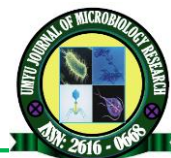





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Antifungal Activity of *Allium sativum* (Garlic) and *Zingiber officinale* (Ginger) Extracts against Dermatophytes Isolated from *Tinea Capitis* in Children

Temilola C. Otegwu^{1*} , Ocholi J. Adamu¹, Haruna Adamu¹, Patience M. Danzaria¹ and Aliyu, Abdulwahab¹

¹Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Gombe State University, Gombe, Nigeria.

Correspondence: +234 8035921523, temitegwu@gmail.com.

Abstract

Tinea capitis, or dermatophytosis, is a prevalent infection in school-age children worldwide, leading to school absenteeism and educational setbacks. Ginger (*Zingiber officinale*) and garlic (*Allium sativum*) have demonstrated antifungal properties. This study aimed to assess the efficacy of three extracts (aqueous, ethanolic 70%, and methanolic 70%) of *Zingiber officinale* and *Allium sativum* against dermatophytic fungi isolated from the hair scrapings of 60 elementary school students with clinical signs of *Tinea capitis* in Balanga LGA Gombe State, North-East Nigeria. The antifungal susceptibility was determined using the cup plate method and compared with griseofulvin at 1 mg/mL. The dermatophytes isolated included *Trichophyton mentagrophytes* (25%), *Microsporum canis* (20%), *Microsporum gypseum* (12%), *Trichophyton rubrum* (14%), *Trichophyton verrucosum* (10%), *Trichophyton schoeleinii* (8%), and *Trichophyton tonsurans* (8%). The efficacy of garlic and ginger varied among the dermatophyte species. *Trichophyton rubrum* showed the highest susceptibility to the methanolic garlic extract, followed by *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Microsporum canis*, *Trichophyton verrucosum*, *Trichophyton schoeleinii*, and *Trichophyton tonsurans*. For ginger, *Trichophyton mentagrophytes* was most susceptible, followed by *Microsporum gypseum*, *Trichophyton schoeleinii*, *Trichophyton verrucosum*, *Trichophyton tonsurans*, *Trichophyton rubrum*, and *Microsporum canis*. The methanolic garlic extract and the ethanolic ginger extract showed inhibition zones ranging from 12.93 to 25.87 mm and 12.0 to 24.9 mm, respectively. Aqueous extracts of both herbs exhibited the lowest inhibition zones. *Trichophyton mentagrophytes* were identified as the primary agent of *Tinea capitis* in the study area, caused by both anthropophilic and zoophilic dermatophytes. The study confirmed that ginger and garlic extracts significantly inhibited the growth of isolated dermatophytes, supporting their potential as sources of antifungal medications for managing dermatophytic diseases.

Keywords: *Allium sativum*, *Zingiber officinale*, *Tinea capitis*, methanolic, ethanolic

INTRODUCTION

Fungi are one of the most neglected disease-causing organisms in society, presenting a significant increase in frequency and cause of morbidity and mortality (Koushlesh *et al.*, 2020; Rayens and Norris, 2022). In certain regions of the globe, such as Nigeria, there has been notable evidence showcasing a significant occurrence of severe fungal infections. Oladele and Denning (2014) highlighted that approximately 11.8% of Nigerians are believed to have experienced a serious fungal infection within a single year, resulting in an estimated 960,000 fatalities. *Tinea capitis* also referred to as "Herpes tonsurans," "ringworm of the hair," "ringworm of the scalp," "scalp ringworm," and "Tinea tonsurans," is a globally problematic fungal infection (dermatophytosis) of the scalp (Al Aboud and Crane, 2023). Mycotic fungi that tend to colonize and infect the surface layers of

the skin, hair, or nails are referred to as dermatoses (Smith and McGinnis, 2011). Walker and McGinnis (2014) have classified these dermatophytes into three groups: trichophyton, microsporum, and epidermophyton. Dermatophytes are a class of fungi that infect the tissue of the living host's skin, hair, and nails in the stratum corneum. These dermatophytes are classified into anthropophilic species (like *Trichophyton violaceum*, *Trichophyton rubrum*, and *Microsporum audouinii*), geophilic species (such as *Microsporum gypseum* and *Microsporum nanum*), and zoophilic species (including *Trichophyton mentagrophytes*, *Trichophyton verrucosum*, and *Microsporum canis*), based on their ecological preferences; however, some dermatophytes change their ecological niche (Achterman and White 2012). Numerous prescription medications have been used to treat tinea.

