Unizik Journal of Educational Research, Science and Vocational Studies (UJERSVOCS)
Volume 3 (1); Sept.-December, 2025; https://unilaws.org/ujervs
E-ISSN (Online): 1595-6199 ISSN (Print): 1595-6202

COMPARATIVE STUDY OF THE ANTIBACTERIAL AND ANTIFUNGAL EFFECTS OF SNAIL MUCIN ON *STAPHYLOCOCCUS AUREUS* AND *CANDIDA*

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ABSTRACT
This study examined the Comparative Antibacterial and Antifungal Effects of Snail Mucin on Staphylococcus aureus and Candida albicans. *The work was motivated by the growing problem of antimicrobial resistance, which has reduced the efficacy of conventional antibiotics and antifungal agents. Snail mucin (*Achatina fulica*) was extracted and tested using the agar well diffusion and broth dilution methods to assess its inhibitory potential against* S. aureus *and* C. albicans. *Results revealed concentration-dependent antimicrobial activity, with the highest inhibition zones at 100% mucin concentration for both organisms.* Staphylococcus aureus *showed greater susceptibility (22.4 ± 0.5 mm) compared to* Candida albicans *(19.6 ± 0.3 mm). The minimum inhibitory concentrations (MIC) were 25% for* S. aureus *and 50% for* C. albicans, *while the minimum bactericidal and fungicidal concentrations (MBC/MFC) were 50% and 75%, respectively. These findings confirm that snail mucin possesses bioactive compounds such as peptides and glycoproteins responsible for broad-spectrum antimicrobial activity. The study concludes that snail mucin could serve as a natural alternative or complementary therapy in combating resistant bacterial and fungal infections. Further purification and* in vivo *evaluation of its active constituents are recommended.*

Keywords: snail mucin, *Staphylococcus aureus*, *Candida albicans*, antibacterial, antifungal, antimicrobial resistance

Introduction
Infectious diseases caused by bacteria and fungi remain a major global health challenge, particularly due to the increasing prevalence of antimicrobial resistance. Among the most common pathogenic microorganisms implicated in human infections are *Staphylococcus aureus* and *Candida* species. *Staphylococcus aureus* is a Gram-positive bacterium responsible for a wide range of infections, including skin abscesses, pneumonia, osteomyelitis, and endocarditis. *Candida* species, especially *Candida albicans*, are opportunistic fungal pathogens known to cause candidiasis, particularly in immunocompromised individuals (Kumar *et al.*, 2020). The development of multidrug-resistant strains of these microorganisms has prompted the search for alternative and natural antimicrobial agents with fewer side effects and better biocompatibility (Ali *et al.*, 2019).

Snail mucin, a secretion produced by the mucous glands of terrestrial snails, has gained scientific attention for its biological and therapeutic properties. It is composed of bioactive molecules such as glycoproteins, glycolic acid, hyaluronic acid, peptides, and antimicrobial compounds that promote wound healing and inhibit microbial growth (Igwe *et al.*, 2021). Traditionally, snail slime has been used in African and Asian medicine for treating inflammation, burns, and microbial infections (Oluwole & Okon, 2017). Modern research has expanded its use in pharmacology and cosmetics, particularly in formulations for skin repair and infection control (Obasi *et al.*, 2023).
Given its biochemical composition, snail mucin may possess dual antimicrobial properties, acting against both bacterial and fungal pathogens. Several studies have reported its inhibitory effect on Gram-positive and Gram-negative bacteria, as well as certain fungal strains (Okoro *et al.*, 2018; Nwachukwu & Ogbunugafor, 2022). However, there is limited comparative data on its efficacy against both *Staphylococcus aureus* and *Candida* species. Understanding the extent of its antibacterial and antifungal activity will contribute to the scientific basis for its potential use in developing alternative therapeutic agents.

Statement of the Problem

Despite the availability of conventional antibiotics and antifungal drugs, the emergence of resistant microbial strains poses a serious public health concern. *Staphylococcus aureus* has developed resistance to β-lactam antibiotics, leading to methicillin-resistant *S. aureus* (MRSA) infections, while *Candida* species exhibit resistance to azole antifungals (Chowdhary et al., 2020). These resistances increase treatment costs, prolong hospital stays, and contribute to high morbidity and mortality rates.

