract against tinea infection is well documented. However, limited studies have been conducted on the comparative studies of commercial antifungal drugs and traditional herbs.* ***Objective:*** *The current study was aimed to pinpoint the prevalence of tinea infection-causing pathogens, a comparative study of commercial antifungal drugs and traditional herbs against two fungal species, Trichophyton rubrum, and Aspergillus fumigatus isolated from dermatophytosis patients in Swat.* ***Methodology:*** *190 samples were collected from infected peoples' skin, nails, and hairs in different hospitals and private medical facilities. The samples were cultured on potato dextrose medium, labeled carefully, and incubated. Moreover, the growths were observed under a microscope, and species were identified based on morphological characteristics.* ***Results:*** *A total of 12 different fungal species were isolated. Among all T. rubrum species, 25% was recorded, followed by Candida (19.4%) and Penicillium spp (16.6%). The minimum rate was recorded for Aureobasidium pullulans, Epidermphyton floccosum, Trichophyton basicola, T. verrucosum, T. tonsurans, and T. consultants with 2.78% each. A total of six anti-fungals were examined, and fluconazole and clotrimazole showed the best results against T. rubrum and A. fumigatus. Eight traditional herbs were studied against T. rubrum and A. fumigatus. Ethyl acetate extract showed the best results against both species, followed by methanol extract. n Hexane extract was found to be less effective.* ***Conclusion:*** *The study concluded that fluconazole, clotrimazole, and Ethyl acetate extract of medicinal plants were more effective against T. rubrum and A. fumigatus.*

Keywords: Tinea infection, dermatophytic infections, Anti-fungal, Traditional herbs.

Introduction

Dermatophytosis, especially Tinea infections, is caused by more than 30 species of dermatophytes (1). Tinea infections are usually restricted to superficial skin, but it has the potential for systemic infection, particularly in immune-compromised patients (2). According to WHO, about 25% of the world population is affected by dermatophytes (3). Clinical mycosis of tinea infection is mainly related to Trichophyton, Microsporium, and Epidermophyton. Different types of tinea infection depend on infection sites and types of host. Trichophyton rubrum is the primary causative agent isolates from skin infection (4-6). Various commercial antifungal drugs are used to control these pathogens. However, their uses are restricted nowadays due to their elevated toxicity and residue problems (7).In the recent past, the interest in the use of natural plant extracts as a therapeutic has increased. Several plants, herbs and their components have been recognized since the late 19th century as having antimicrobial and antitoxin properties. The plant extract is more secure to humans and the ecosystem than chemical drugs. It can be used without any trouble by communities that have used plant extract for thousands of years to improve the taste and odor of foods plus economic value (8-10). The use of plant extract against tinea infection is well documented (11). However, limited studies have been conducted on the comparative studies of commercial antifungal drugs and traditional herbs.

To fill 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.

Methodology

The ethical committee of the Centre for Biotechnology and Microbiology, University of Swat, Pakistan, approved the study. One hundred ninety samples were collected from infected people's skin, nails, and hairs visited/admitted in different hospitals and private medical facilities in district Swat, Pakistan. Each individual signed a proper consent form before sampling. The samples were inoculated directly on Potato Dextrose Agar (PDA) medium under the biosafety cabinet (12). The plates were examined and identified under microscopic observation for various taxonomic features, i.e., colony color, colony shape, types of mycelia, types of fungal spores, and fruiting bodies. The observed traits were compared to the critical monographs on dermatophytes. The identified fungal species were again grown on media separately, and pure cultures were obtained

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and maintained for further study.

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. Well-diffusion methods apply the solution of drugs. A wells were made in ager plates by punching with a sterile crock borer of 4 mm, and 100µl of each drug solution was poured into the well. The plates were allowed to stand by for about 30 minutes. The plates were then incubated at 28ºC, and the inhibition zones were measured after 24 to 168 hours.

