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Evaluation of Antifungal Activity of Plant Extract Against Fungal Pathogen Associated with Superficial Infection

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Abstract

Superficial fungal infections represent a major public health problem all over the world especially middle and low income countries. This study aimed to evaluate the antifungal activity of plant extracts against pathogenic fungi infecting the skin of school children in Gilgit-Baltistan. Various plant extracts were obtained from locally available plant species and tested against selected pathogenic fungi using standard in vitro antifungal assays (the food poison technique). The results revealed varying degrees of antifungal activity among the plant extracts with some showing promising inhibitory effects against the targeted fungi. Statistical analysis indicated significant differences in antifungal efficacy among the tested extracts. Three fungal species, namely *Tinea capitis*, *Tinea corporis*, and *Tinea pedis* and five plant extracts *Allium sativum*, *Thymus vulgaris*, *Azadirachta indica*, *Mentha piperita* and *Capparis spinosa* at 8, 16 and 24% concentration were tested against respective species. Results showed a concentration-dependent inhibition pattern across all extracts. At 24% concentration, *A. sativum* exhibited the highest average inhibition across all fungi (81.2%), followed by *T. vulgaris* (75.9%), and *A. indica* (71.8%). In contrast, *C. spinosa* showed poor efficacy, averaging below 40% inhibition even at the highest concentration. Overall, extracts from *A. sativum*, *T. vulgaris*, and *A. indica* demonstrated consistent and potent antifungal activity across all tested species and concentrations, supporting their potential for safe, plant-based antifungal formulations. These findings advocate the use of accessible herbal alternatives for managing paediatric fungal infections, particularly in resource-limited school settings. Further research involving clinical validation and formulation development is strongly recommended.

Author Details

Keywords:

Fungal Pathogen; school children; plant extracts; antifungal potentials

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INTRODUCTION

Fungal infections are among the most difficult diseases to manage in humans. According to reports, fungal infections generate high rates of morbidity and mortality (CDC, 2020). Fungal pathogens have largely been neglected by the public and public health experts despite having an irreversible impact on human health (Rodrigues and Nosanchuk, 2020). Despite being confined to the epidermis, dermatophyte infections can be invasive and result in dangerous, widespread infections in immunocompromised patients (Trottier et al., 2020). HIV treatment in AIDS patients, cytotoxic chemotherapy in cancer patients, immunosuppressive treatments when innate defenses have been compromised, and the presence of catheters and other indwelling devices are among the major risk factors for the development of invasive fungal infections (Li et al., 2020). Fungal infections can range in clinical severity from asymptomatic to moderate skin infections to major invasive infections. Global Action Fund for Fungal Infectious (GAFFI) estimates that each year, approximately 135 million women experience vulvovaginal candidiasis (thrush), nearly one million people experience invasive candidiasis, 60,000–100,000 cases of Candida peritonitis occur, over 300,000 patients develop invasive aspergillosis, 400,000 cases of pneumocystis pneumonia are observed, and there are approximately 500,000 new cases of histoplasmosis (Havlickova et al., 2018). About one billion people worldwide have cutaneous fungal infections of the skin, hair, and nails (Nweze and Eke, 2018). Patients who also have other coexisting clinical disorders, such as immunological malfunction, chemotherapy, cancer, and long-term chronic diseases, have considerably greater rates of morbidity and mortality from infection. It is a serious public health issue that contributes to, among other things, reduced school enrollment among children in low- and middle-income countries. School-aged children are more susceptible to fungal infections due to factors such as inadequate personal cleanliness, frequent human interaction, unhygienic living conditions, crowding, and low socioeconomic position. According to reports, 20 to 25% of people globally are afflicted with the disorders (Havlickova et al., 2018). The frequency of superficial skin fungal infections among kids in underdeveloped countries ranges between 20 and 90% (Nweze

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and Eke, 2018). Numerous studies have been conducted on the secondary metabolites of medicinal plants and their antifungal efficacy against various fungal infections (Chahal et al., 2021). Numerous studies have been undertaken on various medicinal plant extracts in the hopes of identifying new and more effective antifungal chemicals. According to the World Health Organization, medicinal plants would be the best source of a variety of medications (Scorzoni et al., 2017).

