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Antibacterial Activity of *Achatina fulica* Shell Powder against Selected Bacterial Pathogens Associated with Wound Infections

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Abstract

Wound infections remain a major public health concern due to the increasing prevalence of multidrug-resistant bacterial pathogens, which reduce the effectiveness of conventional antibiotics. This study evaluated the antibacterial activity of *Achatina fulica* shell powder against selected bacterial pathogens associated with wound infections**.** Shells of *A. fulica* were cleaned, dried, pulverized, and suspended in absolute ethanol to prepare a stock concentration of 200 mg/mL. The antibacterial activity of the shell powder was evaluated against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Escherichia coli* using the agar disc diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth tube dilution method. *Achatina fulica* shell powder exhibited antibacterial activity against the Gram-positive bacteria *S. aureus* and *S. pyogenes*, with mean inhibition zones of 8.00 ± 2.00 mm and 9.00 ± 1.00 mm, respectively. No antibacterial activity was observed against *P. aeruginosa* or *E. coli*. The MIC for both susceptible isolates was 200 mg/mL, while no MBC was detected within the concentration range tested. *Achatina fulica* shell powder possesses selective but modest antibacterial activity against Gram-positive wound pathogens and demonstrates bacteriostatic properties at relatively high concentrations. Therefore, further studies are needed to investigate in-vivo antibacterial activity.

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1.0 Introduction

Wound infections are still a major global public health issue, contributing to delayed wound healing, longer hospitalization, higher health-care expenditures, and high morbidity and death (Murray *et al*., 2022; Uberoi *et al*., 2024; World Health Organization, 2024). Pathogenic bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli*are largely responsible for the persistence of wound infections

(Nyangono *et al.*, 2021). The fast growth of multidrug-resistant (MDR) bacteria has hampered wound infection therapy, highlighting the urgent need for new antimicrobial medicines that are effective, safe, inexpensive, and ecologically sustainable (Murray *et al*., 2022; WHO, 2024).

Antimicrobial resistance (AMR) is recognized by the World Health Organization as one of the greatest threats to global health. The widespread misuse and overuse of antibiotics have accelerated the evolution of resistant bacterial pathogens, reducing the effectiveness of available antimicrobial therapies (WHO, 2024). Furthermore, bacteria embedded within biofilms are highly tolerant to antibiotics and host immune responses, making chronic wound infections particularly difficult to eradicate (Malone *et al.,* 2017; Wang *et al*., 2024).

A. fulica, commonly known as the Giant African Land Snail, is a fascinating gastropod mollusc that has attracted attention for its distinctive features and widespread distribution (Alogna *et al*., 2025). The shell of *A. fulica* consists primarily of calcium carbonate together with calcium phosphate, magnesium, trace minerals, and an organic matrix containing proteins and polysaccharides (Senthilkumar *et al*., 2024; Alogna *et al.,* 2025).

Abdul-Khaliq *et al.* (2020) investigated the antibacterial activity of *Lymnaeaauricularia* (freshwater snail) shell powder collected from Baghdad, Iraq, against three clinically important bacterial pathogens: *Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*. The antibacterial activity was evaluated using the agar well diffusion method at four different concentrations (75, 100, 125, and 150 mg/mL). At the highest concentration tested (150 mg/mL), the shell powder produced the largest zone of inhibition against *E. coli* (18 mm), followed by *P. aeruginosa* (16.5 mm) and *K. pneumoniae* (12 mm).

Despite growing interest in snail-derived biomaterials, studies investigating the antibacterial activity of *A. fulica* shell powder against clinically important wound pathogens remain limited, particularly in low- and middle-income countries where wound infections and antimicrobial resistance continue to impose a significant healthcare burden. This study therefore evaluated the in vitro antibacterial activity of *A. fulica* shell powder against selected wound-associated bacterial pathogens and determined its MIC and MBC under the conditions tested.

2.0 Materials and methods

2.1 Study Area

The study was conducted in the Microbiology Laboratory, Department of Microbiology, Confluence University of Science and Technology, Osara, Kogi State, Nigeria.

