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Evaluating the Antifungal Potential of Herbal Extracts against Clinically-Derived, Drug-Resistant Dermatophytes

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Abstract:
Background:Dermatophyte-induced superficial mycoses are widespread dermatological conditions that affect a substantial population worldwide. The emergence of drug-resistant strains of these fungi has become a significant impediment to effective treatment, necessitating the search for novel therapeutic options. This investigation aims to assess the prevalence of drug-resistant dermatophytes in clinical specimens from India and to evaluate the antifungal properties of specific herbal extracts against these resistant strains.Methods: A comprehensive collection of over 150 dermatological samples, including skin, hair, and nails, was amassed from patients presenting with suspected superficial mycoses at a public healthcare facility in Tamil Nadu, India, over the span of one year. These samples underwent rigorous dermatophyte identification and subsequent antifungal susceptibility testing. The in vitro antifungal potential of various herbal extracts, notably aloe vera gel, Annona reticulata leaf extract, and Acalypha indica leaf extract, was meticulously assessed against the dermatophyte isolates.Results: The investigation revealed a significant incidence of

dermatophytes, with a prevalence rate of 74.67%. The most commonly isolated species included Trichophyton spp., Microsporum spp., Epidermophyton spp., and Aspergillus spp. A striking observation was that over 98% of these isolates were resistant to multiple antifungal drugs. In terms of herbal efficacy, aloe vera gel, Annona reticulata leaf extract, and Acalypha indica leaf extract exhibited notable antifungal activity against the multidrug-resistant dermatophytes. Conversely, extracts from Achyranthus aspera, Azadirachta indica, and pine were found to be ineffective.Conclusion: The findings of this study underscore the promising role of certain herbal extracts as viable alternative or adjunctive treatments for managing drug-resistant dermatophyte infections. It advocates for further investigative research to elucidate the mechanisms of action, ascertain safety profiles, and develop optimal administration strategies for these herbal candidates.

Keywords:Causative agents, Demographic trends, Antifungal susceptibility, Superficial fungal infections, Textile and dye industry, Clotrimazole, Nystatin, Multidrug resistance (MDR)

Introduction:

Superficial mycoses, fungal infections of the skin, hair, and nails, plague millions worldwide. These persistent infections, primarily caused by dermatophyte fungi, pose a significant global health burden[1,2,3].The estimated lifetime risk for these infections hovers around 10-20%, with developing countries like India bearing a disproportionate brunt. In regions like Tamil Nadu, India, the incidence of superficial mycoses can soar as high as 36%.While conventional antifungal agents like griseofulvin, terbinafine, and azole antifungals offer treatment options, their long-term use often incurs side effects. More alarmingly, the emergence of drug-resistant dermatophyte strains renders these once-effective therapies ineffective. This necessitates a proactive search for alternative solutions, particularly in resource-limited settings[4,5].Enter the realm of herbal medicine. Natural plant extracts have long been valued for their therapeutic properties, and their potential against fungal infections is increasingly recognized. This study delves into the efficacy of specific herbal extracts against drug-resistant dermatophytes isolated from clinical patients in Tamil Nadu, India.Over 150 skin, hair, and nail samples were collected from suspected cases, revealing a positive culture rate of 74.67%. The culprit? Dermatophyte species belonging to Trichophyton, Microsporum,Epidermophyton, and even Aspergillus genera. Disturbingly, over 98% of these isolates exhibited resistance to multiple conventional antifungal drugs[6,7,8].

But amidst this grim scenario, a glimmer of hope emerges. The study evaluated the antifungal prowess of various herbal extracts against these resilient fungal foes. The results were promising:

- **Aloe vera gel:** This ubiquitous plant's gel extract displayed potent antifungal activity against the drug-resistant dermatophytes[9,10].
- **Annona reticulata leaf extract:** The leaves of this tropical fruit tree yielded an extract with significant antifungal properties.

- **Acalypha indica leaf extract:** This traditional Indian medicinal plant's leaves, too, proved effective against the fungal invaders.