The need for novel, natural, and effective antimicrobial agents has therefore become imperative. Snail mucin, though used traditionally for healing wounds and treating infections, lacks sufficient comparative scientific validation regarding its efficacy against both bacterial and fungal pathogens. This study seeks to fill that gap by investigating and comparing the antibacterial and antifungal effects of snail mucin on *Staphylococcus aureus* and *Candida* species.

Aim of the Study

The main aim of this study is to compare the antibacterial and antifungal effects of snail mucin on *Staphylococcus aureus* and *Candida*.

Specific Objectives

The specific objectives of this study are to:

1. Extract and purify mucin from snail species.
2. Determine the antibacterial effect of snail mucin on *Staphylococcus aureus*.
3. Compare the inhibitory zones and minimum inhibitory concentrations (MICs) of snail mucin against the two organisms.

Research Questions

1. Does snail mucin exhibit antibacterial activity against *Staphylococcus aureus*?

2. Does snail mucin possess antifungal activity against *Candida* species?
3. How do the antibacterial and antifungal effects of snail mucin compare?

Significance of the Study

This study contributes to the growing body of knowledge on natural antimicrobial agents derived from animal secretions. The findings will provide scientific evidence supporting the traditional use of snail mucin in infection control and wound management. Moreover, the study could pave the way for developing natural, biodegradable, and less toxic alternatives to synthetic drugs, particularly in the face of rising antimicrobial resistance. Pharmaceutical industries, microbiologists, and researchers may benefit from these findings in developing topical formulations, disinfectants, or wound-healing agents based on snail mucin.

Methodology

Research Design

This study adopted an experimental research design to evaluate and compare the antibacterial and antifungal activities of snail mucin against *Staphylococcus aureus* and *Candida albicans*. This design allowed observation of cause-and-effect relationships between snail mucin concentrations and microbial inhibition under controlled laboratory conditions (Cheesbrough, 2016; Kumar*et al.*, 2020).

Collection and Identification of Samples

Live snails (*Achatina achatina*) were collected from local markets and garden areas in Awka, Anambra State, Nigeria. The snails were identified and authenticated by a zoologist in the Department of Biological Sciences, Nnamdi Azikiwe University, Awka. The bacterial (*S. aureus*) and fungal (*C. albicans*) isolates were obtained from existing culture collections in the microbiology laboratory of the same institution. Their identities were confirmed through standard biochemical, Gram staining, and morphological tests (Prescott *et al.*, 2017).

Extraction of Snail Mucin

The snails were rinsed with distilled water to remove impurities. The soft bodies were removed, and mucus was induced by stimulating the snail's foot on a clean glass surface. The collected mucin was filtered using sterile muslin cloth and centrifuged at 5000 rpm for 10 minutes. The clear supernatant was stored at 4°C until use (Okoro *et al.*, 2018; Obasi *et al.*, 2023).

Test Microorganisms and Standardization

Pure cultures of *S. aureus* and *C. albicans* were maintained on nutrient agar and Sabouraud dextrose agar (SDA), respectively. The suspensions were adjusted to 0.5 McFarland standard (~1 × 10^8 CFU/mL for bacteria, ~1 × 10^6 CFU/mL for fungi) (CLSI, 2021).

Preparation of Culture Media

Nutrient agar and SDA were prepared, sterilized at 121°C for 15 minutes, and cooled to 45–50°C before pouring into Petri dishes (Cappuccino & Sherman, 2014).

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Antibacterial and Antifungal Assay

The agar well diffusion method was used to assess antimicrobial effects (Bauer *et al.*, 2018). Wells (6 mm) were filled with 0.1 mL of snail mucin at 25%, 50%, 75%, and 100% concentrations. Distilled water served as negative control; ampicillin (for *S. aureus*) and fluconazole (for *C. albicans*) as positive controls. Plates were pre-diffused for 30 minutes before incubation.

Incubation of Plates

Bacterial plates: 37°C for 24 h; fungal plates: 28°C for 48 h. Zones of inhibition were measured in millimeters and averaged from triplicate readings (Femi-Adepoju *et al.*,, 2020).

Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined using the broth dilution method. Serial dilutions of mucin were inoculated with standardized microbial suspensions. The lowest concentration showing no visible growth was recorded as the MIC (Ali *et al.*, 2019).

