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RESEARCH ARTICLE

Antibacterial and antibiofilm properties of *Helix aspersa* mucus towards multidrug resistant *Staphylococcus aureus*

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ABSTRACT

The increasing prevalence of multidrug-resistant bacteria necessitates the exploration of novel antimicrobial agents. This study aims to investigate the antibacterial and antibiofilm properties of mucus from *Helix aspersa*, a species of terrestrial snail, against multidrug resistant *Staphylococcus aureus* strains. The antibacterial effect was assessed using well diffusion, microdilution, and time kill assays. The antibiofilm effect was assessed using crystal violet staining. Protein profiling was conducted through SDS-PAGE Electrophosis to determine the molecular weights of the mucus proteins. The results show that *Helix aspersa* mucus has potent anibacterial properties with inhibitory diameters ranging from 18.67±1.53 to 25.16±1.04 mm, and MIC and MBC values ranging from 3.12 to 6.25 (v/v). The MBC/MIC values from 1 to 2 and the time kill curve demonstrated that *Helix aspersa* mucus has a bactericidal effect. It has also a significant antibiofilm effect as it could inhibit the formation of biofilms at percentages ranging from 88.46±0.96% to 94.93±1.48% and eliminate biofilm at percentages ranging from 79.69±1.36% to 91.38±1.80%. Protein profiling of the mucus revealed the presence of three distinct proteins with molecular weights of 29.0, 81.1, and 106.4 kDa, which are likely responsible for these bioactive properties. *Helix aspersa* mucus could serve as a promising natural antimicrobial agent, offering potential applications in treating infected wounds caused by multidrug resistant bacteria.

Keywords : Antibacterial effect; *Helix aspersa*; infected wounds; mucus; *Staphylococcus aureus*.

INTRODUCTION

Since Fleming discovered penicillin in 1928, many antibiotics have been discovered, synthesized, and used therapeutically in antimicrobial pharmacology (Arunachalam *et al*., 2023). However, the treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antibiotics, which is due to various factors, including the misuse and the increased use of antibiotics in biomedical and agricultural settings (Bouacha *et al*., 2018; Merah *et al*., 2022). The Gram-positive *Staphylococcus aureus* is one of the most prevalent opportunistic bacteria affecting human. It is the causative agent of many infections, ranging from common skin conditions like impetigo and abscesses to severe and occasionally fatal illnesses, including sepsis, pneumonia, endocarditis, osteomyelitis, and toxic shock syndrome (Madhuri & Khandait, 2021; Bashabsheh *et al*., 2023; Mukherjee *et al*., 2024). To enhance its pathogenicity and dissemination within the host, *S. aureus* uses enzymes including coagulase, hyaluronidase, deoxyribonuclease, and lipase. It can also synthesize extracellular toxins to enhance its pathogenicity, including enterotoxins, toxic shock syndrome toxin 1, exfoliative toxins, hemolysins, epidermal cell differentiation inhibitors, and Panton–Valentine leucocidin (Tuon *et al*., 2023). Although penicillin initially revolutionized the treatment of serious *S. aureus* infections, resistance expanded quickly as a result of the acquisition of resistance genes. The emergence of multidrug-resistant (MDR) *S. aureus* has made the situation worse, increasing mortality, morbidity, and healthcare costs (Bouacha *et al*., 2022). One of the most crucial processes used by MDR *S.aureus* to evade antibiotic therapy is genetic adaptation, which changes the antibiotic target sites through genetic modification (Guo *et al*., 2020). This mechanism enables the bacteria to produce modified proteins with low affinity to antibiotics. MDR *S. aureus* may also acquire resistance genes from other bacteria through bacteriophages, transposons, and plasmids (Nandhini *et al*., 2022). Moreover, MDR *S. aureus* may use efflux pumps to eject antibiotics to the exterior of the bacterial cell, significantly reducing the intracellular concentration of antibiotics. Another key process to increase resistance to antibiotics is the formation of biofilms, which are surface-attached microbial communities of bacterium enclosed in a protective polysaccharide matrix (Suárez *et al*., 2021; Razdan *et al*., 2022; Tuon *et al*., 2023). Currently, available antibiotics are unable to completely eliminate biofilms. Therefore, the development of novel and potent antimicrobial drugs is a crucial strategy to improve the management and treatment of infected wounds. One of the most important strategies in this context is the use of the snail mucus.

