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Isolation and Identification of Dermatophytes and Evaluation the Inhibitory Effect of Nanoparticles Synthesized by *Pseudomonas Aeruginosa* on Isolated Fungi in Al-Shirqat

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Annotation: This study was conducted to investigate the prevalence of dermatophytes in the Al-Sharqat District and evaluate the inhibitory effects of nanoparticles synthesized by *Pseudomonas aeruginosa* against isolated dermatophytes. This study was performed during the period from November 2023 to June 2024. A total of 160 clinically diagnosed skin samples were collected from patients aged between 1 month to 70 years from both sexes. Direct microscopic examination using 10% KOH revealed that 103 skin samples (64.4%) were positive and 57 (35.6%) were negative. Culturing on Sabourad dextrose agar showed fungal growth in 112 samples (70%) and no growth in 48 (30%). Among the identified dermatophytes, Trichophyton strains accounted for 43 (38.4%) and *Microsporum* strains 7(6.2%). Trichophyton mentagrophytes was the most prevalent 24 (%) followed by *Microsporum audouinii* 5 (%). Additionally, 28 (25%) *Aspergillus* isolates and 34 (30.4%) *Candida* species were also detected. Males had a higher susceptibility to

dermatophyte infections (76.8%) compared to females (23.2%), with the 21-30 age group (24.1%) being the most affected. The isolates were treated with nanoparticles synthesized by *P. aeruginosa*, showing significant inhibitory effects on *T. mentagrophytes* and *Microsporum canis*, with sensitivity rates of 65% and 60%, respectively, at a concentration of 4 mM.

Introduction

The skin serves as the body's primary defense barrier. However, despite its structural integrity and protective mechanisms, various factors can lead to pathological conditions. These include exposure to burns and wounds, compromised immunity in individuals with diabetes, immunodeficiency viruses, and cancer. Such conditions increase vulnerability to infections, particularly by keratinophilic organisms such as dermatophytes. These fungi are the causative agents of dermatomycosis, one of the most prevalent skin diseases globally. Dermatomycosis affects the skin, nails, and hair, and is caused by dermatophytes and certain non-dermatophyte fungi. In recent years, there has been a noticeable and gradual increase in the prevalence of these infections, which are now estimated to affect approximately 20-25% of the global population. Common infections include dermatomycosis, candidiasis, and pityriasis versicolor (Mohammad Al-Daami, 2012),(Khurana, 2019) Dermatophytes belong to three primary genera: *Microsporum*, *Trichophyton*, and *Epidermophyton* (Reddy, 2017). The fungi have become resistant to antifungals, so researchers have resorted to other methods of treatment, including extracting nanobodies from plants or bacteria. Recent studies have proven the possibility of using pathogenic bacteria to manufacture nanoparticles and study their effect on fungi.

Materials and Methods

1. Isolation of Fungi

During the study, 160 samples were collected from patients attending Dermatology Clinic at Al-Sharqat General Hospital, Salah al-Din Governorate, between November 2023 to June 2024. The collection was carried out with the assistance of dermatology specialists. Clinical data, including patients' self-reported symptoms, were recorded using a standardized questionnaire. Samples were taken from infected areas of the skin and hair, covering a range of ages and both genders. Skin samples were obtained by scraping; the affected area was first disinfected with 70% ethanol, and scales were gently scraped from the edges of the infection site using a sterile blade. Hair samples were collected from the infected regions with sterile forceps. All samples were plac in sterile test tubes and immediately transported to laboratory for examination and culturing.

2. Identification of fungal Isolates

The fungal samples were identified based on their macroscopic and microscopic features after culturing on Sabouraud Dextrose Agar (SDA) medium for 3 to 14 days. Macroscopic examination included observing colony characteristics such as texture, shape, color, pigmentation, and the appearance of the colony's reverse side. Microscopic analysis centered on fungal structures, particularly conidia (macroconidia and microconidia) in terms of shape, length, range, and wall thickness. Other capabilities which includes conidial septa, conidiophores, and hyphae had been additionally examined, following the protocol defined by way of Ellis et al. (2007).

3. Hair Penetration Test

The identity of Trichophyton mentagrophytes was showed the usage of hair penetration approach, a diagnostic method mainly used to affirm the presence of this fungus (James et al., 2019).

