

Study of the Prevalence of dermatophyte in Wasit Governorate and Evaluation of the Efficacy of Extracts of Garlic (*Allium sativum*)

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ABSTRACT

This study assessed the prevalence of dermatophyte infections among patients at Al-Karama Teaching Hospital in Wasit Governorate, Iraq. A total of 100 samples were collected, with 88 positive for dermatophytes, yielding an 88% positivity rate. The predominant genera identified were Trichophyton (64.77%), Microsporium (23.86%), and Epidermophyton (11.36%), with Trichophyton rubrum being the most common species (39.77%). The most affected sites were Tinea manum (28.6%), Tinea pedis (22.9%), and Tinea facies (17.1%). Infection rates were higher in females (56.8%) and peaked in the 21-30 age group (51.4%). The antifungal activity of aqueous and alcoholic garlic extracts was evaluated, showing the alcoholic extract's inhibition rate against *T. rubrum* (35.96 mm) to be comparable to Clotrimazole (46.94 mm). High-Performance Liquid Chromatography (HPLC) identified eight phenolic compounds in the extracts, indicating garlic's potential therapeutic properties.

Keywords: Garlic (*Allium sativum*), Dermatophytosis, Dermatophytes, Trichophyton, Trichophyton rubrum

INTRODUCTION

Medicinal plants have played a crucial role in traditional medicine worldwide, with about 80% of the global population relying on them for their accessibility, affordability, and effectiveness, while minimizing the side effects of synthetic drugs. Despite the safety of many medicinal plants, some can be highly toxic (Okoye et al., 2014). The increased use of antifungal medications has led to resistance among fungal pathogens (Ghannoum and Rice, 1999), sparking interest in antimicrobial plants as potential therapeutic agents. Numerous studies have investigated the antifungal efficacy of plant extracts (Bereksi et al., 2018; Shiota et al., 2004; Mann et al., 2008; Hussain et al., 2012). Dermatophytes, which primarily infect keratinized tissues, cause significant skin infections, especially in warm, humid environments, with Epidermophyton, Microsporium, and Trichophyton being the main genera (Nweze, 2010). These fungi degrade keratinous tissues by secreting enzymes like endo- and exo-proteases (Monod, 2008). Treating these infections poses a major health challenge, particularly in developing regions, as synthetic drugs face resistance and adverse effects (Malheiros et al., 2005). This study focuses on garlic (*Allium sativum*) as a potentially safe and effective alternative for treating dermatophyte infections.

MATERIALS AND METHODS

Plant Materials Used in the Study

Plant samples were collected from local markets, cleaned, dried at room temperature, and ground using an electric grinder. They were stored in clean, dry plastic bags until laboratory use.

Preparation of Aqueous Extracts

For aqueous extracts, 20 grams of garlic powder were mixed with 400 mL of distilled water in a 1000 mL volumetric flask. This mixture was shaken in a water bath at 40°C for 24 hours, then filtered through medical gauze and sterilized. The filtrate was placed in a glass dish in an oven at 40°C for 48 hours until it formed a powder, which was collected in an airtight container and refrigerated until use.

Preparation of Alcoholic Extracts

Alcoholic extracts were prepared using 95% ethanol, following the same procedure as the aqueous extracts.

Sample Collection

Samples were collected from Al-Karama Teaching Hospital between November 20, 2023, and February 8, 2024. A total of 100 samples, including skin scrapings, hair, and nail clippings from dermatology patients aged 5 to 57 years, were gathered. The cohort included 57 females and 43 males. Direct examination and culture diagnostics were performed alongside a questionnaire on demographics and living conditions. Statistical analysis was conducted to track recurrent fungal infections over three months.

Direct Examination

Samples were placed on glass slides with a drop of 10% potassium hydroxide (KOH) and allowed to dissolve for 30 minutes. Microscopic examination followed to observe fungal hyphae and spores.