Table 1. List of commercial antifungal drugs, groups, and concentrations

S. No.	Antifungal	Group	Concentration/well
1	Fluconazole	Triazole	60 µl
2	Ketoconazloe	Imidazole	40 µl
3	Nystatin	Polyene anti-fungal	30 µl
4	Clotrimazole	Imidazole	40 µl
5	Candazole	Imidazole	30 µl
6	Econazole	Imidazole	60 µl

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 plant materials were then finally powdered after complete dryness. The powdered plant material was mixed with methanol, ethyl acetate, and n-hexane solvents in an extraction flask and placed in a shaking incubator for about seven days. A rotary evaporator then evaporated the filtrate solvent at 37 ºC. A detailed extraction methodology can be viewed in our recent publication (13). Well-diffusion methods were applied to check the antifungal sensitivity assay of the herbal extracts. The exact size of the 4mm well was made in ager plates by sterilized cork borer. Each extract solution was prepared in DMSO (Dimethyl sulfoxide), and 0.5 mg was prepared in 3ml of DMSO mixed in a solution. The zone of inhibition (ZI) was measured by how 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

S. No.	Local names	Botanical names	Parts used
1	Papra	*Fumariaindica*	Whole plant
2	Sumbal	*Adiantumincisum*	Leaves
3	Kwarai	*Berberis lyceum*	Roots bark
4	Neem	*Azadirachtindica*	Leaves
5	Turmeric	*Curcuma longa*	Rhizome
6	Butey	*Ajugabracteosa*	Whole plant
7	Ajlai	*Debregeasiasaeneb*	Leaves
8	Azghake	*Fagoniaindica*	Whole plant

Results

Among the total 190 collected samples, in 72 samples, fungal growth was observed. A total of 12 fungal isolates growth were obtained in these positive samples. The most prevalent fungal pathogen was *T. rubrum*, n= 18 (25%). The occurrence of other fungal pathogens is presented in Table 3. To assess the antifungal activity of commercial drugs and traditional plants, two fungal isolates were selected, including *T. rubrum* and *A. fumigatus*.

Table 3. Tinea infection-causing fungi isolated from different samples collected in Swat

S. No.	Pathogens	Frequency	Percentage %
1	*T. rubrum*	18	25.00
2	*Candida spp.*	14	19.44
3	*Penicillium spp.*	12	16.67
4	*Aspergillus fumigatus*	8	11.11

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5	*Alternaria spp.*	4	5.56
6	*Microsporumcanis*	4	5.56
7	*Aureobasidium pullans*	2	2.78
8	*Epidermphyton floccosum*	2	2.78
9	*Trichophyton basicola*	2	2.78
10	*T. verrucosum*	2	2.78
11	*T. tonsurans*	2	2.78
12	*T. tonsultans*	2	2.78
	Total samples	72	100

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

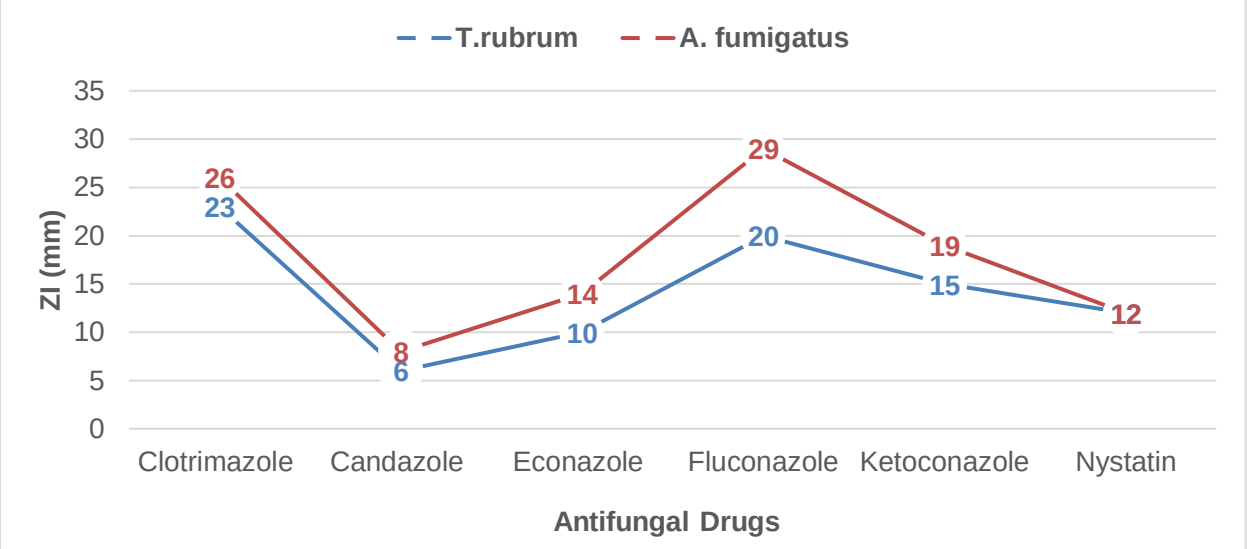

Figure 1. Susceptibility of antifungal drugs against *T. rubrum* and *A. fumigates* after 96 hours