Not all herbal warriors emerged victorious, however. Extracts from Achyranthus aspera, Azadirachta indica (neem), and pine trees showed no antifungal activity against the resistant strains[11,12].These findings illuminate the potential of specific herbal extracts as alternative or complementary therapies for managing drug-resistant dermatophyte infections[13,14,15]. While further research is necessary to elucidate their mechanisms of action, optimize delivery methods, and ensure patient safety, these natural options offer a beacon of hope in the face of escalating drug resistance.Continued research on these promising herbal extracts, alongside exploration of other natural antifungal agents, can pave the way for safer, more effective, and potentially more affordable treatment options for superficial mycoses[16,17]. This is particularly crucial in resource-constrained settings where access to conventional antifungals may be limited. By harnessing the power of nature, we can combat the rising tide of drug-resistant fungal infections and protect the health of millions worldwide.

Materials and Methods

Chemicals and reagents: All chemicals and reagents used in this study were obtained from Hi Media Laboratories (Mumbai, India).

Participants:

- **Sample size:** A total of 150 participants with suspected dermatophytosis were recruited from the Dermatology outpatient department of the Government hospital in Erode city, Tamil Nadu, India.
- **Inclusion criteria:** All participants presented with clinical symptoms suggestive of dermatophytosis.
- **Exclusion criteria:** Individuals with confirmed non-dermatophytic fungal infections, on topical or systemic antifungal therapy within the past 2 weeks, or with immunocompromised status were excluded.

Study design and procedure:

- **Ethical approval:** The study was approved by the Institutional Ethics Committee of Dermatology outpatient department, Government hospital, Erode city, TamilNadu, India and conducted in accordance with the Declaration of Helsinki.
- **Consent:** Written informed consent was obtained from all participants before participation.
- **Sample collection:** Specimens were collected from each participant by scraping the edge of the affected skin, nail scraping, and clipping the infected hair. Data related to participants' sex, age, and socioeconomic status was recorded in a pre-designed questionnaire. Collected samples were transported to the laboratory within one hour and processed without delay.

Microbiological investigation:

- **Culture:** Samples were inoculated onto Sabouraud Dextrose Agar and further subcultured onto Potato Dextrose Agar, Rose Bengal Agar, and Dermatophyte Test Medium.
- **Identification:** Morphological identification of dermatophytic fungi was performed using lactophenol cotton blue mount.
- **Differentiation:** Urease test on Christensen's agar medium was used to differentiate between species.
- **Antibiogram:** The susceptibility of isolated fungi to antifungal agents (nystatin, clotrimazole, ketoconazole,miconazole, itraconazole, fluconazole, and amphotericin B) was determined using the disc diffusion method according to CLSI guidelines.

Plant extract preparation:

- Fresh leaf materials from Acalypha indica, Achyranthus aspera, Azadirachta indica, Annona reticulata, and Aloe vera gel were collected, shade-dried, and powdered.
- Aqueous extracts were prepared by soaking 1.0 g of powder in 100 ml of sterile distilled water for 24 hours. The filtrate was dried at 50°C.
- The antifungal activity of these extracts was assessed using the well diffusion method.

Statistical analysis: Data was tabulated and analyzed using IBM SPSS software version 21.0 (Chicago, USA). Chi-square test and one-way ANOVA test were used with a significance level of $p < 0.05$.

Results:

Prevalence of Superficial Fungal Infections:

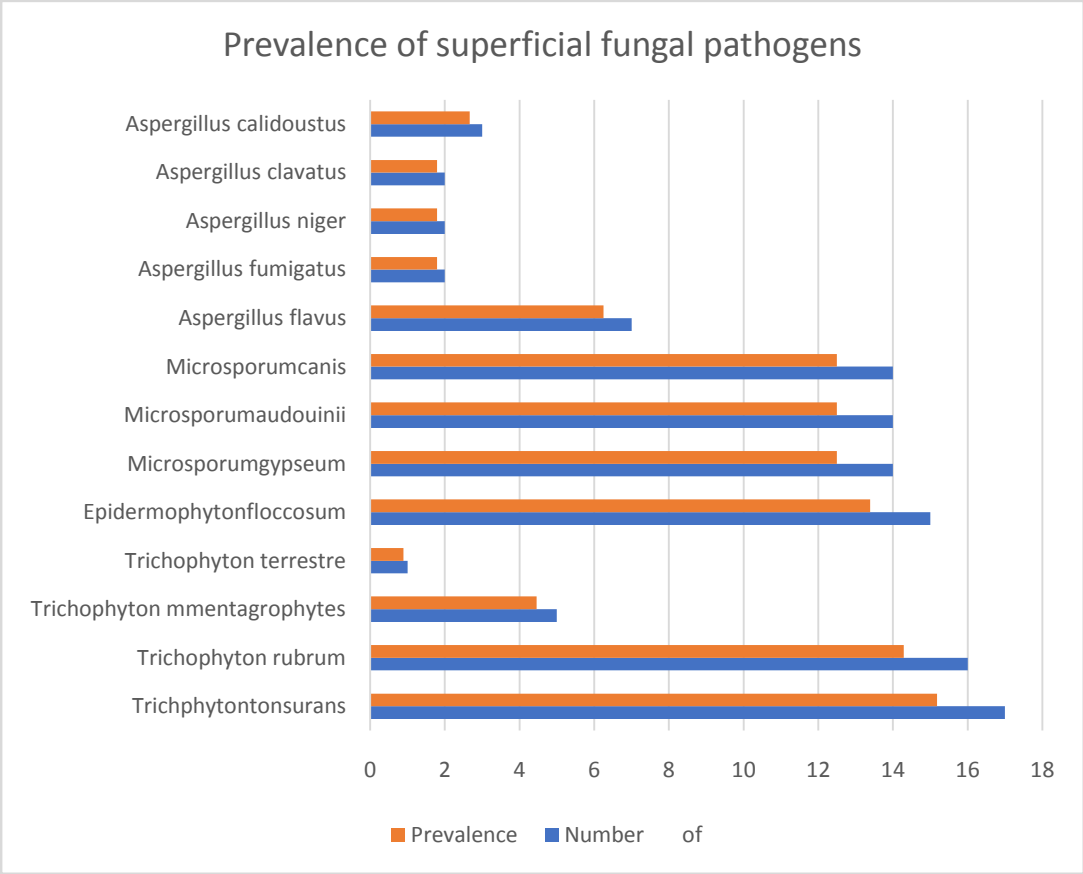
Table 1. Sexwisedistributionofskininfection

Sex	Percentage	Count
Male	36%	36
Female	64%	64
Total	100%	100

- Dermatophytic fungal infections were confirmed in 96 out of 150 participants (64%), with an overall prevalence of 74.66%.
- Non-dermatophytic fungi were identified in 16 cases (10.67%), while 38 samples (25.33%) showed no fungal growth.
- Among the identified dermatophytes, Microsporum species were the most common (37.5%), followed by Trichophyton species (34.82%) and Epidermophyton species (13.39%).

- Aspergillus species were the most common non-dermatophyticmold identified (14.29%).