Material and Methods

Collection of Clinical Specimens: During fieldwork, samples were collected from infected school children. Sample collection for dermatophyte infections varies according to the site of infection. In cases of Tinea capitis, infected hair shafts along with scalp scrapings are collected using sterile forceps or a scalpel, with preference given to broken hairs from the active margins of the lesion where fungal activity is highest. For Tinea corporis, skin scrapings are obtained from the active, advancing edge of the lesion after cleaning the area with 70% alcohol to reduce contamination. In the case of *Tinea pedis*, samples are collected from affected areas such as toe webs, soles, or other lesion sites, and if nails are involved, nail clippings or scrapings. The collected samples were transported to the laboratory of Biological Sciences, Karakoram International University, Gilgit for isolation.

Tinea capitis

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Figure. 1 Clinical presentation of fungal infections in sample school children

Culturing of Specimens

Sabouraud's Dextrose Agar (SDA) medium was prepared according to the manufacturer's instructions (Oxoid) by dissolving 65g in 1000 ml of distilled water, followed by sterilization through autoclaving. After cooling to approximately 45°C, 0.5g of cycloheximide was added to inhibit the growth of non-pathogenic saprophytic fungi, and 0.05g of chloramphenicol was incorporated to suppress bacterial contamination. The clinical specimens were inoculated onto the prepared SDA plates and incubated at 27±2°C for a period of 2–4 weeks. The culture plates were examined periodically for the appearance of fungal growth (Kwon-Chung and Bennett, 1992). For detailed identification, a small portion of the fungal colony was aseptically transferred using a sterile needle onto a clean glass slide containing a drop of lactophenol cotton blue (LPCB) stain. The preparation was gently covered with a coverslip and examined under a light microscope at 40× magnification to observe morphological characteristics for the identification of the respective fungal pathogens.

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Plant materials used in the experiments. Five plants were collected during the field visit and brought to the laboratory. These samples were washed with water and shade-dried. After drying, ground to make fined power. Fifty grams of powder were mixed with ethanol and distilled water at ratio of 20:80 v/v for twenty minutes, then left in dark glass bottles for three days. Filter the extract through thin cheesecloth sheets. The final extracts were exposed to 60°C in a water bath for 30 min for ethanol evaporation. The extracts were then stored in a refrigerator at 5°C until use.

In vitro bioassay

Food poison technique was applied to evaluate the antifungal potential of the plant extract as described by Manmohan and Govindaiah (2012). Three concentrations (8, 16 and 24%) with three replications of each extract were used. Sabouraud dextrose agar (SDA) as nutrient medium. The vigorously growing culture of fungal species was prudently cut using a gel cutter and moved aseptically to the center of each Petri dish comprising the poisoned solid medium. Control was sustained by growing the cultures on SDA without the botanical extract. All the petri dishes were incubated at 27 ±2°C for seven days. After an incubation period, bio-efficacy was calculated in terms of percentage growth inhibition according to the following formula [Jayshree et al., 2012)

$$I\% = [(dc - dt)/dc] \times 100$$

(I%=Inhibition percentage; dc=colony diameter in control, dt=colony diameter in treatment)

All experiments were carried out in triplicate.

