Al-Qadisiyah Journal of Pure Science Vol. (31) Issue (Special) (2026) pp. 262– 269
Al-Qadisiyah Journal of Pure Science
ISSN(Printed): 1997-2490 ISSN(Online): 2411-3514
DOI: 10.29350/jops
Al-Qadisiyah Journal of Pure Science

Fungal melanin is an effective alternative to some bacterial pathogens associated with multidrug-resistant skin infections.

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Received: 17/04/2026; Revised: 22/04/2026; Accepted: 07/05/2026.

Abstract
The escalating global challenge of bacterial skin infections is significantly compounded by the rapid emergence of antimicrobial resistance (AMR), which undermines the efficacy of conventional therapeutic protocols. This investigation was conducted to isolate and characterize prevalent bacterial pathogens from diverse skin manifestations and to evaluate their susceptibility profiles against commonly prescribed antibiotics. In this cross-sectional study, 145 clinical specimens were collected and subjected to rigorous microbiological and biochemical identification. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method against five standard antibiotics. The results revealed a high isolation rate, with confirmed bacterial growth in 115 specimens (79.3%). *Staphylococcus aureus* was identified as the predominant pathogen, followed by *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. Notably, four specific isolates exhibited multi-drug resistance to the tested antibiotics. To address this, the study explored the bioactivity of fungal melanin as a natural antimicrobial alternative. Fungal melanin demonstrated potent antibacterial efficacy against these resistant strains, achieving a Minimum Inhibitory Concentration (MIC) of 16 µg/mL and a Minimum Bactericidal Concentration (MBC) of 32 µg/mL. These findings highlight the critical prevalence of Staphylococcal species in dermatological infections and the alarming trajectory of resistance development. The study concludes that fungal melanin possesses significant potential as a robust natural antimicrobial agent or adjunct therapy, offering a promising strategy to combat resistant bacterial skin infections in the face of dwindling antibiotic effectiveness.
Key words- Antimicrobial resistance, Fungal melanin, Natural antimicrobial agents, *A. niger*

Introduction

Millions of clinical visits are caused by bacterial skin and soft tissue infections (SSTIs), which are a significant global health concern. Numerous pathogens can cause these infections, but the most commonly implicated ones are *Pseudomonas aeruginosa* and *Staphylococcus aureus*. [1]. The rapid emergence of multidrug-resistant (MDR) strains, such as Methicillin-resistant S. aureus (MRSA), poses a major threat to current treatment protocols, despite the historical efficacy of conventional antibiotics. [2].

Clinical outcomes are further complicated by these resistant pathogens' persistence within biofilms, which results in chronic infections and higher medical expenses. [3]. Research has turned to finding new, bio-based antimicrobial agents in response to the declining effectiveness of conventional antibiotics. Melanin, a naturally occurring pigment present in all biological kingdoms, has drawn a lot of attention because of its special physicochemical characteristics. Particularly, fungal melanin has shown impressive biological activities, such as strong antimicrobial, anti-inflammatory, and antioxidant properties. [4]. Melanin, in contrast to synthetic antibiotics, exerts antimicrobial pressure through a variety of mechanisms, including membrane disruption and the induction of oxidative stress, which prevents bacteria from rapidly developing resistance. [5]. Melanin has been shown to have potential, but research on its specific effectiveness against clinical isolates from various skin diseases is still ongoing. By separating bacterial pathogens from clinical cases and assessing the susceptibility of resistant strains to fungal melanin, this study aims to close this gap. Our goal is to provide a quantitative foundation for the possible application of fungal melanin as a next-generation therapeutic agent in dermatology by calculating the Minimum Inhibitory Concentration (MIC).

Material and Methods

Extraction of melanin pigment

The method described by Rajagopal et al. [6]. was used to extract melanin pigment from *A. niger* (that was isolated from soil by dilution method).