Snails are animals that exist almost everywhere in the world; most are terrestrial. They are mollusks, gastropods, hermaphrodite, and members of the order of stylommatophora. In general,

snails are phytophagous. The ground is also part of its food, and constitutes a contribution in calcium essential to the formation of its shell and influencing its growth. Snails produce large quantities of mucus, often called "slime". (Gabriel *et al*., 2011; Zhong *et al*., 2013; Belouhova *et al*., 2022). Snail mucus is composed of several bioactive compounds and has several therapeutic effects for the treatment of skin diseases. It is mainly composed of water and a mixture of proteoglycans, glycosaminoglycans, glycoprotein enzymes, hyaluronic acid, copper peptides, antimicrobial peptides, and metal ions (Gabriel *et al*., 2011; Cilia & Fratini, 2018; Topalova *et al*., 2022). It also contains allantoin, collagen, elastin, and glycolic acid. Snail mucus has long been known to have a range of beneficial properties, including wound healing, anti-inflammatory effects, and the ability to protect against UV radiation. The antibacterial effect of snail mucus is attributed to the presence of various bioactive compounds such as glycoproteins, enzymes, peptides, and hyaluronic acid. The antimicrobial activity of snail mucus was first reported by researchers in Japan in 1982 in *Achatina fulica* mucus (Iguchi *et al*., 1982). Since then, numerous researches have been carried out on the composition of *Achatina fulica* mucus (Berniyanti *et al*., 2007; Zhong *et al*., 2013; Nugrahananto *et al*., 2014) and the snail mucus of various other species (Dolashki *et al*., 2018; Nantarat *et al*., 2019; Noothuan *et al*., 2021; Abimbola Okeniyi *et al*., 2022). However, few studies have been conducted on the composition of *Helix aspersa* mucus (Gabriel *et al*., 2011; Abimbola Okeniyi *et al*., 2022). *Helix aspersa* is a species of terrestrial snail commonly found in various regions, including Europe and North Africa. It is also known as the garden snail, and is a terrestrial gastropod mollusk belonging to the family Helicidae. It is one of the most widespread snail species and is easily recognizable by its medium-sized, globular shell with a brownish color and spiral bands. *Helix aspersa* mucus is rich in bioactive compounds such as glycoproteins, allantoin, and hyaluronic acid. This makes the snail an excellent candidate for research into natural antimicrobial agents, particularly in the context of rising antibiotic resistance (Gabriel *et al*., 2011, Merah *et al*., 2022). Beyond its therapeutic properties, snail mucus has significant economic potential, particularly in the cosmetics and pharmaceutical industries. The snail farming industry (heliciculture) has seen growth due to the demand for snail mucus-based products, which are used in skin care for their anti-aging and regenerative properties. Exploring the antibacterial effects of snail mucus can open new avenues for its application, potentially boosting local economies through the development of new products. Even though Algeria has a rich abundance of the species *Helix aspersa*, the composition and therapeutic properties of its mucus have never been studied. To the best of our knowledge, this is the first study in Algeria that explore the protein profile, antibacterial, and antibiofilm properties of *Helix aspersa* mucus towards MDR *S.aureus* isolated from infected wounds.

MATERIAL AND METHODS

Snail collection

Thirty wild individuals of *Helix aspersa* were collected during Mars 2023 from the region of Bouthelja, El Taref (East of Algeria). The selected animals have an average size ranging from 30.12±3.42 to 35.78±3.67 mm and an average weight between 13.71±2.16 and 15.63±2.39g. The animals were placed in three plastic containers measuring 30 cm wide, 20 cm high, and 35 cm long. The containers contained a wet sponge to maintain humidity and were filled with sandy soil. Wells were made in the lids of the containers to ensure good aeration. The selected animals were fed daily with fresh lettuce leaves, cabbage, and spinach, and supplied with water. The containers were kept at room temperature (22±4°C) and in ambient light cycles.