Tinea capitis is treated with oral antifungal medications such as terbinafine, fluconazole, and itraconazole (Gupta *et al.*, 2001; Shemer *et al.*, 2012). Over time, the efficacy of griseofulvin, the established treatment for tinea capitis, seems to be diminishing. (Shemer *et al.*, 2012; Alkwesani *et al.*, 2019).

Aspirin, quinine, digitalis, and opium are only a few of the medications that have been used traditionally as herbal remedies. Their purity and quantification have made them more predictable (Dhupe, 2020). Herbal treatments frequently contain a more nuanced and sophisticated combination of ingredients, and occasionally, they can provide access to substances that the pharmaceutical industry has not yet fully utilized. According to Pfaller *et al.* (2017), some of the most recently approved antifungal medications are made from natural compounds that fungi create. These treatments have demonstrated good efficacy against fungal infections. Due to their excellent activity and far fewer adverse effects than synthetic medications, herbal formulations have always drawn a lot of interest (Chakraborty, 2018). Resistance strains have emerged as a result of the widespread use of antifungal medicines, which have numerous side effects in terms of toxicity, efficacy, and cost (Campoy and Adrio, 2017). Thus, it is necessary to create new antifungal medications that are less expensive, have fewer adverse effects, and have lower dose requirements (Taha *et al.*, 2016).

These days, organically prepared herbs are used to cure a variety of illnesses, including infectious ones. The field of traditional herbal medicine is expanding and has a rich history. Conventional medicine encompasses all areas of knowledge, expertise, and health maintenance, as well as the prevention, diagnosis, and treatment of mental and physical illnesses (WHO, 2022). The World Health Organization (WHO, 2002) has acknowledged traditional medicines as a fundamental component of primary healthcare despite the remarkable progress made in conventional medicine (Patil and Saini, 2012). This recognition stems in part from the fact that some conventional drugs have proven to be ineffective or have severe side effects (Njoroge and Bussmann, 2006; Bhadauria and Kumar, 2011). Plant parts such as stems, leaves, roots, seeds, fruits, and flowers all contain phytochemicals. But a lot of phytochemicals also have antioxidant, chemopreventive, antibacterial, antifungal, and anti-allergic qualities (Amlan, 2012).

Garlic is one of the plants with the strongest antifungal and antibacterial effects, and ginger has excellent antifungal qualities as well. A paste made from crushed garlic and olive oil has

been used to treat fungal infections (Bayan *et al.*, 2014).

To the best of our knowledge, Balanga LGA in Gombe, North-East Nigeria, has not had a comprehensive assessment of tinea infections among primary school students. Thus, using the cup plate diffusion method, the common forms of Tinea capitis among students in Balanga LGA, Gombe, North-East Nigeria, were examined, and their susceptibility patterns to aqueous, methanolic, and ethanolic extracts of garlic and ginger were established against a conventional antifungal agent (griseofulvin).

MATERIALS AND METHODS

Study Area

The research was carried out in Balanga LGA, located in Gombe, North-East Nigeria, focusing on primary school children within the community. The primary sources of livelihood in this area are farming and low-income trading. Sixty (60) primary school students were chosen at random from schools in easily reachable areas for the research. The selection was based on observable signs of Tinea infections, such as erythema, alopecia, scaling, crusting, circinate lesions, follicular inflammation, and purities.