Results

Table 1 presents the antifungal efficacy of different phytoextracts against *Tinea capitis* in terms of percentage inhibition at three concentrations (8, 16, and 24%). The results clearly demonstrate a concentration-dependent increase in antifungal activity for all tested plant extracts. Among the evaluated phytoextracts, *A. sativum* exhibited the highest inhibitory effect at all concentrations, with inhibition percentages of 48.11, 66.43, and 81.74% at 8, 16, and 24%, respectively. These values were significantly higher ($p<0.05$) compared to other treatments, indicating their strong antifungal potential. *T. vulgaris* (thyme) ranked second, showing inhibition of 42.32, 60.43, and

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76.64% across the respective concentrations, followed by *A. indica*, which demonstrated moderate antifungal activity (40.51, 58.04, and 74.30%). In contrast, *M. piperita* and *C. spinosa* exhibited comparatively lower inhibitory effects. Statistical analysis using ANOVA followed by Tukey's HSD test confirmed that the differences among treatments were significant ($p<0.05$), as indicated by different letter groupings within each column. The low standard error of the mean (0.70, 0.40, and 0.27) and coefficient of variation (1.61, 0.94, and 0.64) suggest high precision and reliability of the experimental data. Figure 2 illustrates a clear concentration-dependent increase in inhibition of *Tinea capitis* by all plant extracts. *Allium sativum* exhibited the highest antifungal activity, followed by *Thymus vulgaris* and *Azadirachta indica*. *Mentha piperita* and *Capparis spinosa* showed comparatively lower inhibition across all tested concentrations. More or less similar behaviour of plant extract was observed against the pathogen *Tinea corporis and Tinea pedis* (Table 2-3 and Figure 3-4). Table 4 presents the average inhibition rates of different plant extracts across all tested fungi and concentrations. A clear trend of increasing antifungal activity with rising concentration was observed for all extracts. *A sativum* showed the highest inhibition at all levels (48.1%, 67.1%, and 81.2%), with the greatest overall mean (65.5), indicating its superior antifungal efficacy. *Thymus vulgaris* ranked second (overall mean 59.7), followed by *A. indica* (55.5), both demonstrating strong inhibitory potential. In contrast, *M. piperita* (36.7) and *C. spinosa* (27.1) exhibited comparatively lower antifungal activity. Table 5 further supports these findings, where *A. sativum* recorded the lowest LC_{50} value (12.18%), indicating the highest potency. *T. vulgaris* and *A. indica* showed moderate LC_{50} values (15.28 and 15.59%), while *M. piperita* required a much higher concentration (25.15%). LC_{50} for *Capparis spinosa* was unreliable due to insufficient data.

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Table 1. In vitro screening of plant extracts against *T capitis* by poison food technique.

Scientific name	Common name	Con...(8%)	Con...(16%)	Con...(24%)
Allium sativum	Garlic	48.11±0.39[a]	66.43±0.42[a]	81.74±0.62[a]
Thymus vulgaris	Thyme	42.32±1.80[b]	60.43±1.04[b]	76.64±0.44[b]
Azadirachta indica	Neem	40.51±0.45[c]	58.04±0.50[c]	74.30±0.46[c]
Mentha piperita	Peppermint	22.36±0.31[d]	35.34±0.33[d]	52.63±0.34[d]
Capparis spinosa	Caper bush	16.47±0.28[e]	25.36±0.08[e]	38.40±0.12[e]
	St.Err.com.	0.70	0.40	0.27
	CVC	1.61	0.94	0.64

Mean±SD within a column followed by the same letter does not differ significantly (ANOVA followed by Tukey's HSD test, $p<0.05$). Each value is the mean of 3 replications.
Con...=Concentration