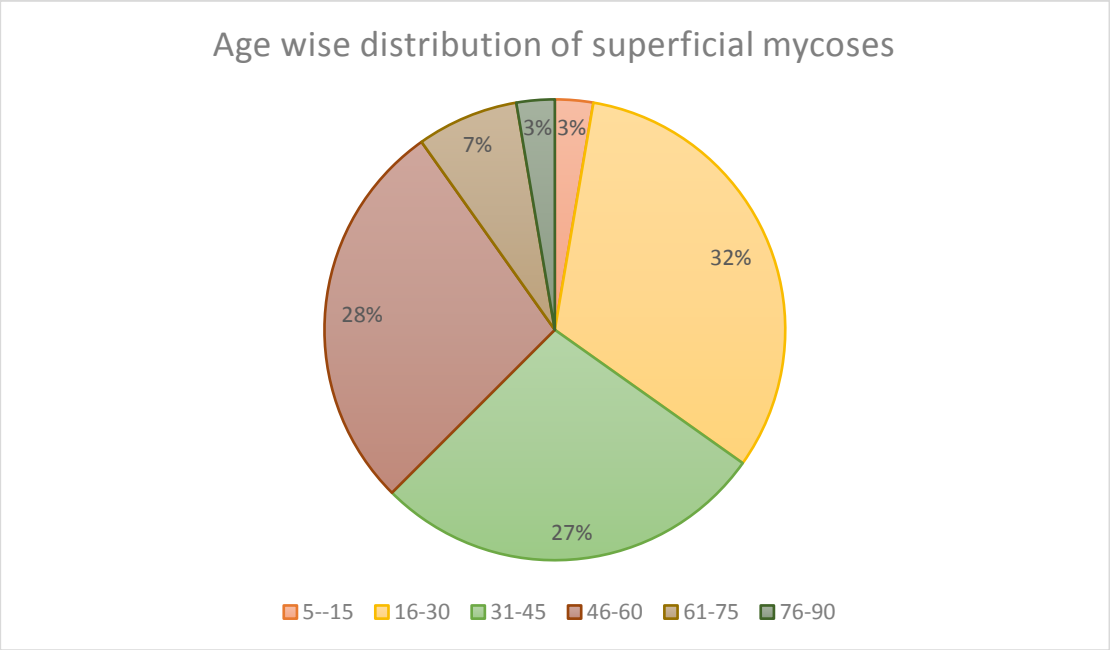
Fig : 1.Prevalence of superficial fungal pathogens



Demographics:

- Sex distribution revealed a higher prevalence in males (64%) compared to females (36%). This may be due to occupational factors in the study area, which has a large textile and dye industry.
- Age distribution showed a higher incidence in individuals aged 16-60 years compared to children and older adults. This could be related to increased workplace exposure in adults.
- Socioeconomic status analysis indicated a higher prevalence among lower and middle-class individuals (48 and 52 cases, respectively) compared to higher-class individuals (12 cases). This may be linked to hygiene practices and healthcare access.

Fig 2. **Age wise distribution of superficial mycoses**



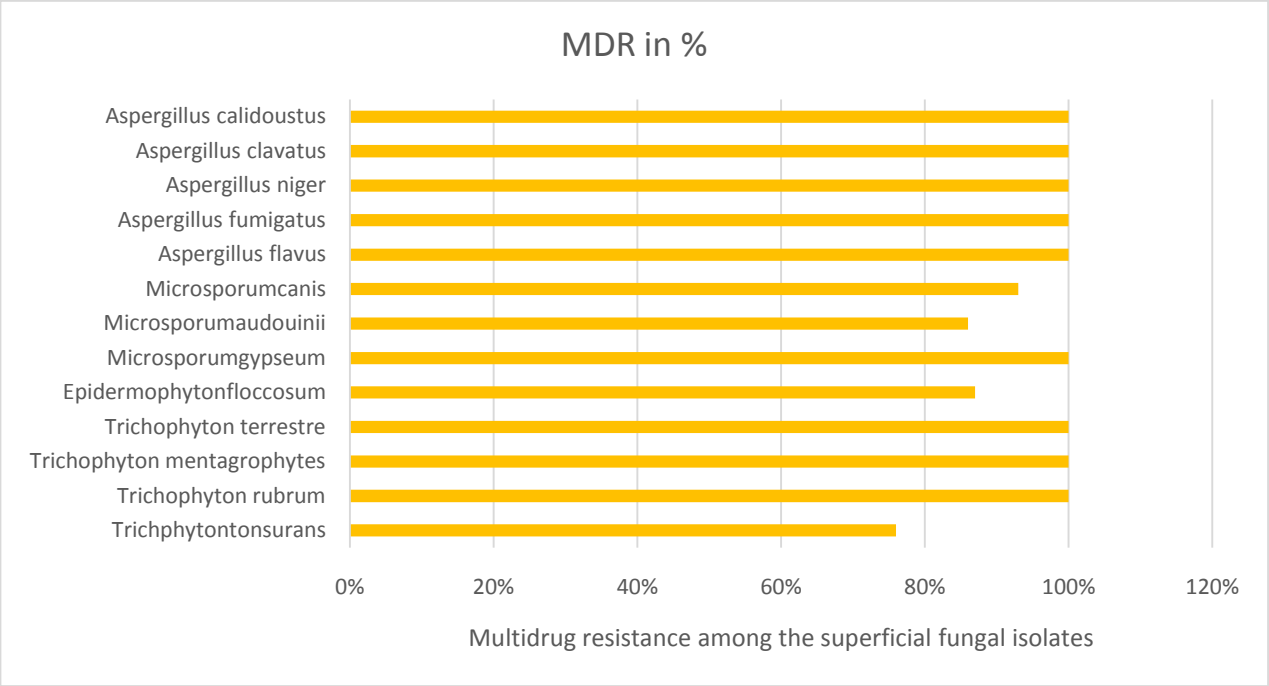
Antifungal Susceptibility:

- The drug sensitivity of isolated dermatophytic strains varied significantly.
- Trichophyton tonsurans exhibited high sensitivity to clotrimazole (65%) but resistance to ketoconazole (100%) and itraconazole (82%).
- Similar trends were observed in Trichophyton rubrum and Trichophyton mentagrophytes.
- Epidermophyton floccosum showed resistance to multiple drugs, including clotrimazole (73%), fluconazole (73%), amphotericin B (100%), ketoconazole (100%), and miconazole (100%).
- Microsporum species were generally sensitive to clotrimazole and nystatin but resistant to amphotericin B, ketoconazole, miconazole, and itraconazole.
- Microsporumgypseum exhibited 100% resistance to fluconazole.
- These findings suggest that clotrimazole and nystatin may be effective treatment options for dermatophytic infections in this region, while other antifungal agents may have limited efficacy.

Multidrug Resistance:

- Multidrug resistance (MDR) was observed in several isolates, with Trichophyton rubrum, Trichophyton mentagrophytes, and Microsporumgypseum exhibiting 100% resistance to the tested drugs.
- Trichophyton tonsurans and Epidermophyton floccosum showed MDR rates of 76% and 87%, respectively.

Fig : 3 Multidrugresistanceamongthesuperficialfungalisolates



Herbal Extracts:

- Preliminary results suggest that some herbal extracts, such as Aloe vera and Annona reticulata, may have antifungal activity against MDR isolates of superficial fungi.
- Acalypha indica showed moderate activity, while other tested extracts were not effective.
- Further investigation is needed to confirm the efficacy and potential clinical applications of these herbal extracts.

Table 2: Antibiogramofthedermatophyticfungalisolates

Isolates	**Antibiotics (Conc.)**													
	Clotrimazole (10µg)		**Fluconazole (10µg)**		**Amphotericin B (20µg)**		**Ketoconazole (30µg)**		**Miconazole (30µg)**		**Itraconazole (30µg)**		**Nystatin (50µg)**	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Trichphytontonsurans	11 (65%)	6 (35%)	6 (35%)	11 (65%)	4 (24%)	13 (76%)	0 (0%)	17 (100%)	0 (0%)	17 (100%)	3 (18%)	14 (82%)	10 (59%)	7 (41%)
Trichophyton rubrum)	11 (69%)	5 (31%)	1 (6%)	15 (94%)	3 (19%)	13 (81%)	0 (0%)	16 (100%)	0 (0%)	16 (100%)	1 (6%)	15 (94%)	8 (50%)	8 (50%)
Trichophyton entagrophyt	4 (80%)	1 (20%)	0 (0%)	5 (100%)	1 (20%)	4 (80%)	0 (0%)	5 (100%)	0 (0%)	5 (100%)	0 (0%)	5 (100%)	3 (60%)	2 (0%)
Trichophyton terrestre	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (10%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)
Epidermophytonfloccosum	4 (27%)	11 (73%)	4 (27%)	11 (73%)	0 (0%)	15 (100%)	0 (0%)	15 (100%)	0 (0%)	15 (100%)	8 (53%)	7 (47%)	8 (53%)	7 (47%)
Microsporumgypseum	9 (64%)	5 (36%)	0 (0%)	14 (100%)	0 (0%)	14 (100%)	0 (0%)	14 (100%)	0 (0%)	14 (100%)	4 (29%)	10 (71%)	9 (64%)	5 (36%)
Microsporumaudouinii	8 (57%)	6 (43%)	3 (21%)	11 (79%)	0 (0%)	13 (93%)	0 (0%)	14 (100%)	0 (0%)	14 (100%)	3 (21%)	11 (79%)	11 (79%)	3 (21%)
Microsporumcanis	8 (57%)	6 (43%)	3 (21%)	11 (79%)	1 (7%)	7 (100%)	0 (0%)	14 (100%)	0 (0%)	14 (100%)	0 (0%)	14 (100%)	11 (79%)	3 (21%)