Specimens Collection and Ethical Approval

At AL-Shamaa Hospital, 145 clinical specimens (swabs) from patients with different kinds of skin infections were gathered. Every participant gave their informed consent, and the study was carried out in compliance with the ethical guidelines established by the science college's ethical committee. And every individual participant in the study gave their informed consent. Patients were made aware of the study's objectives and the privacy of their microbiological information.

Isolation and Identification of Bacteria

Based mostly on morphological characteristics, the Gram-staining method, and biochemical reactions, all isolates were diagnosed in accordance with well-known, established microbiological methods [7].

Antimicrobial susceptibility testing

This was carried out on a pure culture that demonstrated notable growth in accordance with the Kirby-Bauer disc diffusion method's standard criteria [8]. Muller Hinton Agar (MHA) was used as the medium, and it was aerobically incubated at 37°C. The inoculum density needed for susceptibility testing was 0.5%, McFarland. Amoxicillin, tetracycline, neomycin, cefixime, and gentamicin were the five commonly used antibiotics that were tested.

Antibacterial activity of extracted melanin

Test microorganisms, were separately cultivated on the Muller-Hinton agar plates; swab inoculations of the microbes were made on the surface to produce a lawn culture. Antimicrobial activity of (4, 8,16 ,32) µg /ml concentration of pigment extract was tested by well diffusion method [9]. The plates were incubated at 37°C for 24 hrs. and checked out for halo zone apparition. Presence of halo zone around the well indicated a positive antimicrobial property**.**

Statistical Analysis

Every experiment was carried out three times. One-way ANOVA and Tukey's test ($p < 0.05$) was used to analyze the data in order to identify any significant differences.

Results and Discussion

Extraction of melanin pigment

The extracted melanin in this study has an uneven size and shape and appears as an amorphous solid. It forms chunks or rough fragments and lacks a distinct, crystalline structure. Figure 1.

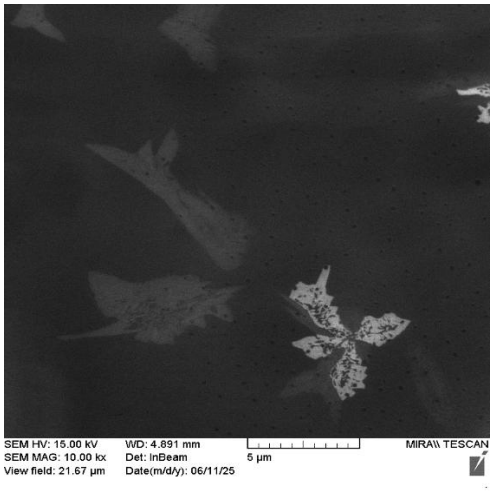


Figure 1. SEM of the extracted Melanin

Bacterial isolates

Analysis of the 115 clinical specimens from 145 specimens table1, revealed the presence of both Gram positive and Gram-negative bacterial pathogens. Table2. Gram-positive bacteria constituted the majority of the isolates. The most frequently identified organism was *Staphylococcus aureus*, which is a cause of skin infections such as abscesses, impetigo, and wound infections [10]. Other Gram-positive isolates included *Staphylococcus epidermidis*, commonly associated with wound and device-related infections [11], and *Streptococcus pyogenes,* particularly in cases of cellulitis and erysipelas [12]. Gram-negative bacteria were also isolated from a considerable number of samples, especially from chronic wounds and hospital-acquired infections. These included *Pseudomonas aeruginosa*, frequently associated with burn and ulcer infections [13], as well as *Escherichia coli, Klebsiella spp., Proteus spp., and Acinetobacter spp*., which are commonly implicated in contaminated wounds and immunocompromised patients. [14].