2.2 Collection, Identification and Processing of snail shell

Live snails were purchased from Okene Central market in Kogi State, Nigeria in May, 2026. The snails were identified as *Achatina fulica* by Professor O. S. Omowaye, a zoologist at the Federal University of Lokoja, Nigeria. After identification, fifteen (15) snails were processed following the method described by Abdul-Khaliq *et al*. (2020) and Nyangono *et al.* (2021) with minor modifications. The shells were gently broken with a small hammer to separate the meat from the shell. They were then thoroughly washed with clean water to remove any remaining dirt before being dried in a hot-air incubator at 55°C until completely dry. The dried shells were ground into a fine powder using a commercial milling machine and sieved with a sterile muslin cloth (160 µm sieve) to obtain a uniform particle size. The resulting shell powder was transferred into sterile, airtight containers and stored at room temperature until it was used for subsequent analyses.

2.3 Culture media preparation

Culture media were prepared according to the manufacturer's instructions. The prepared media were sterilized by autoclaving at 121°C for 15 minutes under a pressure of 15 psi. After

sterilization, the media were allowed to cool to approximately 45°C before being aseptically dispensed into sterile Petri dishes. The poured plates were incubated at 37°C for 24 hours to confirm sterility. Sterile plates showing no microbial growth were stored at 4°C until required for the study.

2.4 The test bacteria

The bacterial isolates used in this study were pathogenic strains obtained from the culture collection of the Vaccine Laboratory, Step B, Federal University of Technology, Minna, Niger State, Nigeria. The test organisms included *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, and *Escherichia coli*.

2.5 Preparation of Snail Shell Powder Suspension

A stock suspension of *Achatina fulica* shell powder was prepared by suspending 1 g of the powder in 5 mL of absolute ethanol to obtain a nominal concentration of 200 mg/mL. The suspension was thoroughly vortexed to ensure even dispersion of the powder before use. Absolute ethanol was used as the negative control to confirm that the solvent had no antibacterial effect, while chloramphenicol served as the positive control for comparison with the antibacterial activity of the shell powder against the test bacterial isolates.

2.6 Antibacterial Activity of Snail Shell Powder

The antibacterial activity of the snail shell powder was evaluated using the agar disc diffusion method as previously described by Voleti (2022), with slight modifications. Fresh bacterial cultures were standardized to 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/mL). Sterile nutrient agar plates were uniformly inoculated by swabbing the standardized bacterial suspension over the entire agar surface.

Sterile paper discs (6 mm in diameter) were impregnated with the prepared snail shell suspension (200 mg/mL) and allowed to dry at 40°C for approximately 20 minutes. Thereafter, each disc was carefully placed aseptically at the center of the inoculated agar plate. The plates were left undisturbed at room temperature for approximately 30 minutes to allow the snail shell constituents to diffuse into the agar medium before incubation.

The plates were subsequently incubated at 37°C for 18 - 24 hours. Following incubation, the antibacterial activity was assessed by measuring the diameter of the clear zones of inhibition surrounding each disc using a transparent ruler. The inhibition zones were recorded in millimetres (mm), and the mean values from replicate determinations were calculated. Chloramphenicol (25 mg/mL) was included as the positive control, while ethanol served as the negative control to validate the assay as described by Attah *et al*. (2021).

2.6 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of *A. fulica* shell powder against the bacterial isolates was determined using the broth tube dilution method as described by Voleti (2022) with minor modifications. One gram of the shell powder was suspended in 5 mL of ethanol to prepare a stock solution of 200 mg/mL concentration. Two-fold serial dilutions were then made in sterile nutrient broth to obtain final concentrations of 200, 100, 50, and 25 mg/mL. Each tube was inoculated with 0.1 mL of a standardized bacterial suspension adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). A fifth tube containing only sterile nutrient broth without bacterial inoculum served as the sterility control. The tubes were incubated at 37°C for 24 h and subsequently examined for visible turbidity. The MIC was recorded as the lowest concentration of the shell powder that completely inhibited visible bacterial growth when compared with the sterile control (Voleti, 2022; Musa *et al*., 2022).

2.7 Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) was determined from the tubes that showed no

visible growth in the MIC assay. A loopful of broth from each clear tube was aseptically streaked onto freshly prepared nutrient agar plates and incubated at 37°C for 24 h. After incubation, the plates were examined for bacterial growth. The lowest concentration of the shell powder that produced no visible colony growth on the agar plates was recorded as the MBC (Musa *et al*., 2022).