Mucus preparation

Snails were cleaned with sterile distilled water before inducing mucus production. Then, a sterile Pasteur pipette was used to gently stimulate each snail and a sterile syringe was used to collect the produced mucus. Mucus from all 30 snails was combined into a single sample before testing. A volume of 10 mL of sterile distilled water was added to 5 mL of snail mucus. The mixture was incubated for 24 hours with shaking (at 150 rpm) and then centrifuged at 8000 g for 30 min at 4°C, producing a water-soluble fraction. 2 volumes of ethanol (70%) were added to this solution and centrifuged again at 3000 g for 30 min to obtain a precipitated fraction. The precipitated fraction of the snail mucus was specifically selected to identify the protein content and assess its antibacterial effect (Nugrahananto *et al*., 2014). The precipitated fraction was freshly diluted with distilled water to obtain the following dilutions: 100, 80, 40, 20, 10, 5, and 2.5% (v/v).

Bacteria selection

The Multidrug-resistant bacteria were isolated from infected wounds of patients at Ibn Sina Hospital, Annaba, Algeria. The wound area was gently cleaned with a sterile saline solution (0.9% NaCl). To collect pus, sterile swabs were rotated over the infected area and then incubated in 5 mL of nutrient broth (Difco, MD, USA) at 37°C for 24 hours. The broth was inoculated on Chapman agar plates (Difco, MD, USA). Bacterial identification was performed using Gram staining, oxidase, catalase, coagulase, and API STAPH. The inoculum was prepared by selecting 10 colonies from stock culture and suspended in 20 mL of sterile in tryptic soy broth (Difco, MD, USA). The bacterial suspension was incubated for 24 hours at 37°C and then adjusted to 10^6 colony-forming units (CFU)/mL.

According to the Clinical and Laboratory Standards Institute (CLSI, 2017), an antibiogram of the isolated bacteria was performed in Mueller Hinton agar medium (Difco, MD, USA) using the disk diffusion method. In Table 1, only strains resistant to most of the following antibiotics were selected: amoxicillin–clavulanic-acid (2/1 µg), clindamycin (2 µg), colistin (10 µg), doxycycline (30 µg), fosfomycin (200 µg), oxacillin (1 µg), ofloxacin (5 µg), pristinamycin (15 µg), sulfamethoxazole-trimethoprim (1.25/23.75 µg), and vancomycin (5 µg) (BioRad, France).

The MDR *S. aureus* were also assessed for their adhesion profile to the microtiter plate wells using the previously described methods (Bouacha *et al*., 2022). A volume of 200 µL of the bacterial suspension was transferred to the sterile 96-well microtitre plates (Fisher Scientific, UK). A well containing 200 µL of tryptic soy broth without bacteria was used as a negative control. After 24 hours of incubation, planktonic bacteria were removed by washing the wells three times with 200 µL of phosphate-buffered saline. After drying the plates in the air, they were stained for 10 min using 200 µL of crystal violet (1%), dried at room temperature, and carefully rinsed under running tap water to remove any residual stain. The biofilms were dissolved in 200 µL of ethanol (95%) and characterized by measuring the optical density (OD) at 570 nm. A bacterial strain was considered a strong adherent if its OD was higher than four times that of the negative control. From all the isolated bacteria, thirteen strains were identified as MDR strains and were found to produce biofilms. These strains, along with two standard strains, *S. aureus* ATCC 29213 and *S. aureus* ATCC 43300, were utilized to assess the antibacterial and antibiofilm effects of snail mucus.