Study Population

Participants chosen for the study were under 14 years old. The focus was solely on primary school children due to the higher prevalence of Tinea infections among this demographic (Adesiji *et al.*, 2019; Ezomike *et al.*, 2021). Pupils without evident signs of Tinea infections, non-primary school children, and those undergoing antifungal treatment, whether conventional or traditional, within 2 weeks after the sample collection period were excluded.

Ethical Approval

Ethical approval was obtained from the departmental board of Pharmaceuticals Microbiology and Biotechnology and the Ministry of Health Gombe state with reference number MOH/ADM/621/V.1/447.

Consent

Written informed consent was obtained from parents or guardians of the children, and assent of each child was obtained before recruitment and maintain confidentiality.

Specimen Collection

Scalp hair samples were obtained from sixty schoolchildren by scraping the affected areas with a sterile scalpel, one per child. Before collection, the sampling site was cleaned with 70% (v/v) ethanol.

The scrapings were placed on sterile paper, labelled, and sealed in envelopes to protect them during transport to the lab. Each envelope was marked with the student's details—name, age, sex, and collection date. Adequate samples were collected to facilitate all necessary analyses.

Each sample was divided into two parts: one for direct microscopy and the other for culture.

All specimens were cultured using a pair of MycoselR agar plates per sample. Through sub-culturing onto plain Sabouraud Dextrose Agar (SDA) from the original plate growth, identification and susceptibility tests were carried out.

Direct Microscopic Examination of Specimens

The method employed was adapted from [Ayodele et al. \(2021\)](#) with modifications. A wet mount was prepared for each specimen by transferring the scrapings to a grease-free microscope slide using sterile forceps to place the material onto two drops of 20% KOH solution. Subsequently, a cover slip measuring 22mm by 22mm was applied, and the slide was gently warmed over a low Bunsen flame to dissolve keratin and release fungal elements. Following this, the slides were left to stand for five minutes before being examined under low (10x) and high (40x) magnification. Identification of the etiologic agents was done based on the microscopic characterization of their spores and hyphae.

Identification of Dermatophytes by Culture

Each specimen was inoculated onto a pair of MycoselR agar plates by placing a portion of the scrapings centrally onto the surface of the medium using a sterile wire loop or forceps. One plate per sample was incubated at room temperature (25-30 °C), while the other was placed in an incubator set at 35-37 °C. Culture plates were inspected every other day for signs of growth, with cultures deemed negative only after four weeks of incubation. Once dermatophyte growth was confirmed, sub-culturing onto SDA was performed for further identification and susceptibility testing, According to [Baron et al., 2003](#). Colonies displaying cottony, wooly, powdery, or fluffy characteristics were indicative of dermatophyte growth.

Herb Collection and Identification

Fresh garlic bulbs (*Allium sativum*) and ginger rhizomes (*Zingiber officinale*) were purchased from a local market in Gombe, Gombe State, Nigeria. These specimens were assigned identification codes PCG/GSU/00091 and PCG/GSU/00054, respectively, at the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical

Sciences, Gombe State University, Gombe, Gombe State, Nigeria.

Herb Extraction Procedure

Garlic (*Allium sativum*): After being dehusked, the bulbs were cleaned with sterile distilled water, allowed to dry naturally, and then pulverized into a powder using mortar and pestle. The procedure outlined by [Doherty et al. \(2010\)](#) was used to perform the extraction. The powder sample was extracted using water, ethanol, and methanol as solvents. 100 g of powdered sample was extracted with 1000 ml distilled water, 70 % ethanol, and methanol, respectively. The sample was soaked overnight for 24 hours and filtered with muslin cloth. The extract was collected in a round bottom flask, concentrated using a rotary evaporator and then oven dried at 40 °C.

Ginger rhizome extracts were prepared according to the method reported by [Tanweer et al., 2020](#). 50 kg healthy ginger rhizomes were washed with distilled water, cut into pieces, and dried for 72 hours at 60°C. The dried rhizome pieces were pulverized into powder and filtered through a 100-mesh sieve. The filtered powder (5g) was dissolved in 5mL of 70% ethanol and 5mL of methanol and then was extracted in an extractor at 80°C for 9 hours. All extracts were collected, and the alcohol evaporated using a rotary evaporator at 60°C for 1 hour to obtain a paste.