Culture of Samples

Samples were cultured on Sabouraud Dextrose Agar (SDA) with 0.05 grams of chloramphenicol added post-sterilization. A drop of 30% ammonium hydroxide was used to inhibit opportunistic fungi. Plates were incubated at 28°C for one to three weeks and monitored daily, and fungal colonies were identified using lactophenol cotton blue stain.

Assessment of Inhibitory Activity of Plant Extracts on Fungal Growth

The disc diffusion method evaluated the inhibitory effects of garlic extracts (aqueous and alcoholic) on dermatophyte growth. Sterilized 5 mm paper discs were soaked in varying concentrations (25%, 50%, 75%, and 100%) and placed on SDA inoculated with fungi. Plates were incubated at 25°C, and inhibition zones were measured in millimeters (Muthomi et al., 2008). Clotrimazole's effects were evaluated similarly (Jebara et al., 2022).

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was assessed to find the lowest concentration at which fungal growth is inhibited.

Active Ingredient Analysis in Garlic Extracts

Fifty microliters of each extract were analyzed using High-Performance Liquid Chromatography (HPLC Shimadzu-C-6A) (Table 1) following Hartley and Buchan's procedures. Peaks were detected with a UV detector, and standards were run for comparison. The analysis took place at the Ministry of Science and Technology, determining the concentration of isolated compounds (Hartley, D., & Buchan, J. (2010)).

Statistical Analysis

Statistical-Package-for-Social-Science, version 25.0 for windows was used to do statistical analysis on all data. All findings with a significant level ($P \leq 0.05$)

Table 1. Conditions of High-Performance Liquid Chromatograph used for separation of phenolic compounds from garlic

Parameter	Characteristic
Column dimensions	50 length × 2.6 mm diameter
Diatomite	Supleco wax 10
Attenuation	0.01 ppm
Rate of recorder	10 mm / minutes
Detector	UV – 280 nm at flow rate 1.0 ml /min
Volume injection sample	50 µl
Type of Column	C18-ODS (25 cm 4.6 mm)
Mobile face	(Methanol : D.W : formic acid) (70 : 25 : 5) , the column is C18-ODS (25 cm 4.6 mm)
Temperature	25 °C

RESULTS

A total of 100 samples were collected from Al-Karama Teaching Hospital for fungal culture and identification. Of these, 88 samples tested positive, yielding a positivity rate of 88%, while 12 were negative.

Among the positive cases, there were approximately 50 females and 38 males, resulting in infection rates of 56.8% for females and 43.18% for males (Figure 1). The most commonly isolated fungus was *Trichophyton rubrum*, accounting for 39.77% of cases, followed by *T. mentagrophytes* at 25% (Table 3). The site of infection for *T. rubrum* was predominantly *Tinea manuum* (28.6%), followed by *Tinea pedis* (22.9%), *Tinea facies*

(17.1%), Tinea cruris (11.4%), Tinea corporis (8.6%), and Tinea unguium and Tinea barbae (5.7% each) Table 4.