4. Isolation and Identification of Pseudomonas aeruginosa

A overall of 15 Pseudomonas aeruginosa samples had been gathered from various body sites, consisting of blood, urine, pus, and diabetic foot infections. Initial identification become based on the remark of colonial traits on selective media. Pseudomonas aeruginosa isolates were further confirmed the use of the VITEK-2 system (Hassan et al., 2024).

5. Preparation of Nanoparticles from *Pseudomonas aeruginosa* Bacteria

Nanoparticles were prepared from *Pseudomonas aeruginosa* using protocols described by (Katva *et al*., 2017; Klaus *et al*,.1999).

6. Inhibitory Activity Test of Silver Nanoparticles Against Fungi

The antifungal hobby of silver nanoparticles was evaluated using the agar properly diffusion approach on Sabouraud Dextrose Agar (SDA) medium. Wells with a diameter of five mm have been made at the medium, and one-week-vintage fungal isolates were inoculated into the wells. The plates were then incubated at 25°C for one week. After incubation, the diameters of the inhibition zones had been measured in millimeters. This test became repeated in triplicate to ensure reliability of the effects (Shinkafi & Dauda, 2013).

Results and Discussion

The results of the direct microscopic examination using 10% KOH revealed that 103 skin samples were positive, accounting for 64.4% of the total samples, while 57 skin samples were negative, representing 35.6%. In contrast, laboratory cultures on SDA medium showed 112 positive samples (70%) and 48 negative samples (30%) (Table 1). The discrepancy between the results of the KOH test and culture may be attributed to the fact that KOH microscopy is a simple and rapid diagnostic technique that provides a preliminary diagnosis, whereas laboratory culture is more sensitive and capable of detecting fungal growth accurately (Nagar, 2023).

Table (1): Microscopic examination and laboratory culture of fungal isolates

Test	Positive Samples		Negative Samples		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
Direct KOH Test	133	83.1 %	27	16.9 %	160	100 %
Culture	112	70 %	48	30 %		

10. Hair Perforation Test

To confirm the identity of *T. mentagrophytes*, the hair perforation test was performed. The results confirmed the ability of *T. mentagrophytes* to penetrate the hair shaft, confirming its identity (James *et al*., 2019) as illustrated in Figure 2.

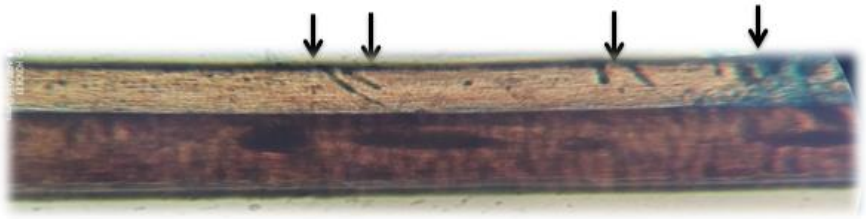

Figure 2: Hair perforation by *T. mentagrophytes*

Types of Fungi Isolated During the Study

The identification of samples cultured on Sabouraud Dextrose Agar (SDA) revealed that 112 out of 160 samples (70%) were positive for fungal growth, as indicated by the colonial morphology of the growing colonies. The dermatophytic fungi identified included 43 isolates of *Trichophyton* (38.4%), 7 isolates of *Microsporum* (6.2%), and 28 isolates of *Aspergillus* (25%).

In addition, *Candida* species were also identified as causative agents of skin infections, with 34 isolates accounting for 30.4% of the positive cases. These results are presented in Table (2). These findings align with those of Ahmed (2022), who reported that dermatophyte isolates comprising 33% and *Candida* isolates making up 25% of the cases.

The isolated dermatophytes were identified through their cultural characteristics, including colony color, morphology, size, reverse side appearance, and microscopic features such as the shape and size of conidia and hyphae. Among the dermatophytes, *T. mentagrophytes* was the most prevalent species, with 24 isolates accounting for 21.4% of cases. Its presence was confirmed using hair perforation test, which yielded positive results. Other species included 7 isolates of *T. interdigitale* (6.2%), 6 isolates of *T. rubrum* (5.4%), 4 isolates of *T. schoenleinii* (3.6%), and 2 isolates of *T. verrucosum* (1.8%).

The genus *Microsporum* was represented by *M. canis* (4.5%) and *M. audouinii* (1.8%), with a total of 7 isolates from both species. Overall, the genus *Trichophyton* was more common than *Microsporum*.