Table1- specimen's distribution

No. of specimens		%
growth	115	79.3
No growth	30	20.7
total	145	100

Table 2. No. of isolates and percentage

Bacterial Species	Number of Isolates (n)	Percentage (%)
Staphylococcus aureus	54	47
Staphylococcus epidermidis	18	15.7
Streptococcus pyogenes	10	8.7

Pseudomonas aeruginosa	9	7.8
Escherichia coli	8	6.9
Proteus spp.	6	5.2
Enterococcus spp.	4	3.5
Klebsiella spp.	4	3.5
Acinetobacter spp.	2	1.7
Total	**115**	100

Antimicrobial susceptibility

The susceptibility patterns of the 115 clinical isolates Table 3, reveal a significant variance in therapeutic efficacy, with aminoglycosides emerging as the most potent candidates for clinical intervention. Gentamicin exhibited the highest sensitivity rate at 92.1% (106 isolates), followed closely by Neomycin. This robust efficacy is attributed to the superior ability of aminoglycosides to penetrate bacterial membranes and irreversibly bind to the 30S ribosomal subunit. Recent literature underscores that despite global resistance trends, aminoglycosides maintain high bactericidal activity against clinical isolates that have not yet transitioned into advanced biofilm-forming stages [15,16]. A critical finding in this study is the substantial number of isolates categorized as Intermediate (I), particularly for Tetracycline (n=26) and Cefixime (n=23). From a clinical standpoint, the "Intermediate" category represents a dangerous "buffer zone" and a precursor to full resistance. Modern research (2024) suggests that isolates displaying intermediate susceptibility to third-generation cephalosporin like Cefixime often harbor low-level expression of Extended-Spectrum β-Lactamases (ESBLs). This necessitates aggressive dosing regimens or combination therapies to prevent therapeutic failure and the subsequent selection of fully resistant clones. [17,18].

Table 3. Antibiotic Susceptibility Pattern of bacterial isolates

Antibiotic	Sensitive	Intermediate	Resistant
Amoxicillin	90	21	4
Tetracycline	85	26	4
Neomycin	93	18	4
Cefixime	88	23	4
Gentamicin	106	5	4

While the number of fully Resistant (R) isolates remained low at 4 (3.4%) for all tested agents, the presence of cross-resistance across five distinct antibiotic classes is a hallmark of Multidrug-Resistant (MDR) strains. Recent genomic studies (2023) indicate that even a small percentage of MDR isolates in a clinical setting can act as a

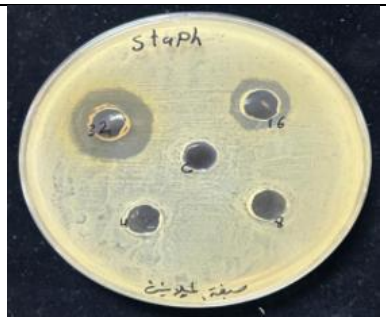
genetic reservoir. These strains often carry mobile genetic elements, such as plasmids or integrons, which facilitate the rapid horizontal transfer of resistance genes (e.g., bla or aac genes) to susceptible populations, posing a severe threat to hospital infection control. [19,20].

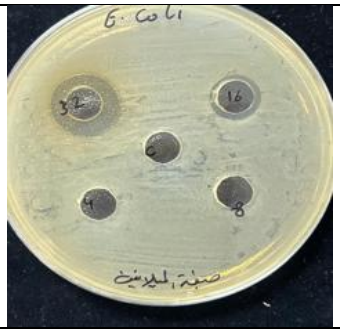
Antibacterial activity of extracted melanin

The antibacterial evaluation of melanin against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) clinical isolates demonstrated a clear Table 5,figure 2, statistically significant dose-dependent response. At the lowest tested concentration (4 µg/mL), the inhibition zones were minimal (6.0 mm for *S. aureus* and 5.0 mm for E. coli). However, as the concentration increased to 32 µg g/mL, the zones expanded significantly to 22.0 ± 1.3 mm and 18.0 ± 1.0 mm, respectively. Growth Inhibition Percentage: The total growth inhibition climbed from 34.7% at the lowest dose to a peak of 78.4% at 32 µg g/mL. Statistical Significance: The inhibitory effect became statistically significant at 8 µg g/mL ($P < 0.05$) and reached high significance ($P < 0.001$) at the 32 µg g/mL concentration compared to the negative control.