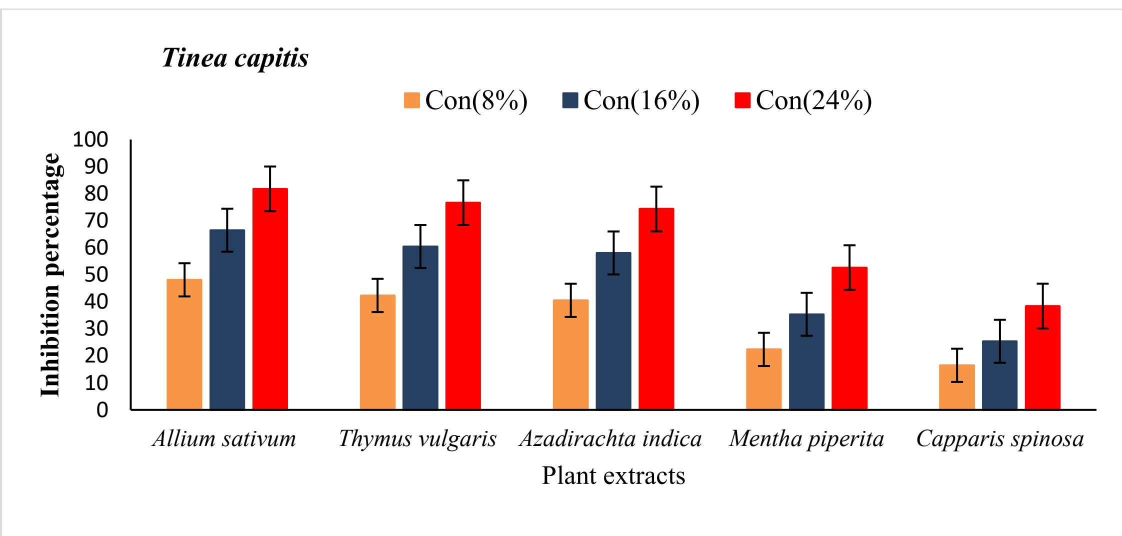

Figure 2. Mean inhibition percentage of botanical extract against the pathogen *T. capitis*

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Table 2. In vitro screening of plant extracts against *T corporis* by the poison food technique

Scientific name	Common name	Con...(8%)	Con...(16%)	Con...(24%)
Allium sativum	Garlic	48.82±1.32[a]	64.16±2.00[a]	79.71±1.60[a]
Thymus vulgaris	Thyme	43.86±3.21[b]	62.66±1.99[a]	77.40±1.11[ab]
Azadirachta indica	Neem	42.97±2.51[b]	56.56±3.96[b]	73.90±3.01[b]
Mentha piperita	Peppermint	26.00±2.09[c]	33.83±2.41[c]	52.29±2.29[c]
Capparis spinosa	Caper bush	20.09±2.53[d]	26.96± 0.23[d]	41.04 ±1.38[d]
	St.Err.com.	1.35	2.16	1.71
	CVC	3.13	4.98	3.94

Mean ± SD within a column followed by the same letter does not differ significantly (ANOVA followed by Tukey's HSD test, p<0.05). Each value is the mean of 3 replications.
Con...=Concentration

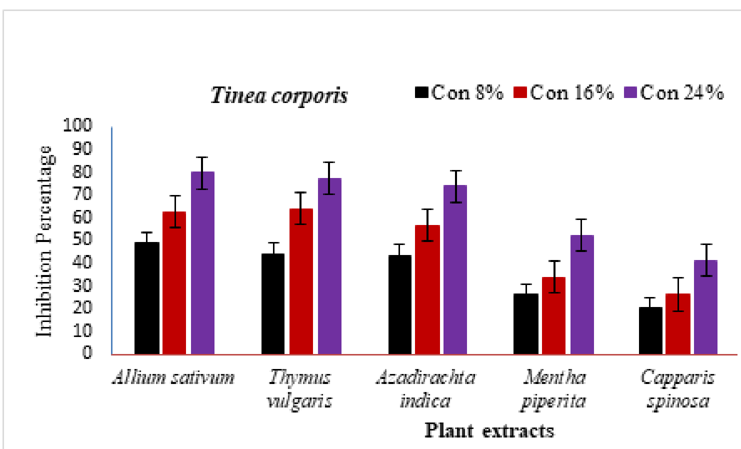

Figure 3. Mean inhibition percentage of botanical extract against the pathogen *T. corporis.*

Table 3. In vitro screening of plant extracts against *T. pedis* by poison food technique.