For the genus *Aspergillus*, 28 isolates were recorded, including 17 isolates of *A. niger* (15.1%) and 11 isolates of *A. flavus* (9.8%).

The genus *Candida* accounted for 30.4% of infections, with 34 isolates out of 112 positive samples. These were distributed as follows: 13 isolates of *C. albicans* (11.6%), 9 isolates of *C. tropicalis* (8.0%), and 6 isolates each of *C. parapsilosis* and *C. krusei* (5.4%).

Table (2): Fungal species identified in this study

Fungus	Number of Samples	Percentage
Trichophyton	**43**	**%38.4**
Microsporum	**7**	**% 6.2**
Candida	**34**	**%30.4**
Aspergillus	**28**	**% 25**
Total	**112**	**100%**

Table (3): Distribution of fungal species

Fungal Type	Number of Isolates	Percentage (%)
T.mentagrophytes	**24**	**% 21.4**
T.rubrum	**6**	**% 5.4**
T.interdigitale	**7**	**% 6.2**
T.verrucosum	**2**	**% 1.8**
T.schoenleinii	**4**	**% 3.6**
M.canis	**5**	**% 4.5**
M.audouinii	**2**	**% 1.8**
C.albicans	**13**	**% 11.6**
C.tropicals	**9**	**% 8.0**
C.parasilosis	**6**	**% 5.4**
C.krusi	**6**	**% 5.4**
A.niger	**17**	**% 15.1**
A.flavus	**11**	**% 9.8**
Total	**112**	**% 100**

11. Association Between Gender and Dermatophyte Infections

As presented in Table 3, the results indicated a significantly higher infection rate among males (76.8%) compared to females (23.2%). These differences were statistically significant ($P \leq 0.05$). These findings are consistent with Ahmed (2022), who reported male infection rate of 65.51% and female rate of 34.48%. Similar trends were observed in studies conducted by Mohammed et al. (2017) and Ali et al. (2017), which also documented higher dermatophyte infection rates among males. The increased prevalence of dermatophyte infections in males is attributed to factors such as excessive sweating, extended hours in damp clothing, prolonged exposure to physical labor, and inadequate hygiene. Indirect transmission via wet surfaces, such as pathways, locker rooms, and foot-washing areas at swimming facilities, also significantly contributes to the spread of infections (Jazdarehee *et al*., 2022).

Table (3): Distribution of Dermatophyte based on sexes

Category	Total	Positive (No.)	%	Negative (No.)	%
Males	**113**	**86**	**76.8**	**27**	**56.3%**
Females	**47**	**26**	**23.2**	**21**	**43.7%**
Total	**160**	**112**	**70%**	**48**	**30%**
Statistical Analysis		**Chi-square=6.830 P-value =0.0329**			

Isolation and Identification of Pseudomonas aeruginosa

Fifteen positive samples of Pseudomonas aeruginosa were successfully isolated on culture media. The bacterial isolates were further confirmed using Vitek 2 system, which confirmed the initial identification with a high accuracy of 98%. These findings are consistent with the study conducted by Sudhakar et al. (2015). On MacConkey agar, the colonies appeared large, flat, and pale due to non-fermentation of lactose, accompanied by a characteristic grape-like odor. On Cetrimide agar, a selective medium for P. aeruginosa, colonies appeared yellow-green pigmentation in some isolates due to production of fluorescent pigment pyoverdine, while others exhibited a blue-green coloration resulting from the pigment pyocyanin.

13. Synthesis of Silver Nanoparticles (AgNPs)

13-1 Visual Observation of Nanoparticles

Silver nanoparticles were synthesized from bacterial cultures using a modified method that incorporated gradual pH adjustment, ultrasonic exposure, and shaking, as described by Ghadi et al. (2014). Both pH changes and ultrasound exposure were essential in promoting nanoparticle formation. Adjusting the pH facilitated interactions among compounds in the bacterial extract or with other ions, as highlighted in previous studies. The pH change affects the nature molecular bonding and bond strength by altering electrical charges. Additionally, ultrasonic exposure played a critical role in breaking down larger particles into smaller nanoparticles (Huang et al., 2024). The process was visually monitored through changes in the solution's color after adding silver nitrate. Initially, the solution transitioned from light yellow to milky white. After one hour of incubation, the color shifted to light brown, and following 24 hours of incubation, the solution turned dark brown, confirming the formation of silver nanoparticles As shown in Figure(3)