Aqueous Extract preparation was done by weighing 40g of ginger powder and 120 ml of distilled water in a beaker. This was set on a hot plate and vigorously stirred at 30°C for 20 minutes to achieve a uniform mixture, and a paper filter was used to extract the pure filtrate. To get the crude extract, excess water was evaporated by placing sample tubes in a hot air oven set at 80 °C for 24 hours.

Reconstitution of Extract

The dried extracts were reconstituted by dissolving 1 g of extract in 100 ml of water. 1 mL of this mixture was then added to 9 mL of sterile distilled water to give a concentration of 1 mg/ml.

Preparation of standard

Griseofulvin, initially dissolved in sterile distilled water at a concentration of 50 mg/mL, underwent dilution steps to achieve a final working concentration of 1 mg/ml.

Specimen inoculation

Sabouraud Dextrose Agar (SDA) was prepared and distributed into sixty sterile Petri dishes. Each dish received the respective specimen and was then left to incubate at room temperature to encourage growth.

Antifungal susceptibility test

Using the well-in-agar method, the anti-dermatophytic activity of garlic's ethanolic, methanolic, and aqueous extracts was investigated. After being added to sterilized petri dishes, SDA was left to crystallize. Each plate was swabbed with a standard inoculum of the fungal isolate, which was produced in normal saline and left to stand for three minutes. Using a sterile cork borer, wells of 6 mm in diameter were created on the agar medium's surface. One well was filled with griseofulvin at a concentration of 1 mg/ml after the base was sealed with 1-2 drops of melted SDA. Each well

contained 0.2 ml of the extracts of ginger and garlic. For twenty-four hours, the plates were incubated at 28 °C. The agar wells' surrounding inhibition zones were measured in millimeters. These measurements were then compared with breakpoint tables obtained from the Clinical Laboratory Standard Institute 2020 edition.

RESULTS

A total of 60 children were recruited as participants from two primary schools. Figure 1 shows the distribution of the participants from Pilot Primary School Cham (66.67%) and Kwasi Primary School (33.3%).

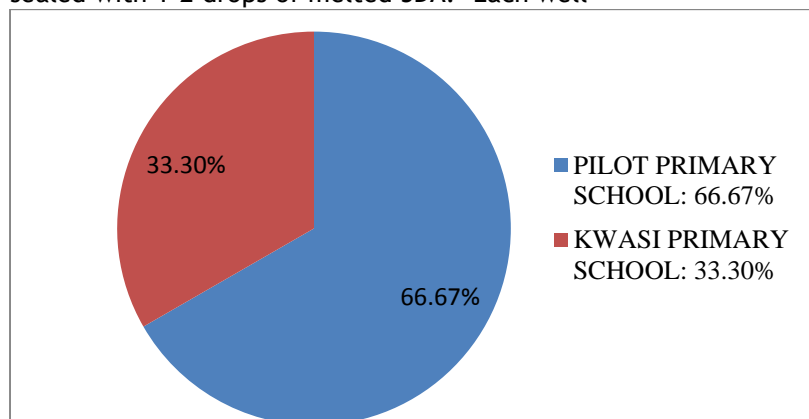


Figure 1: Distribution of Pupils in Primary Schools

The results of the microscopy presented in Figure 2 indicate dermatophytes were isolated from 58 (96.33%) of the participants, while 2 (3.33%) participants were infected with *Aspergillus niger*, which is not implicated in *Tinea capitis* infection. *Trichophyton mentagrophytes* were the most prevalent dermatophyte isolated (15%). The other dermatophytes isolated were *Trichophyton mentagrophyte*, *Mricosporum canis*, *Trichophyton rubrum*, *Microsporium gypseum*, *Trichophyton schoenleinii*, *Trichophyton verrucosum*, and *Trichophyton tonsurans*.