Determination of Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC)

Aliquots from MIC tubes showing no turbidity were sub-cultured on nutrient agar or SDA. The lowest concentration yielding no visible growth was recorded as MBC or MFC (Nwachukwu & Ogbunugafor, 2022).

Data Analysis

All experiments were conducted in triplicate. Data are presented as mean ± SD. ANOVA was used to determine significant differences ($p < 0.05$) (Singh & Kumar, 2020).

Results and Discussion

Antibacterial Activity of Snail Mucin on *Staphylococcus aureus*

Table 1: Antibacterial Activity of Snail Mucin on *Staphylococcus aureus*

sample	**Concentration of Snail Mucin (%) Mean Zone of Inhibition (mm) ± SD**				Concentration of Positive Control (Ampicillin)**(%)Mean Zone of Inhibition (mm) ± SD**				Concentration of Negative Control (distilled water) **(%) Mean Zone of Inhibition (mm) ± SD**			
	100%	75%	50%	25%	100 %	75%	50%	25%	100%	75%	50%	25%
Staphylococcus aureus	22.4 ± 0.5	18.2 ± 0.4	14.5 ± 0.6	9.3 ± 0.5	25.8 ± 0.4	19.4 ± 0.3	12.9 ± 0.2	6.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

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Antifungal Activity of Snail Mucin on *Candida albicans*

Table 2: Antifungal Activity of *Snail Mucin on* Candida albicans

sample	**Concentration of Snail Mucin (%) Mean Zone of Inhibition (mm) ± SD**				Concentration of Positive Control (Ampicillin) **(%) Mean Zone of Inhibition (mm) ± SD**				Concentration of Negative Control (distilled water) **(%) Mean Zone of Inhibition (mm) ± SD**			
	100%	75%	50%	25%	100 %	75%	50%	25%	100%	75%	50%	25%
Candida albicans	19.6 ± 0.3	15.8 ± 0.5	11.4 ± 0.6	7.3 ± 0.4	23.1 ± 0.3	19.4 ± 0.3	12.9 ± 0.2	6.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Comparative Analysis of Minimum Inhibitory, Bactericidal, and Fungicidal Concentrations

Table 3: Comparative analysis of Minimum Inhibitory and Bactericidal/Fungicidal Concentrations of Snail Mucin

Microorganism	**MIC (%)**	**MBC/MFC (%)**
Staphylococcus aureus	25	50
Candida albicans	50	75

Discussion

The present study investigated the antibacterial and antifungal activities of snail mucin extracted from *Achatina fulica* against *Staphylococcus aureus* and *Candida albicans*. The antimicrobial potentials were determined using the agar well diffusion method at different concentrations (100%, 75%, 50%, and 25%), with ampicillin as the positive control and distilled water as the negative control. The results presented in Table 1 reveal that snail mucin exhibited considerable antibacterial activity against *Staphylococcus aureus*, producing inhibition zones of 22.4 ± 0.5 mm at 100%, 18.2 ± 0.4 mm at 75%, 14.5 ± 0.6 mm at 50%, and 9.3 ± 0.5 mm at 25% concentrations. The positive control (ampicillin) produced a higher inhibition zone (25.8 ± 0.4 mm), while the negative control showed no inhibition. These findings demonstrate that the antibacterial activity of snail mucin is concentration-dependent, with the highest activity observed at full strength (100%). This observation agrees with the findings of Pitt *et al.* (2015), who reported that mucus secretions from *Helix aspersa* exhibited strong antibacterial effects against *S. aureus* and *Pseudomonas aeruginosa*. Similarly, Dolashki *et al.* (2018) and Velkova *et al.* (2024) found that peptide fractions isolated from *Cornu aspersum* mucus possessed significant antibacterial activity against both Gram-positive and Gram-negative bacteria.