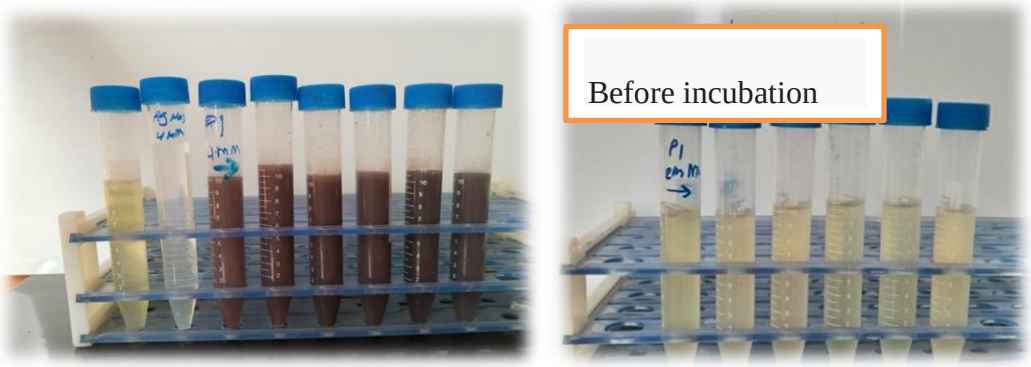

Figure (3) Stages of Formation and Color Change of Silver Nanoparticles (AgNPs)

14. Confirmatory Tests for the Synthesis of Silver Nanoparticles

Analysis of Bio-Synthesized Silver Nanoparticles

The purity and structure of silver nanoparticles synthesized using bacterial extract were evaluated through X-ray diffraction (XRD). The XRD analysis displayed a clear diffraction pattern within the range of 10–80 degrees, confirming the crystallization of nanoparticles in a face-centered cubic (FCC) crystal system. At a concentration of 2 mM, clear diffraction peaks were observed at 420, 660, and 910, corresponding to angles of 25°, 28°, and 32°, respectively. At a concentration of 4 mM, peaks were noted at 800, 4100, and 600, corresponding to angles of 38°, 32°, and 46°, respectively.

The size of the nanoparticles was calculated using the Scherrer equation, demonstrating that the biosynthesis process using *Pseudomonas aeruginosa* bacterial extract successfully produced pure silver nanoparticles with a well-defined crystalline structure, as depicted in Figure 4.