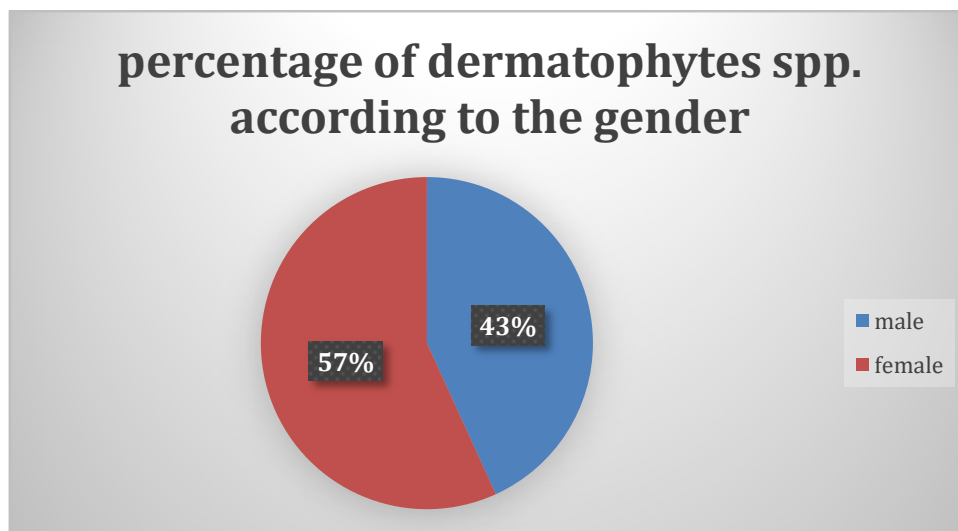


Figure 1. Type and percentage of dermatophytes spp. according to the gender

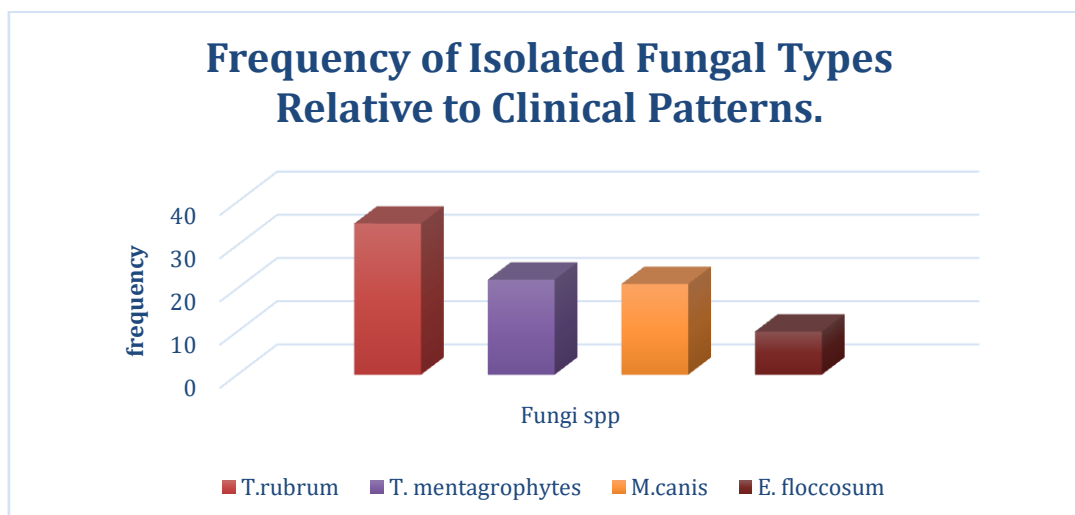


Figure 2. Frequency of Isolated Fungal Types Relative to Clinical Diagnosis .

Table 2. Distribution of T. rubrum Dermatophyte Infections Based on Site of Infection

Fungi spp	infection	Frequency	percent
T. rubrum	Tinea Pedis	8	22.9
	Tinea unguium	2	5.7
	Tinea corporis	3	8.6
	Tinea cruris	4	11.4
	Tinea manuum	10	28.6
	Tinea faciei	6	17.1
	Tinea barbae	2	5.7

The infection rate for T. rubrum was higher in males (60%) compared to females (40%). The highest infection rate occurred in the 21-30 age group (51.4%) Table 5. Additionally, Microsporumcanis accounted for 23.86% of infections, while Epidermophyton floccosum had the lowest at 11.36%.