Aouji *et al.* (2017) also noted that crude mucus extract from *Helix aspersa* displayed effective bacteriostatic properties attributed to the presence of glycoproteins and low-molecular-weight peptides. The mechanism behind this antibacterial activity has been associated with the presence of antimicrobial peptides (AMPs) and glycoproteins in snail mucin, which disrupt bacterial cell walls, leading to cytoplasmic leakage and eventual cell death (Cilia & Fratini, 2018; Dolashki *et*

al., 2020; Wong, 2025). Furthermore, Topalova *et al.* (2022) demonstrated that peptide fractions from *Cornu aspersum* mucus alter membrane permeability and interfere with protein synthesis in *Escherichia coli* and *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) obtained for *S. aureus* were 25% and 50%, respectively (Table 3). This indicates that a higher concentration is required for bactericidal effects compared to inhibition. Similar findings were reported by Dolashka *et al.* (2020), who observed MIC values of 25–50 µg/mL for mucus peptide fractions against *S. aureus*, suggesting that the antibacterial effect is both dose- and purity-dependent. In comparison to ampicillin, snail mucin exhibited slightly lower zones of inhibition, which may be due to the complex composition of the crude extract. Purified fractions, as reported by Dolashki *et al.* (2018), often show higher potency because purification concentrates the active components. The absence of inhibition in the negative control confirms that the activity observed is inherent to the mucin constituents and not an artefact of solvent interference (Pitt *et al.*, 2015).

The antifungal assay results in Table 2 indicate that snail mucin demonstrated inhibitory activity against *Candida albicans*, with inhibition zones of 19.6 ± 0.3 mm (100%), 15.8 ± 0.5 mm (75%), 11.4 ± 0.6 mm (50%), and 7.3 ± 0.4 mm (25%). The positive control (ampicillin) yielded a larger inhibition zone (23.1 ± 0.3 mm), while the negative control produced none. These findings are consistent with the work of Abd-El Azeem *et al.* (2016), who reported antifungal effects of snail tissue extract on *Candida albicans* and *Aspergillus niger*. Similarly, Cilia and Fratini (2018) and Dolashki *et al.* (2020) documented that peptide fractions from snail mucus significantly inhibited *Candida* species. Noothuan *et al.* (2021) found that mucus from *Lissachatina fulica* and *Hemiplecta distincta* contained proteins with antifungal activity, supporting the present result. The minimum inhibitory and fungicidal concentrations (MIC = 50%; MFC = 75%) suggest that *C. albicans* is slightly less sensitive to snail mucin compared to *S. aureus*. This may be attributed to the structural complexity of fungal cell walls, which contain chitin and glucans that confer additional resistance to antimicrobial penetration (Cilia & Fratini, 2018). Nevertheless, the dose-dependent inhibition observed indicates that active antifungal components are present in the mucin. The overall findings correspond with earlier reports that snail mucin possesses broad-spectrum antimicrobial properties**,** which vary with snail species, environmental conditions, and extraction techniques. Greistorfer *et al.* (2017) emphasized that mucus composition changes with diet and habitat, influencing biological activity. Suárez *et al.* (2021) confirmed that *Achatina fulica* mucus fractions exhibit antibacterial and antifungal activities and inhibit virulence factors in resistant *S. aureus* strains.

The present study's MIC and MBC/MFC values fall within ranges reported by Dolashki *et al.* (2018) and Velkova *et al.* (2024), suggesting that the crude extract retains sufficient bioactivity without purification. However, the relatively high concentration required for complete inhibition highlights the potential benefits of fractionation and peptide isolation, as proposed by Topalova *et al.* (2022). These results confirm that snail mucin contains bioactive compounds capable of inhibiting both bacterial and fungal pathogens. The dose-dependent response implies that higher concentrations are more effective, aligning with standard antimicrobial kinetics. Given the global

challenge of antibiotic resistance, snail mucin and its derived peptides could serve as promising alternatives or adjuncts to conventional antibiotics **(**Wong, 2025**)**.

Conclusion

This study investigated the antimicrobial activity of snail mucin from *Achatina fulica* against *Staphylococcus aureus* and *Candida albicans*. The results showed that snail mucin exhibited significant, concentration-dependent antibacterial and antifungal effects, with the highest inhibition at 100% concentration. *Staphylococcus aureus* was more sensitive to the mucin than *Candida albicans*, while the negative control showed no effect, confirming that the antimicrobial activity came from the mucin itself. These findings agree with earlier studies that attribute such activity to peptides, glycoproteins, and other bioactive compounds present in snail mucus. Snail mucin possesses notable natural antimicrobial properties and could serve as an alternative or complementary therapy against microbial infections. Further studies should isolate and purify its active components and evaluate their safety and effectiveness in vivo.

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