2.8 Data Analysis

The statistical evaluation of antimicrobial activity results was performed using IBM SPSS Statistics software, version 23. Data obtained were presented as Mean ± Standard Error of Mean (SEM) from two independent replicates. Within the same column, Values with different superscripts along column are significantly different at $p < 0.05$.

3.0 Result

3.1 Antibacterial activity of *Achatina fulica* shell powder

The result of antibacterial activity of *A. fulica* shell powder at a concentration of 200 mg/mL against four bacterial isolates, with the positive control and negative control were presented in Figure 1. The shell powder exhibited antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes*, producing mean zones of inhibition of 8.00 ± 2.00 mm and 9.00 ± 1.00 mm, respectively. However, no inhibitory activity was observed against *Pseudomonas aeruginosa* or *Escherichia coli*, as both organisms recorded 0.00 ± 0.00 mm. The positive control demonstrated antibacterial activity against all the test organisms, with inhibition zones of 25.50 ± 1.50 mm for *P. aeruginosa*, 8.00 ± 1.00 mm for *S. aureus*, 9.50 ± 0.50 mm for *S. pyogenes*, and 24.00 ± 2.00 mm for *E. coli*. In contrast, the negative control showed no inhibitory effect against any of the bacterial isolates, with all values recorded as 0.00 ± 0.00 mm.

3.2 Minimum inhibitory and minimum bactericidal concentrations of *Achatina fulica* shell powder

The results of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Achatina fulica* shell powder against the tested bacterial isolates were presented in Table 1. The shell powder exhibited an MIC of 200 mg/mL against both *Staphylococcus aureus* and *Streptococcus pyogenes*, indicating that visible bacterial growth was inhibited at this concentration. However, no MBC was recorded for either bacterial isolate, as indicated by Nil.

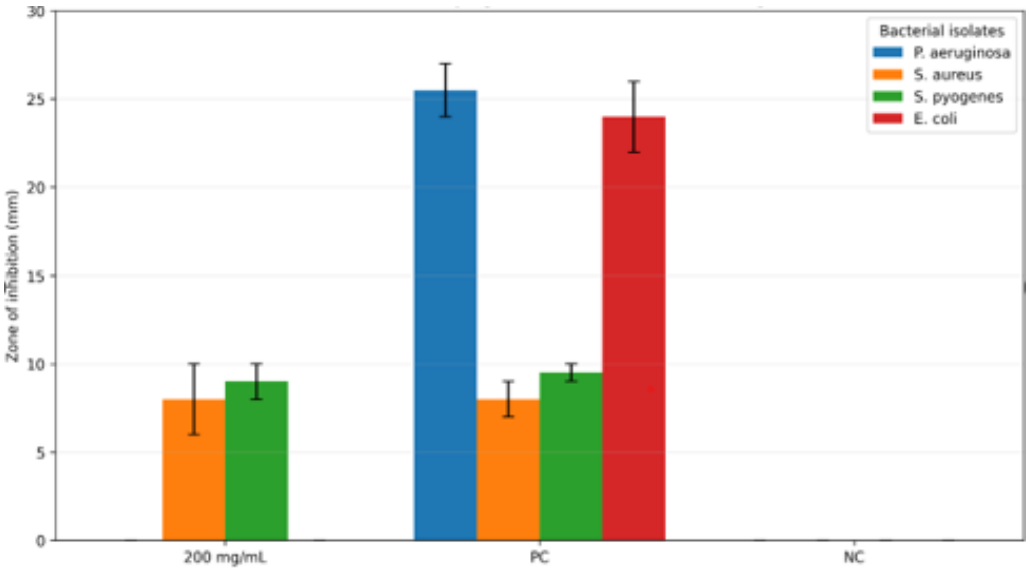

Figure 1: Antibacterial activity of *Achatina fulica* powder

Keys: PC = Positive control, NC = Negative control, **X-axis:** 200 mg/mL, PC, and NC, **Y-axis:** Zone of inhibition (mm), Error **bars:** ± SD

Table 1: The Minimum Inhibitory MIC Concentration, and Minimum Bactericidal Concentration MBC of *Achatina fulica* powder

Bacteria	MIC	MBC
S. aureus	200 mg/mL	Nil
S. pyogenes	200 mg/mL	Nil

Key: Nil, no MBC

4.0 Discussion

In recent years, considerable attention has been directed toward the bioactivity of natural compounds and their potential pharmaceutical applications. Among invertebrates, molluscs have been recognized as important sources of bioactive compounds with diverse therapeutic properties (Giftson & Patterson, 2014; Ali *et al*., 2024; Akilimali *et al*., 2025).