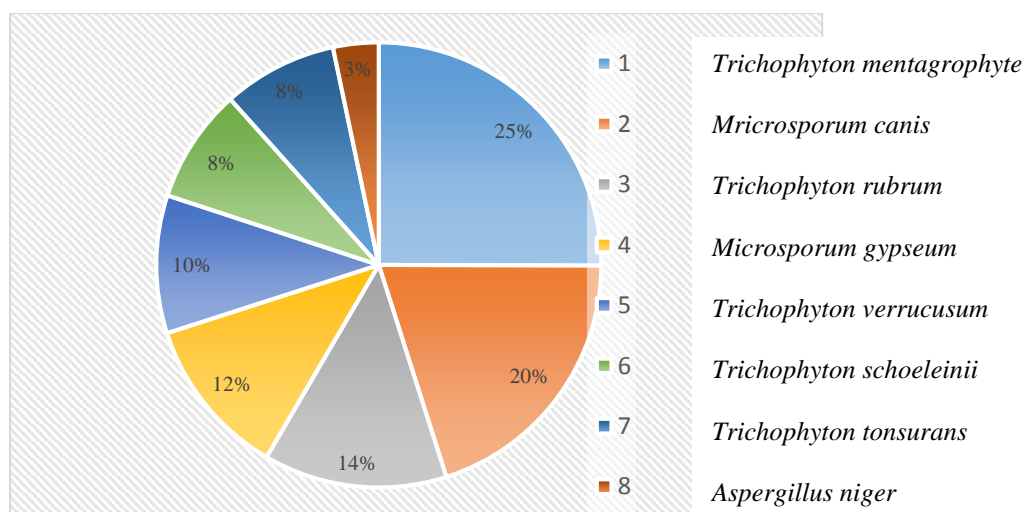


Figure 2: Direct Result of Fungal Isolates

Antifungal efficacy

Table 1 represents the diameter zones of inhibition obtained for methanolic, ethanolic, and aqueous crude extracts of garlic (*Alium sativa*). The aqueous, methanolic, and ethanolic extracts of garlic inhibited the fungi, with zones

ranging from 12.93 to 25.87mm. The methanolic extract had the best activity amongst the extracts tested. The largest zone of inhibition was obtained against *Trichophyton rubrum* for garlic methanolic crude extract.

Comparative analysis of the garlic extracts against the dermatophytes isolated shows a better activity for the methanolic extract against four (4) dermatophyte species and a better activity of the ethanolic extracts against the four (4) other dermatophytes. From the results obtained, the activity of both the methanolic and ethanolic crude extracts of *Allium sativum* can be considered to be on par with the dermatophytes isolated. The activity

of the aqueous garlic extract shows smaller diameter zones of inhibition against all dermatophytes compared to both methanolic and ethanolic extracts.

The diameter zone of inhibition of all extracts compared to dilutions of the standard drug Griseofulvin shows better activity of all three (3) extracts to Griseofulvin against all isolates except for *Trichophyton schoenleinii*.

Table 1: Zone of inhibition (mm) for Methanolic, Ethanolic, and Aqueous extract of garlic (*Allium sativum*)

| Dermatophyte isolate | Diameter Zone of Inhibition (mm) | | | |
|-----------------------------------|----------------------------------|-----------|---------|--------------|
| | Methanolic | Ethanolic | Aqueous | Griseofulvin |
| <i>Mricosporum canis</i> | 22.44 | 19.63 | 16.40 | 0.00 |
| <i>Microsporum gypseum</i> | 24.22 | 23.67 | 16.53 | 0.00 |
| <i>Trichophyton tonsurans</i> | 19.88 | 20.95 | 0.00 | 0.00 |
| <i>Trichophyton schoenleinii</i> | 20.07 | 21.82 | 15.66 | 31.00 |
| <i>Trichophyton rubrum</i> | 25.87 | 24.81 | 18.33 | 0.00 |
| <i>Trichophyton verrucosum</i> | 21.33 | 22.33 | 15.67 | 0.00 |
| <i>Trichophyton mentagrophyte</i> | 24.00 | 20.40 | 12.93 | 0.00 |