Table 5: Distribution of T. rubrum Dermatophyte Infections Based Age Groups

Age(years)	Frequency	Percent
≥ 10	5	14.3

11-20	3	8.6
21-30	18	51.4
31-40	5	14.3
41-50	3	8.6
51-60	1	2.9
Total	35	100.0

Morphological Characteristics

T. rubrum colonies appeared white, slightly elevated, and granular on SDA medium, with powdery or fluffy textures and yellow-brown or reddish-brown undersides. Microscopic Characteristics Small microconidia were round and thin-walled, clustered like grape bunches. Macroconidia were club-shaped, multi-septate, and thick-walled, sometimes exhibiting spiral hyphae Figure 1

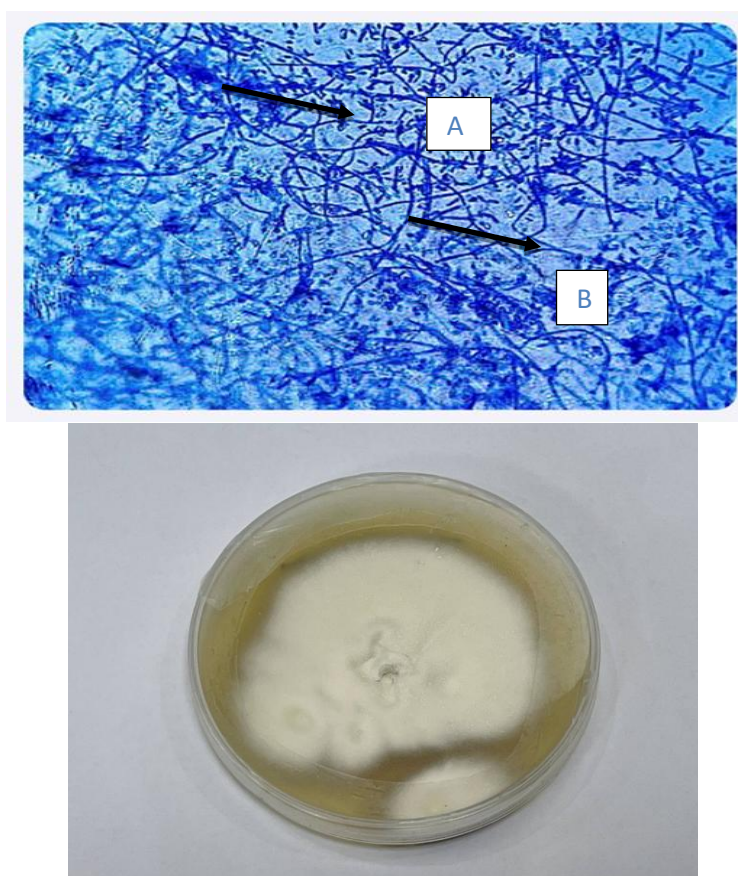


Figure 3: Morphological and Microscopic Characteristics of the fungus Rubrum T grown on SDA nutrient medium after 12 days (A) from incubation at 28°C (A) : C) Small conidia that appear on the fungal threads in a round and alternate arrangement on the hyphae. (B) : Large conidia resembling cigars, segmented with thick, smooth walls (photographed under a light microscope at X40)

Effect of Garlic Extracts on the Growth of *T. rubrum*

Fungal colony diameters for *T. rubrum* exposed to the alcoholic garlic extract were measured at 35.96 mm, 26.67 mm, 17.69 mm, and 8.61 mm for concentrations of 100%, 75%, 50%, and 25%, respectively. The aqueous extract resulted in colony diameters of 25.65 mm, 17.64 mm, 12.76 mm, and 6.67 mm at the same concentrations Table 6.

Table 6: Effect of Aqueous and Alcoholic Extracts of Garlic on Inhibition Zone Diameter (mm) of *T. rubrum* in Culture Medium

Treatment Extract	Type of Extract	Concentration	Zones of inhibition (mm)	
			100	35.96
		75	26.67	e

Allium sativum	Alcoholic	50	17.69	h
		25	8.61	l
	Aqueous	100	25.65	f
		75	17.64	h
		50	12.76	j
		25	6.67	n
Control (water)		0		
Control (Achohi)		0		
LSD 0.05		0.330		
P-value		0.001		

Chemical Detection of Active Components in Garlic Extracts

HPLC analysis revealed the presence of eight phytotoxins in the garlic extract, identified as phenolic compounds with varying retention times. The concentrations were ranked as follows: caffeic acid, ellagic acid, gallic acid, hydroxybenzoic acid, p-coumaric acid, protocatechuic acid, sinapic acid, and syringic acid Table -7