Table 1. Susceptibility of the pathogenic bacteria to antibiotics

S. aureus strains	Susceptibility to antibiotics									
	AMC	CL	CO	DO	FO	OF	OX	PR	SXT	VA
S. aureus ATCC 29213	S	S	S	S	S	S	S	S	S	S
S. aureus ATCC 43300	R	S	S	S	S	S	R	S	S	S
S. aureus 1	R	R	S	R	R	R	R	R	R	S
S. aureus 2	R	R	R	R	S	S	R	R	R	S
S. aureus 3	R	S	R	R	S	R	R	R	S	S
S. aureus 4	R	R	R	R	S	S	R	S	R	S
S. aureus 5	R	S	R	R	R	S	R	S	R	S
S. aureus 6	R	S	R	R	S	S	R	R	R	S
S. aureus 7	R	R	S	S	R	S	R	R	R	R
S. aureus 8	R	R	R	R	R	R	R	R	R	S
S. aureus 9	R	R	R	R	S	R	R	R	R	S
S. aureus 10	R	R	R	R	R	R	R	R	R	S
S. aureus 11	R	R	R	S	R	S	R	R	R	S
S. aureus 12	R	R	R	R	R	R	R	S	R	S
S. aureus 13	R	R	S	R	S	S	R	R	R	S

AMC: amoxicillin–clavulanic-acid, CL: clindamycin, CO: colistin, DO: Doxicycline, FO: fosfomycin, OF: ofloxacin, OX: oxacillin, PR: pristinamycin, SXT: sulfamethoxazole-trimethoprim, VA: vancomycin, R: resistant, S: susceptible.

Antibacerial effect

The antibacterial effect was performed according to the previously described methods (Bouacha *et al*., 2022, 2023; Boudiar *et al*., 2023). Initially, agar well diffusion assay was carried out using Mueller Hinton agar plates with wells in 6 mm diameter. The entire surface of the plates was streaked using swabs and 50 µL of the snail mucus was added to the well. A well filled with 50 µL of sterile water was used as a negative control. The plates were incubated at 37°C for 24 hours. The results of the inhibitory diameters around the wells were performed in triplicate and the mean ± standard deviations (SD) were calculated.

Next, a microdilution assay was performed using 96-well microtitre plates. In each well, a volume of 100 µl of the inoculum was added to 100 µL of snail mucus at various dilutions. A well used as a negative control was filled with nutrient broth; another well used as a positive control was filled with bacterial inoculums. The microtiter plates were incubated at 37°C for 24 hours. MIC values were indicated by the lowest dilution where no growth was detected.

Finally, the minimum bactericidal concentrations (MBC) were identified by streaking on nutrient agar plates 10 µL of the cultures that did not show bacterial growth in MIC assessment. After incubation at 37°C for 24 hours, MBC values were determined as the minimum dilution of mucus extracts with no visible growth on the Petri plates (Bouacha *et al*., 2022).

Time kill assay

Time-kill assay was performed according to (Bouacha *et al*., 2022). A tube containing 40% (v/v) *Helix aspersa* mucus was inoculated with 20 µL of the bacterial suspension at 10^6 CFU/mL. Another tube of bacterial inoculum without snail mucus was used as a positive control. The tubes were incubated at 37°C with shaking continuously at 150 rpm. Broth aliquots of 10 µL were collected, cultivated on nutrient agar, and incubated for 24 hours at 37°C. Total CFU/mL was calculated after every three hours of incubation. Time-kill curves were constructed for each strain by plotting $\log_{10}$ CFU/mL.

Anitibiofilm effect

The antibiofilm effect of the snail slim was performed according to the previously described methods (Bouacha *et al*., 2022). A volume of 100 µL of the inoculum and 100 µL of 40% (v/v) snail mucus were used to inoculate the microtiter plates. Two control wells were used; one filled with inoculum was used as a growth control and another filled with tryptone soy broth was used as a negative control. After incubation for 24 hours at 37°C, the wells were gently aspirated and washed three times with sterile phosphate-buffered saline. Then, 200 µL of ethanol (95%) was added to the wells for 20 minutes and they were dyed with 200 µL of crystal violet (1%) for 20 min. The Excess stain was washed off with running water and dried in the air for 30 min. After dissolving the biofilms with 200 µL of ethanol, the adherent biofilms were quantified spectrophotometrically at 570 nm. The percentage of biofilm reduction was calculated according to this formula:

$$\textit{The percentage of biofilm reduction}\ (\%) = \frac{\textit{OD positive control} - \textit{OD treatment}}{\textit{OD of the positive control}} \times 100$$