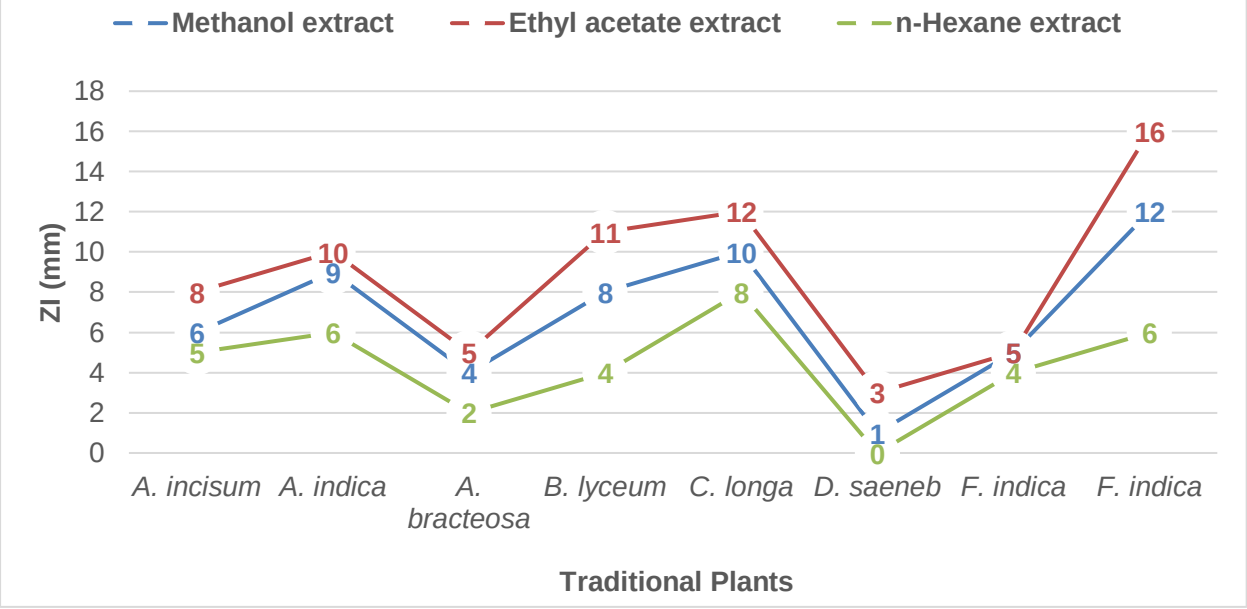

Figure 2. Antifungal activity of traditional plants against *T. rubrum* after 96 hour

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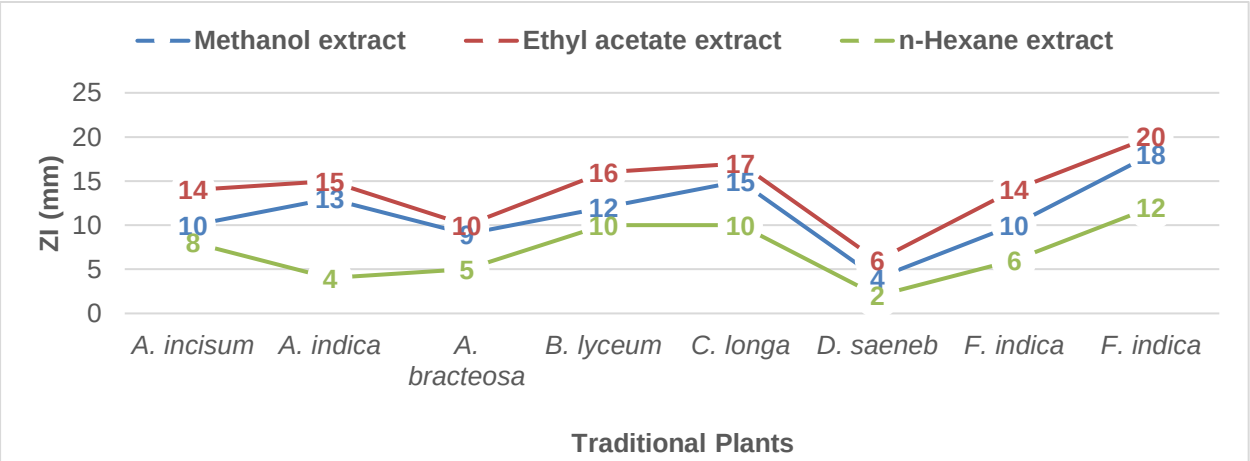