Table 7. The concentration of phytotoxins in the Garlic extract

No	Name	Allium (ppm)
1	Caffeic acid	15.9
2	Ellagic acid	42.5
3	Gallic acid	62.5
4	hydroxybenzoic acid	19.6
5	p-coumaric acid	22.6
6	Protocatechuic acid	18.9
7	Sinapic acid	9.8
8	syringic acid	22.6

DISCUSSION

Dermatophyte infections are a significant global health issue, particularly in developing countries, where warm, humid conditions and inadequate hygiene exacerbate the problem (Karan et al., 2009). This study identified seven species of dermatophytes affecting both males and females across various age groups, with *Tinea manuum* being the most prevalent (28.6%, 10 samples), especially among females aged 21-30. This finding contrasts with prior research that reported higher infection rates in the 31-40 age group (2011). The discrepancy may stem from increased interactions between younger individuals and domestic animals, which can facilitate the transmission of fungal infections.

The predominant dermatophyte in this study was *Trichophyton rubrum*, found in 39.77% of cases, followed by *T. mentagrophytes* (25%) and *Microsporum canis* (23.86%). This aligns with several studies (Saleh, 2010; Aruna, 2006; Janardhan et al., 2017), though *T. mentagrophytes* was reported as more prevalent in some (Singh et al., 2020). The findings underscore the significance of *Trichophyton* species in dermatophytosis (Jebara et al., 2022). The study used traditional methods to diagnose three dermatophyte species in patients at Al-Karama Teaching Hospital, categorized into the genera *Trichophyton*, *Microsporum*, and *Epidermophyton*. Specifically, *T. rubrum* was identified as responsible for multiple tinea infections (e.g., *Tinea pedis*, *Tinea unguium*). The identification relied on morphological and microscopic characteristics (Havlickova et al., 2008).

Notably, the study also reported *E. echinacea*, a rare fungus linked to hedgehogs, causing three types of tinea. This is the first documentation of skin infections by this fungus in Wasit Province, highlighting potential zoonotic transmission (Abarca et al., 2017).

Regarding antifungal activity, the alcoholic extract of garlic (*Allium sativum*) exhibited stronger inhibitory effects than the aqueous extract, attributed to its higher polarity and concentration of bioactive compounds, consistent with Srinivasan et al. (2000). Inhibition correlated with extract concentration, demonstrating garlic's antifungal properties.

Previous studies have shown that garlic, alongside other plants like *Nigella sativa* and henna, effectively inhibits dermatophytes (Gaherbawy, 1996). Research by Singh and Singh (1997) confirmed the antifungal potential of plant extracts against dermatophytes, while Rai et al. (1999) highlighted the roles of various phenolic compounds in this activity. Compounds such as caffeic acid and gallic acid have been noted for their ability to inhibit certain enzymes (Farooq et al., 2007) and disrupt microbial growth (Bular et al., 2010; Suarez et al., 2003).

(Jebara et al., 2022) emphasized the importance of these secondary metabolites in inhibiting fungal growth. Their mechanisms often involve oxidative processes or the generation of stable free radicals (Yao and Bafici, 2005). The extraction method also influences the effectiveness of these compounds (Doughari et al., 2007).

CONCLUSIONS

This study reveals a high prevalence of dermatophyte infections in Wasit Governorate, with an 88% positivity rate from 100 samples collected at Al-Karama Teaching Hospital. The predominant genus was *Trichophyton*, especially *Trichophyton rubrum*, with *Tinea manuum* being the most affected area. The findings emphasize the need for better awareness of personal hygiene and prevention. Additionally, garlic (*Allium sativum*) extracts showed significant antifungal activity, particularly the alcoholic extracts, highlighting the potential of natural remedies as alternative treatments amid increasing resistance to conventional antifungals.

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