Scientific Name	Common name	Con...(8%)	Con...(16%)	Con...(24%)
Allium sativum	Garlic	44.54±2.06[a]	62.52±3.23[a]	80.60±0.66[a]
Thymus vulgaris	Thyme	39.62±1.39[b]	52.40±0.81[b]	69.42±3.72[b]

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Azadirachta indica	Neem	35.44±1.87[c]	51.29±1.55[b]	69.150±1.45[b]
Mentha piperita	Peppermint	20.80±0.64[e]	32.03±1.00[e]	48.87±0.60[e]
Capparis spinosa	Caper bush	16.26±1.06[f]	24.31±0.62[g]	37.29±0.67[g]
	St.Err.com.	1.01	1.48	1.68
	CVC	2.13	3.10	3.52

Mean±SD within a column followed by the same letter does not differ significantly (ANOVA followed by Tukey's HSD test, $p<0.05$). Each value is the mean of 3 replications.
Con…=Concentration

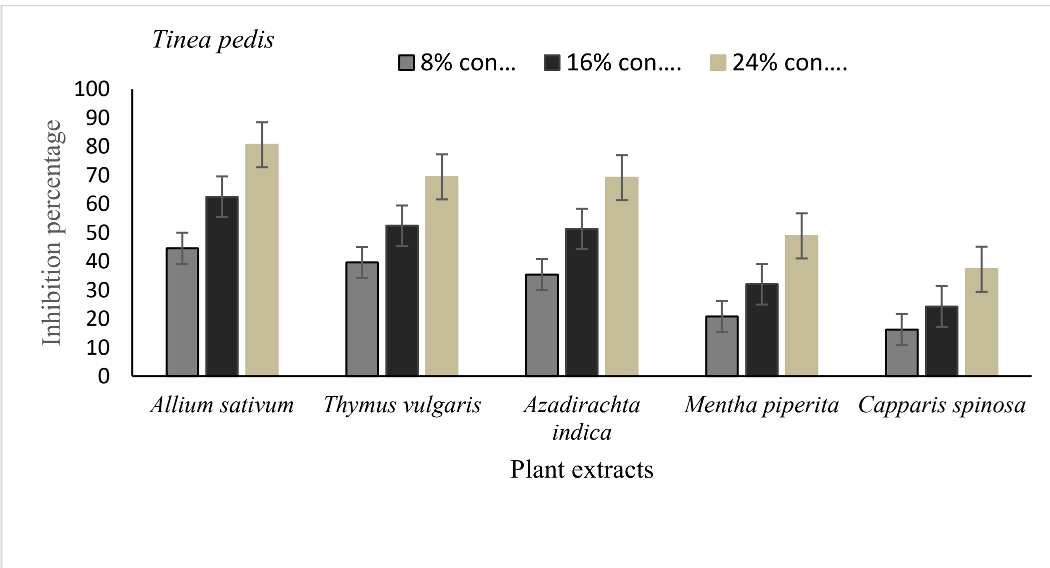

Figure 4. Mean inhibition percentage of botanical extract against pathogen *T. pedis*

Table 4 Average inhibition rate of each plant extract across all pathogens and concentrations.

Scientific Name of Plants	Con…(8%)	Con…(16%)	Con…(24%)	Overall Mean (%)
Allium sativum	48.1	67.1	81.2	65.5
Thymus vulgaris	42.6	60.5	75.9	59.7
Azadirachta indica	38.8	56.0	71.8	55.5

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Mentha piperita	22.5	35.0	52.6	36.7
Capparis spinosa	16.8	25.7	38.9	27.1

Table 5. LC_{50} values of different plant extracts

Plant Extracts	LC_{50} (Concentration %)
Allium sativum	12.18
Thymus vulgaris	15.28
Azadirachta indica	15.59
Mentha piperita	25.15
Capparis spinosa	Not reliable (data insufficient)