Figure 3. Antifungal activity of traditional plants against *A. fumigates* after 96-hour

Discussion

Skin, the outermost layer of the human body, is exposed to different types of pathogenic fungi, including Tinea infection. They are essential to keratinize degrading skin diseases. These infections are distributed all around the world (14). Various types of keratin-degrading fungi have been reported to be associated with Tinea infections. According to a study, *Microsporum, Trichophyton, and epidermophyton species are the major groups where Tinea infections occur (4-12, 14-25).* The present study also indicated that 12 different fungal species *T. rubrum, Candida spp., Penicillium spp., Aspergillus spp., Alternaria spp., Microsporum canis, Aureobasidium pullulans, Epidermphyton floccosum, Trichophyton basicola, T. verrucosum, T. tonsurans and T. consultants* 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 agrees with our results (26).

Treatment of tinea infection is rapidly through anti-fungal drugs. Topical anti-fungal therapy shows more effects. Sometimes, pathogens become resistant to antifungals. Therefore, it is necessary to study the activity of drugs following standardized in vitro test procedures. It is well-documented that Fluconazole was more effective against *T. rubrum* (27). In the present study, fluconazole is recorded as more effective against *T. rubrum*. Our study aligns with Esteban and friends' findings (28).

Antifungal therapy is significant; however, due to toxicity and the problem of antifungal residue agents, people have become interested in using herbs against pathogenic fungi. The present study compared the anti-fungal drugs and traditional herbs extracts against two important selected dermatophytic fungi, i.e., *T. rubrum* and *A. fumigatus*. In our present study ethyl acetate extract has better activity than methanol extract. At the same time, less activity has been shown by n-hexane extract. *F. indica* showed better results against T. rubrum in ethyl acetate, which is followed by *Curcuma long*. Similar results were obtained by (29, 30) in methanol extract. F. indica showed a 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 antifungals, such as fluconazole and clotrimazole, are more effective against T.rubrum and Aspergillus *fumigatus*. Usually, antifungals are of high cost and have some side effects. Our study revealed that Fumaria indica and *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 solvents for extraction due to different polarity and solubility, and ethyl acetate is the best solvent, followed by methanol for plant extraction. Our study revealed that medicinal plants are as effective as commercial anti-fungal drugs. The interest of people in herbs has increased due to their potential, minimum side effects, and being environmentally friendly, cheap, and readily available in society.

Declarations

This paper has been published as a preprint in **researchsqure.com (https://doi.org/10.21203/rs.3.rs-3893652/v1)**

Data Availability statement
All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate.
Approved by the department concerned. (IRB-

Consent for publication
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Conflict of interest

The authors declared an absence of conflict of interest.