Table 5.Effect of Melanin on bacterial strain in different concentration

Melanin Concentration (µg/mL)	S. aureus Zone (mm) (Mean ± SD)	E. coli Zone (mm) (Mean ± SD)	Growth Inhibition (%)	P-value (vs Control)
4	6.0 ± 0.4	5.0 ± 0.3$	34.7%	P > 0.05
8	8.0 ± 0.5$	6.0 ± 0.4	55.7%	P < 0.05
16	18.0 ± 1.1	15.0 ± 0.9	66.2%	P < 0.01
32	**22.0 ± 1.3$**	**18.0 ± 1.0**	**78.4%**	**P < 0.001**
Control (-ve)	4.0 ± 0.2	4.0 ± 0.2	0%	-





S. aureus | **E. coli**

Figure 2 effect of extracted melanin on growth of *S. aureus and E. coli*

The findings of this study highlight the potent antimicrobial properties of melanin, particularly its ability to suppress multidrug-resistant (MDR) clinical isolates that were previously shown to be recalcitrant to standard antibiotics. The achievement of a 22 mm inhibition zone against S. aureus at 32 **µ**g/mL is a major finding, as it suggests that melanin can bypass the resistance mechanisms (such as beta-lactamases) that rendered Amoxicillin and Cefixime ineffective in earlier tests. The superior efficacy observed against *S. aureus* compared to *E. coli* is consistent with the structural differences between Gram-positive and Gram-negative bacteria. Recent research (2022) indicates

that while melanin can disrupt the thick peptidoglycan layer of Gram-positive strains, the complex double-membrane structure of Gram-negative bacteria like E. coli often acts as a more robust permeability barrier [21] Nevertheless, the 18 mm zone for E. coli still represents a significant therapeutic window. The high growth inhibition rate of 78.4% suggests that melanin's mechanism of action involves more than just surface-level contact. According to contemporary studies, natural pigments like melanin can induce the production of Reactive Oxygen Species (ROS), leading to oxidative stress and irreversible damage to bacterial DNA and proteins [22] Furthermore, the dose-response relationship seen here aligns with recent findings that higher concentrations of bio-pigments enhance membrane depolarization, essentially "leaking" the bacterial cytoplasm [23].Given the global crisis of antibiotic resistance, the fact that this melanin extract achieved high statistical significance ($P < 0.001$) against MDR strains positions it as a viable candidate for developing new, bio-based topical treatments for wound and burn infections.

Conclusion

The findings of this study highlight a significant clinical challenge: the high prevalence of multidrug-resistant (MDR) bacterial isolates from skin infections. Our susceptibility testing confirmed that both Gram-positive and Gram-negative pathogens, including S. aureus and P. aeruginosa, have developed robust resistance to a wide spectrum of conventional antibiotics, including beta-lactams and aminoglycosides. However, the application of mold-derived melanin provided a highly effective alternative. Melanin demonstrated a potent, dose-dependent antibacterial activity, achieving a maximum growth inhibition of 78.4% and an impressive 22 mm inhibition zone against S. aureus at a concentration of 32 µg/mL. this research positions melanin as a safe, biocompatible, and powerful antimicrobial candidate. It offers a promising pathway for the development of novel topical therapeutic agents capable of overcoming the limitations of current antibiotic regimens in the treatment of persistent clinical infections.

ETHICAL DECLARATIONS

Acknowledgments

We would like to express our gratitude to the Al-Dewaniyah Hospital's microbiology lab staff for their assistance. Since this study was carried out in accordance with postgraduate research requirements, we also thank the postgraduate research office for their administrative support.and Special thanks also to the patients who consented to have samples taken from them, and we wish them a speedy recovery.

Ethics Approval and Consent to Participate

The study was approved by the Institutional Review Board of the University of Al-Qadisiyah \college of Science (Approval Number 2; Dated 10 July (2023).

Consent for Publication

Not applicable.

Availability of Data and Material

Data generated during this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

Funding
No funding.

Authors' Contributions
Together, the two authors prepared, diagnosed, and reviewed the study.

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