Discussion

The study conducted confirms a high prevalence of superficial fungal infections in the region, with a rate of 74.66% that aligns with previous local reports. Dermatophytes were the dominant isolate, with Microsporum spp. and Trichophyton spp. being the most common, which is like another research from India. This pattern differs slightly from global observations, where Trichophyton rubrum often predominates[18,19]. The sex distribution revealed a higher prevalence in males, potentially due to occupational factors in the textile and dye industry, while the age analysis showed the highest incidence in individuals aged 16-60 years, likely related to increased workplace exposure in adults. Socioeconomic status analysis indicated a higher prevalence among lower and middle-class individuals, possibly linked to hygiene practices and healthcare access[20,21].The study's findings highlight the importance of considering local drug sensitivity patterns, with clotrimazole and nystatin demonstrating the highest efficacy against isolated dermatophytic strains[22,23]. However, a concerning trend of multidrug resistance was observed, with certain species exhibiting 100% resistance to the tested drugs. This underscores the need for ongoing surveillance of antifungal resistance patterns and potentially adopting alternative treatment strategies[24,25].Preliminary results suggest promising antifungal activity of some tested herbal extracts against multidrug-resistant isolates. Aloe vera and Annona reticulata showed significant efficacy, while Acalypha indica produced moderate effects. Further research is warranted to validate these findings, explore the bioactive compounds responsible, and determine the potential clinical applications of these herbal extracts as an alternative or complementary therapy for resistant fungal infections [26,27]. This study contributes to the understanding of the prevalent fungal pathogens and associated drug sensitivities in the region[28]. It highlights the growing concern of multidrug-resistant fungal infections and emphasizes the need for further research and development of alternative treatment options, including the exploration of natural products like medicinal plants[29]. Implementing effective surveillance systems and promoting good hygiene practices remain crucial for controlling the spread of superficial fungal infections.

Conclusion:

Current antifungal therapies for dermatophytosis often face limitations. While effective, synthetic medications can produce undesirable side effects and necessitate extended treatment durations. Additionally, the emergence of drug resistance against existing and newer antifungals poses a significant threat to therapeutic efficacy. Dermatophytes display a concerning adaptability to develop resistance, impacting the long-term effectiveness of available options. Furthermore, antibiogram trends reveal dynamic shifts, highlighting the evolving nature of this challenge. In light of these concerns, exploring alternative treatment options like herbal medicines appears promising. Medicinal plants have a rich history of use in traditional medicine and harbor a vast reservoir of bioactive compounds with potential antifungal activity. Utilizing herbal remedies could offer several advantages:

- Reduced side effects: Compared to synthetic drugs, herbal remedies often exhibit milder adverse effects, improving patient tolerability and compliance.
- Potential for lower resistance development: The complex composition of herbal extracts, often containing multiple bioactive compounds, may hinder the development of fungal resistance compared to single-molecule synthetic drugs.

Therefore, incorporating research and development efforts towards harnessing the antifungal potential of medicinal plants could represent a valuable future direction in tackling dermatophytosis. This approach holds promise for enhancing therapeutic options, mitigating side effects, and potentially curbing the development of antifungal resistance.

Conflict of interest: Nil

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