Table 2 shows the diameter zones of inhibition for methanolic, ethanolic, and aqueous extracts of *Zingiber officinale* (ginger). The antifungal activity of the aqueous, methanolic, and ethanolic extracts of ginger inhibited the fungi, with zones ranging from 12.00 to 24.6mm. The aqueous extract of ginger showed lower activity, while the methanolic extract showed the highest activity against the isolates tested. The largest diameter zone of inhibition was obtained from

the methanolic crude extract activity against *Trichophyton schoenleinii*. The activity of both methanolic and ethanolic extracts against each isolate was similar and comparable.

The standard drug Griseofulvin also showed activity against *Trichophyton schoenleinii* alone, with better activity for this isolate than the three (3) crude extracts tested. It, however, had no effect on the other dermatophytes tested.

Table 2: Zone of inhibition in mm for Methanolic, Ethanolic, and Aqueous extracts of ginger (*Zingiber officinale*)

| Dermatophyte isolate | Diameter Zone of Inhibition (mm) | | | |
|-----------------------------------|----------------------------------|-----------|---------|--------------|
| | Methanolic | Ethanolic | Aqueous | Griseofulvin |
| <i>Mricosporum canis</i> | 20.62 | 19.61 | 15.30 | 0.00 |
| <i>Microsporum gypseum</i> | 21.86 | 23.44 | 15.56 | 0.00 |
| <i>Trichophyton tonsurans</i> | 20.80 | 20.51 | 0.00 | 0.00 |
| <i>Trichophyton schoenleinii</i> | 24.93 | 22.81 | 14.26 | 31.00 |
| <i>Trichophyton rubrum</i> | 21.33 | 20.19 | 12.00 | 0.00 |
| <i>Trichophyton verrucosum</i> | 20.92 | 20.61 | 14.54 | 0.00 |
| <i>Trichophyton mentagrophyte</i> | 23.03 | 24.60 | 0.00 | 0.00 |

DISCUSSION

Tinea capitis is a significant global health issue affecting school children, with a prevalence of 19.7% in developing nations (Sunil et al., 2019). The need for safer, more effective chemotherapeutic agents has increased due to antifungal drug resistance and adverse effects. Despite advancements in prevention and treatment, tinea capitis remains a global public health concern, particularly among elementary school students in economically developing nations. Reports suggest that 10-30% of African primary school students are affected (Ayodele et al., 2021). Cross-sectional studies in Ivory Coast and Nigeria have also shown high prevalence of 11.3% (Menan et al., 2002; Ayabimpe et al.

(2008). Many countries in the world have long been using medicinal plants to treat skin conditions, including mycotic infections (Balakumar et al., 2011).

To the best of our knowledge, no research on Tinea capitis in elementary school students has been done in Gombe State, North-East Nigeria, where this work was conducted. According to the results, of the 60 samples that were clinically suspected of causing tinea capitis, 58 (96.67%) were found to be dermatophytes under a microscope, and 2 (3.33%) were found to be *Aspergillus niger*. *Trichophyton mentagrophyte* was the main cause of Tinea capitis in the children (25%).

According to reports from Adefemi *et al.* (2011) in Kwara, Ayanlowo *et al.* (2014) in Ogun, and Okolo *et al.* (2019) in a rural context in Jos, North Central Nigeria, *Trichophyton mentagrophytes* was the predominant isolate. Furthermore, *Trichophyton mentagrophyte* was identified by Nweze (2001) as the primary cause of Tinea capitis in Anambra State, Nigeria. It is believed that the fungus is widespread and one of the most prevalent dermatophytes that affect both humans and animals (Hainer, 2003). Additional research done in various parts of Nigeria has demonstrated that different places have different causes of tinea capitis. In Borno State, *Trichophyton schoenleinii* was found to be the main cause of Tinea capitis cases.