Authors Contribution

MUHAMMAD NAZIR UDDIN (Associate professor)
Data Analysis

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References

1. White TC, Oliver BG, Gräser Y, Henn MR. Generating and testing molecular hypotheses in the dermatophytes. Eukaryotic cell. 2008;7(8):1238-45.
2. Rodwell GE, Bayles CL, Towersey L, Aly R. The prevalence of dermatophyte infection in patients infected with human immunodeficiency virus. International journal of dermatology. 2008;47(4):339-43.
3. Nigam PK. Antifungal drugs and resistance: Current concepts. Our Dermatology Online. 2015;6(2):212.
4. Banerjee M, Ghosh AK, Basak S, Das KD, Gangopadhyay DN. Comparative evaluation of effectivity and safety of topical amorolfine and clotrimazole in the treatment of tinea corporis. Indian journal of dermatology. 2011;56(6):657-62.
5. Godoy-Martinez P, Nunes FG, Tomimori-Yamashita J, Urrutia M, Zaror L, Silva V, et al. Onychomycosis in São Paulo, Brazil. Mycopathologia. 2009;168:111-6.
6. Graser Y, Kuhnisch J, Presber W. Molecular markers reveal exclusively clonal reproduction in Trichophyton rubrum. Journal of clinical microbiology. 1999;37(11):3713-7.
7. Uddin MN, Shah FA, Muhammad M, Aziz F, Din NU. Occurrence of tinea infection with comparative study of commercial antifungal and traditional herbs in district Swat, Khyber Pakhtunkhwa, Pakistan. 2024.
8. Saadabi AM. Antifungal activity of some saudi plants used in traditional medicine. 2006.
9. Shelef L, Naglik O, Bogen D. Sensitivity of some common food-borne bacteria to the spices sage, rosemary, and allspice. Journal of Food Science. 1980;45(4):1042-4.
10. Shelef L. Antimicrobial effects of spices 1. Journal of food safety. 1984;6(1):29-44.
11. Aly M, Bafeel S. Screening for antimicrobial activity of some medicinal plants in Saudi Arabia. African Journal of Traditional, Complementary and Alternative Medicines. 2009:480-1.
12. Griffith GW, Easton GL, Detheridge A, Roderick K, Edwards A, Worgan HJ, et al. Copper deficiency in potato dextrose agar causes reduced pigmentation in cultures of various fungi. FEMS microbiology letters. 2007;276(2):165-71.
13. Khan W, Bakht J, Shafi M. Antimicrobial potentials of different solvent extracted samples from Physalis ixocarpa. Pakistan Journal of Pharmaceutical Sciences. 2016;29(2).
14. Havlickova B, Czaika VA, Friedrich M. Epidemiological trends in skin mycoses worldwide. Mycoses. 2008;51:2-15.
15. Irum F, Suhail M, Abro H. Keratinophilic fungi from the soil of district, Jamshoro, Sindh, Pakistan. Pakistan Journal of Botany. 2007;39(4):1377.
16. Adamski Z, Batura–Gabryel H. Medical mycology for physicians and students. Scientific Publishing of Poznan Medical University, Poznan, Poland; 2007.
17. Higgins E, Fuller L, Smith C. Guidelines for the management of tinea capitis. British Journal of Dermatology. 2000;143(1):53-8.
18. Paulo UdSPIdMTdS. Revista do Instituto de Medicina Tropical de São Paulo: O Instituto; 2005.
19. Zaugg C, Monod M, Weber J, Harshman K, Pradervand S, Thomas J, et al. Gene expression profiling in the human pathogenic dermatophyte Trichophyton rubrum during growth on proteins. Eukaryotic cell. 2009;8(2):241-50.
20. Youssef N, Wyborn C, Holt G, Noble W, Clayton YM. Antibiotic production by dermatophyte fungi. Microbiology. 1978;105(1):105-11.
21. Piérard GE, Arrese JE, Piérard-Franchimont C. Treatment and prophylaxis of tinea infections. Drugs. 1996;52:209-24.
22. Macura AB. Dermatophyte infections. International journal of dermatology. 1993;32(5).
23. Merlin K, Kilkenny M, Plunkett A, Marks R. The prevalence of common skin conditions in Australian school students: 4 Tinea pedis. British Journal of Dermatology. 1999;140(5):897-901.
24. Gupta AK, Cooper EA. Dermatophytosis (Tinea) and other superficial fungal infections. Diagnosis and treatment of human mycoses: Springer; 2008. p. 355-81.
25. Ameen M. Epidemiology of superficial fungal infections. Clinics in dermatology. 2010;28(2):197-201.
26. Cervelatti EP, Ferreira-Nozawa MS, Aquino-Ferreira R, Fachin AL, Martinez-Rossi NM. Electrophoretic molecular karyotype of the dermatophyte Trichophyton rubrum. Genetics and Molecular Biology. 2004;27:99-102.
27. Fernández-Torres B, Carrillo A, Martın E, Del Palacio A, Moore M, Valverde A, et al. In vitro activities of 10 antifungal drugs against 508 dermatophyte strains. Antimicrobial agents and chemotherapy. 2001;45(9):2524-8.
28. Esteban A, Abarca M, Cabañes FJ. Comparison of disk diffusion method and broth microdilution method for antifungal susceptibility testing of dermatophytes. Medical mycology. 2005;43(1):61-6.
29. Ahmed AA, Bishr MM, El-Shanawany MA, Attia EZ, Ross SA, Paré PW. Rare trisubstituted sesquiterpenes daucanes from the wild Daucus carota. Phytochemistry. 2005;66(14):1680-4.
30. Aiyegoro O, Afolayan A, Okoh A. In vitro time kill assessment of crude methanol extract of Helichrysum pedunculatum leaves. African Journal of Biotechnology. 2008;7(11).

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