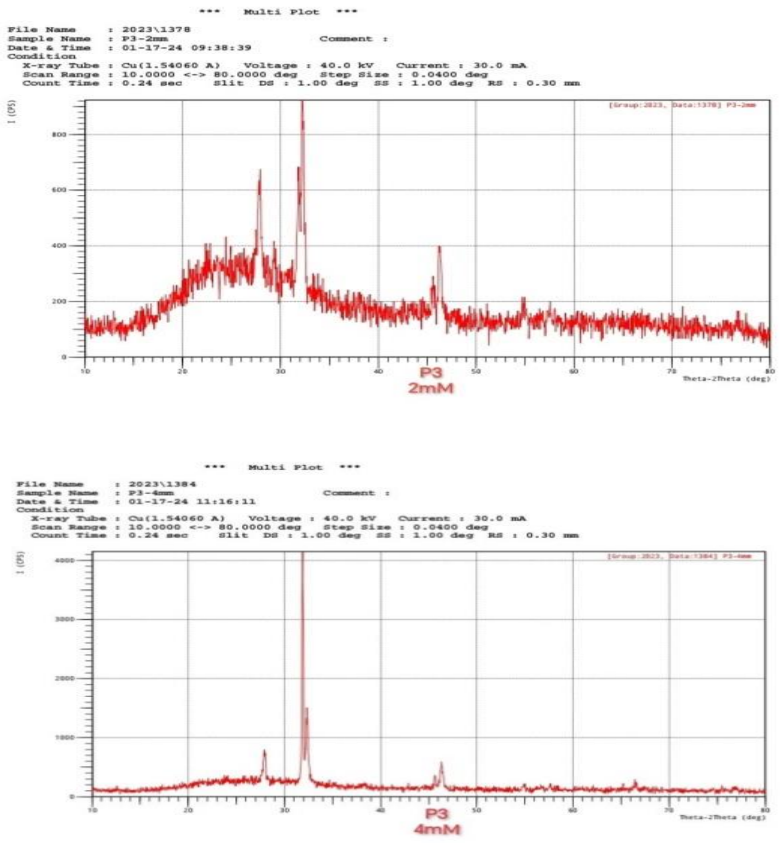

Figure (4:)X-Ray Diffraction (XRD) Analysis of Silver Nanoparticles

Inhibitory Effect of Silver Nanoparticles on Dermatophytes

The results presented in Table 4 demonstrate that *T. mentagrophytes* exhibited moderate sensitivity to silver nanoparticles, with an inhibition rate of 56% at a concentration of 4 mM and 41% at 2 mM. In contrast, *M. canis* showed higher sensitivity, with inhibition rates of 60% at 4 mM and 56% at 2 mM, as illustrated in Figure 5. These findings are consistent with the study performed by Al-Jobory et al. (2020), which reported that *M. canis* had the highest inhibition rate, followed by *T. mentagrophytes* and *T. interdigitale*, while *T. rubrum* exhibited the lowest sensitivity to silver nanoparticles.

Table (5): Effect of Nanoparticles on *T. mentagrophytes* and *M. canis*

Antifungal	Nanobodies in concentration 4 Mm			Nanobodies in concentration 4 Mm		
	Sensitive %	Intermediate %	Resistance %	Sensitive %	Intermediate %	Resistance %
T.mentagrophyt	**10%**	**56%**	**% 39**	**% 27**	**% 41**	**23%**
M. cains	**10%**	**% 60**	**% 30**	**8%**	**56%**	**36%**

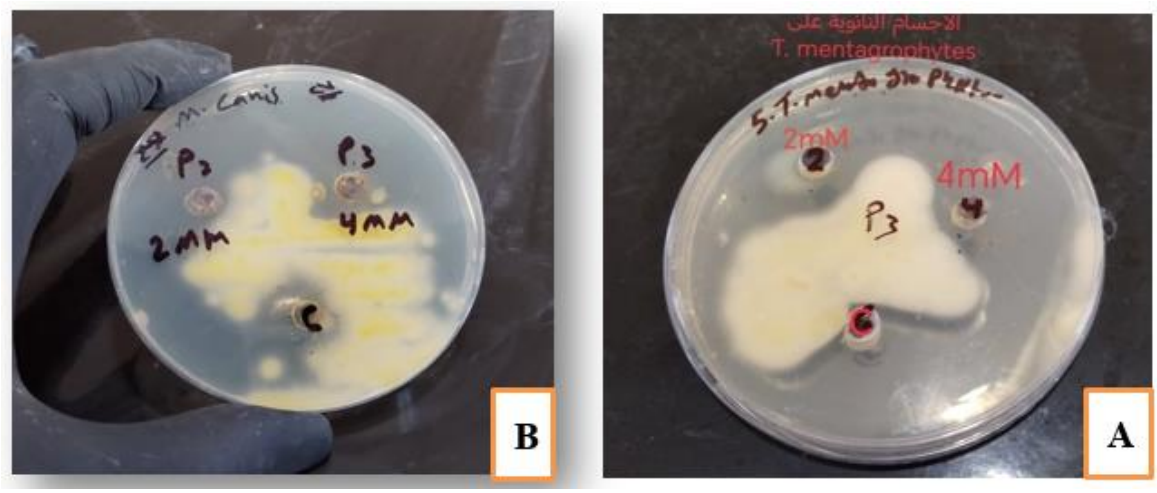

Figure (5): Effect of Nanoparticles on *T. mentagrophytes* (A) and *M. canis (B)* .

Research objectives

The high incidence of skin fungi and the lack of research on inhibiting these fungi using nanobodies manufactured from pathogenic bacteria. Therefore, in the current study, we addressed the isolation and diagnosis of skin fungi and the study of the extent of the effect of nanobodies manufactured from pathogenic bacteria on the isolated fungi.

Conclusions

The study highlighted a high prevalence of dermatophytes in Al-Shirqat, with notable differences between males and females and among different age groups. Males exhibited a higher infection rate compared to females, and the 21-30 age group showed the highest number of infections. *Trichophyton mentagrophytes* was the most frequently isolated species, establishing its role as a major causative agent of fungal infections. Silver nanoparticles demonstrated a significant ability to reduce fungal colony numbers, particularly those of *Trichophyton* species, with the most pronounced effect observed at a concentration of 4 mM

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