To calculate the percentages of biofilm elimination, wells with established biofilms were aseptically washed three times with phosphate-buffered saline to remove planktonic bacteria, and 200 µL of snail mucus at 40% (v/v) was added. After 24 hours of incubation at 37°C, the solutions were removed and each well was washed three times with sterile phosphate-buffered saline. As in the evaluation of biofilm reduction, the biofilms were quantified spectrophotometrically and the percentages of biofilm elimination were estimated.

SDS-PAGE Electrophosis

The precipitated fractions was used to analyse the prtein profil of the mucus. The protein concentration of mucus was estimated using the Bradford assay. Protein content was determined by estimating linear regression standard curve. Then, an SDS-PAGE electrophoresis was performed in 12% polyacrylamide gel to determine the molecular weight of protein content. Briefly, the precipitated fraction was mixed 1/1 with Laemmli buffer (3.55 mL of type I water; 1.25 mL of 0.5 M Tris-HCl at pH 6.8; 2.5 mL of 0.5 M Tris-HCl at pH 6.8; 2.5 mL of 0.5 M Tris-HCl at pH 6.8; 2.5 mL glycerol; 2 mL 10% SDS and 0.2 mg 0.5% bromophenol blue) and heated to 95°C for five minutes. 5 µl of protein molecular weight markers and 5 µl of mucus proteins were added to the wells. The voltage of the gels was first set at 50 V for 20 minutes, and then it was increased to 150 V for an additional 85 minutes. The gel was removed and stained with Coomassie R-250 for an hour and then rinsed with a decolorizing solution (50% methanol and 10% acetic acid) until the bands could be seen as intended. The molecular weight of proteins was estimated by comparison with the molecular markers (14-100 kDa).