Trichophyton soudanese was identified by Menan *et al.* (2002) as the most prevalent etiological agent of Tinea capitis in the distant Ivory Coast. *Microsporum audouinii* was noted as the predominant isolate in Mozambique. According to Sidat *et al.* (2007), these results show changes over time in the etiological agents of Tinea capitis, both within and between geographical areas.

Numerous plant species have had their antibacterial properties studied. Plant extracts with antibacterial, antioxidant, hemostatic, analgesic, and immune-stimulating properties have been used in traditional cultures to treat skin conditions (Jeruto *et al.*, 2016).

Based on the findings of this study, garlic extracts in their aqueous, methanolic, and ethanolic forms have antifungal action against fungi in zones of 12.93 to 25.87 mm. The antifungal activity of the methanolic extract was highest, whereas the aqueous extract displayed the lowest activity. *Allium sativum* (garlic) extract showed the biggest diameter zone of inhibition against *Trichophyton rubrum*. It is not surprising that garlic has been utilized as a folk remedy around the world since ancient times, as evidenced by the research on its anti-dermatophytes action conducted in Abia by Kanu *et al.* (2014) (Bhadoria and Kumar, 2011).

In this investigation, garlic extracts in aqueous, ethanolic, and methanolic forms showed a broad range of anti-dermatophytic effectiveness against seven dermatophytes isolated from sixty primary school students. This study is similar to that of Aala *et al.* (2013), who documented the efficacy of garlic against clinical isolates of dermatophytes, such as *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton verrucosum*, *Microsporum canis*, and *Epidermophyton floccosum*. Garlic extract was shown to be the most efficient natural antifungal agent when Narula and Sareen (2011) examined the effects of antifungals on keratinophilic fungi isolated from soil. *Allium*

sativum extract was the most effective extract, inhibiting growth by 47.5% to 100%.

This study made it evident that the extraction solvent had an impact on the garlic's level of anti-dermatophytic activity. Because they are organic solvents, methanol and ethanol will dissolve organic compounds more effectively, releasing the active ingredient needed for antifungal activity. *Trichophyton rubrum* exhibited the highest sensitivity to garlic extracts, followed by *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Microsporum canis*, *Trichophyton verrucosum*, *Trichophyton schoenleinii*, and *Trichophyton mentagrophyte*, in descending order. These findings, as presented in Table 1, align with the research conducted by Sidat *et al.* (2007), which similarly identified *Trichophyton rubrum* as the most susceptible dermatophyte to garlic plant extract. Conversely, Tadeq *et al.*, (2005) reported *Trichophyton mentagrophytes* as highly susceptible, indicating the distinctiveness of each plant extract and its interaction with specific fungi.

The study revealed that all seven isolates, with the exception of *Trichophyton schoenleinii*, were resistant to Griseofulvin, a medication that has been used for many years to treat Tinea capitis. This resistance may have been partially explained by repeated use. Furthermore, non-adherence resulting from extended use necessary for Griseofulvin treatment may also be a component of its resistance. Al-Refai *et al.* (2007) and Ayodele *et al.* (2021) had previously reported on this observation, pointing to potential causes for treatment failures that might have resulted from the drug's use.

These susceptibility data point to therapeutic difficulties with Griseofulvin; consequently, more susceptibility testing as well as research into novel medications, are needed.

CONCLUSION

The results of this investigation demonstrated that the extracted forms of ginger and garlic significantly inhibited the isolated organisms. Furthermore, the results of this study, when compared positively to earlier research on the antifungal activity of ginger and garlic, indicate that the plants may be a promising source of medications for the treatment of dermatophytic infections and, as such, may be applied topically as an adjuvant to oral therapy for the condition. It is important to support ongoing infection control monitoring, treatment, and evaluation in the local community and elementary schools nationwide. Encouraging good personal hygiene should never stop. This research could lead to the development of a new medication to combat dermatophytosis, which is a serious concern in the current antibiotic resistance period.

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