In the present study, *A. fulica* shell powder demonstrated selective antibacterial activity against the Gram-positive bacteria *S. aureus* and *S. pyogenes*, while no inhibitory effect was observed against the Gram-negative bacteria *P. aeruginosa* and *E. coli*. The inhibition zones of 8.00 ± 2.00 mm and 9.00 ± 1.00 mm against *S. aureus* and *S. pyogenes*, respectively, indicate that the shell powder exhibited only modest antibacterial activity at the tested concentration of 200 mg/mL. This finding contrasts with the report of Abdul-Khaliq *et al*. (2020), who recorded inhibition zones of 16.5 mm and 18.0 mm against *E. coli* and *P. aeruginosa*, respectively, using *Lymnaeaauricularia* (snail) shell powder at a concentration of 150 mg/mL. The difference may be attributable to differences in the snail species used.

The greater susceptibility of Gram-positive bacteria (*S. aureus* and *S. pyogenes*) compared with Gram-negative bacteria (*E. coli* and *P. aeruginosa*) can be explained by fundamental differences in their cell wall architecture. Gram-positive bacteria possess a thick peptidoglycan layer but lack the outer membrane characteristic of Gram - negative bacteria, allowing antimicrobial agents to penetrate the cell more readily (Maher & Hassan, 2023). In contrast, the outer membrane of Gram-negative bacteria can act as an effective permeability barrier, thereby limiting the entry of some antimicrobial agents (Breijyeh *et al.,* 2020).

The minimum inhibitory concentration (MIC) results showed that the growth of *S. aureus* and *S. pyogenes* was inhibited only at the highest concentration tested (200 mg/mL), indicating relatively low antibacterial potency. According to antimicrobial susceptibility principles, lower MIC values generally indicate greater antimicrobial efficacy, whereas higher MIC values suggest weaker inhibitory activity (Wiegand *et al.,* 2008; Younas *et al*., 2025). The findings of the present study are consistent with some of those of Nyangono *et al.* (2021), who evaluated the antibacterial activity of *A. fulica* shell powder against five wound-associated bacterial pathogens (*S. aureus, S. agalactiae, P. aeruginosa, E. coli,* and *Klebsiella pneumoniae*) using the agar diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) analyses demonstrated that all shell preparations exhibited antibacterial activity, although the effect was predominantly bacteriostatic rather than bactericidal.

The requirement of a relatively high concentration to inhibit bacterial growth therefore suggests that crude *A. fulica* shell powder possesses limited antibacterial potency and may require further purification or modification to enhance its antimicrobial effectiveness. Interestingly, no minimum bactericidal concentration

(MBC) was observed against either *S. aureus* or *S. pyogenes*. This finding suggests that although the shell powder inhibited bacterial growth at 200 mg/mL, it was unable to completely eliminate the organisms at the concentrations tested. The absence of a detectable MBC indicates that complete bacterial killing was not achieved within the concentration range tested. Therefore, the preparation demonstrated inhibitory activity, but its bactericidal concentration could not be established under the conditions of this study. Bacteriostatic agents inhibit bacterial multiplication without causing irreversible cell death; consequently, bacterial growth may resume once the antimicrobial agent is removed (Baquero *et al*., 2024).

5.0 Conclusion

The findings of this study indicate that *Achatina fulica* shell powder exhibits modest antibacterial activity, with greater effectiveness against Gram-positive bacteria than against Gram-negative bacteria. It provides scientific support for the traditional use of snail shell-derived materials in the management of bacterial infections. However, further studies, including *in vivo* antibacterial evaluations and toxicological assessments using animal models, are required before its clinical application, particularly in wound care be recommended.

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Conflict of interest

The authors declare no conflict of interest.

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