Discussion

The present study demonstrates that medicinal plant phytoextracts exhibit differential yet consistently concentration-dependent antifungal activity against *Trichophyton* species (*T. capitis, T. corporis,* and *T. pedis*). Across all fungal pathogens tested, inhibition increased markedly with extract concentration, confirming dose–response behavior that is characteristic of biologically active phytochemicals. Among all phytoextracts, Allium sativum (garlic) consistently emerged as the most potent antifungal agent. Its superior performance across fungi, concentrations, and statistical groupings is biologically plausible and aligns with extensive literature attributing garlic's antifungal activity to allicin and other sulfur-containing compounds, which disrupt fungal cell membranes, inhibit thiol-containing enzymes, and interfere with oxidative metabolism (Ankri & Mirelman, 1999; Borlinghaus et al., 2014). The low LC_{50} value recorded for garlic further reinforces its high intrinsic potency, suggesting that relatively small doses are sufficient to achieve meaningful fungal inhibition. Thymus vulgaris (thyme) and Azadirachta indica (neem) consistently ranked second and third in efficacy. The antifungal activity of thyme is commonly attributed to thymol and carvacrol, which cause membrane destabilization and leakage of intracellular contents (Burt, 2004).

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Neem's effectiveness, on the other hand, is linked to compounds such as azadirachtin and nimbidin, which exhibit antifungal, anti-inflammatory, and growth-regulatory effects (Biswas et al., 2002). The clustering of garlic, thyme, and neem into the same high-inhibition groups across dendrograms supports the notion that these extracts share strong and reliable antifungal bioactivity. Their consistent placement as intermediate inhibitors suggests that they may be more suitable as adjuncts rather than stand-alone antifungal agents. In contrast, caper bush repeatedly demonstrated weak antifungal activity and clustering at the lower end of inhibition gradients. The relatively poor performance of these extracts may reflect either a lower abundance of antifungal compounds or reduced extractability using the employed solvent system. Interestingly, despite low absolute inhibition, Pearson's correlation analysis revealed strong positive correlations among nearly all extracts, indicating that even weaker extracts follow similar proportional inhibition trends across concentrations. This suggests that concentration responsiveness is conserved, although efficacy magnitude differs substantially. Despite these strengths, the study has several important limitations. First, all assays were conducted in vitro, which may not fully reflect antifungal performance under clinical or field conditions, where host immunity, skin microenvironment, and microbial interactions play critical roles. Second, crude extracts were used, meaning that active compounds were neither isolated nor quantified, potentially masking synergistic or antagonistic effects among phytochemicals. Third, a single solvent system and limited concentration range were employed, which may have constrained the observable activity of certain extracts. Additionally, cytotoxicity and safety assessments were beyond the scope of this study, limiting immediate translational applicability. Future research should therefore focus on phytochemical profiling, bioassay-guided fractionation, and in vivo validation to confirm efficacy and safety. Exploring synergistic combinations, particularly among garlic, thyme, and neem, may further enhance antifungal effectiveness while reducing required dosages. Such work could contribute meaningfully to the development of eco-friendly, plant-based antifungal formulations, especially relevant for regions with limited access to conventional antifungal drugs.

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Conclusion

This study demonstrated that selected medicinal plant extracts possess significant antifungal activity against *T. corporis* and related fungal isolates, with efficacy strongly dependent on concentration. Garlic extract was the most effective, followed by thyme, and neem highlighting their potential as natural antifungal agents. In contrast, caper bush were the least effective, though they exhibited moderate inhibition at higher doses. The findings support the potential application of garlic, thyme, and neem extracts in developing plant-based antifungal formulations for the management of dermatophyte infections. Further research is recommended to isolate and characterize the specific bioactive compounds, evaluate synergistic effects in combination therapies, and test their efficacy in clinical or field settings. The development of phyto-based antifungal agents offers a promising, eco-friendly, and cost-effective alternative to synthetic antifungal drugs, which are often associated with resistance and side effects.

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Ethical Approval: The Institutional Review Board of Karakoram International University Gilgit approved this study vide letter KIU/BEC/1214.

Conflict of Interest: The authors declare no conflict of interest

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