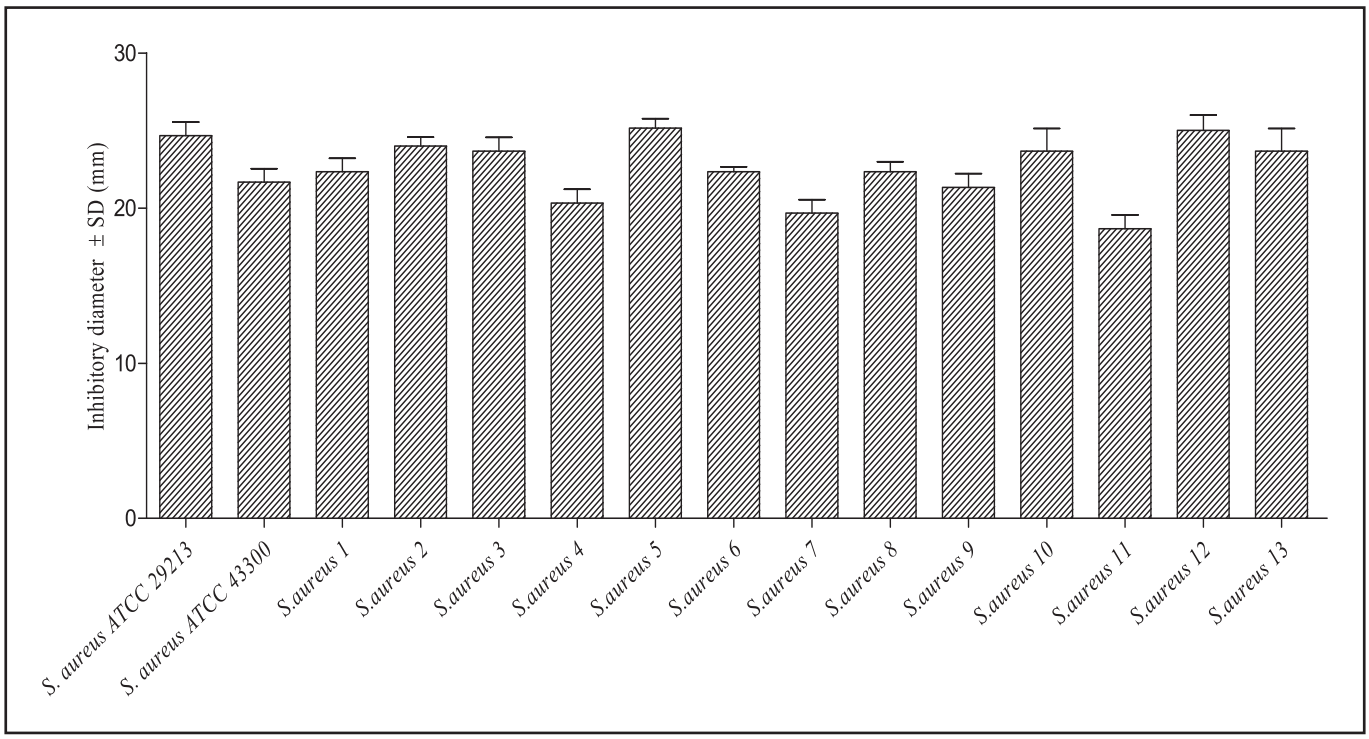

Figure 1. Inhibitory diameters of *Helix aspersa* mucus towards *S. aureus* isolated from infected wounds.

Table 2. MIC, MBC, and MBC/MIC ratio of *Helix aspersa* mucus against *S. aureus* strains

Bacterial strains	MIC (v/v)	MBC (v/v)	MBC/MIC ratio
S. aureus ATCC 29213	3.12	3.12	1.00
S. aureus ATCC 43300	3.12	3.12	1.00
S. aureus 1	3.12	3.12	1.00
S. aureus 2	3.12	6.25	2.00
S. aureus 3	3.12	3.12	1.00
S. aureus 4	3.12	3.12	1.00
S. aureus 5	3.12	6.25	2.00
S. aureus 6	3.12	3.12	1.00
S. aureus 7	3.12	3.12	1.00
S. aureus 8	3.12	3.12	1.00
S. aureus 9	6.25	6.25	1.00
S. aureus 10	3.12	3.12	1.00
S. aureus 11	3.12	3.12	1.00
S. aureus 12	3.12	3.12	1.00
S. aureus 13	3.12	6.25	2.00

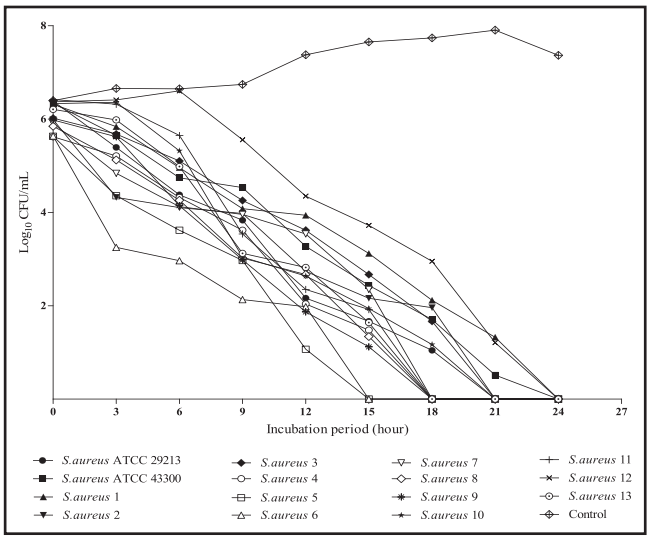

Figure 2. Time kill curve of the effect of *Helix aspersa* mucus on the growth and viability of *S. aureus* strains.

RESULTS

Antibacterial effect

The antibacterial effect of *Helix aspersa* mucus against *S. aureus* strains is demonstrated in Figure 1 and Table 2. The results show that *Helix aspersa* mucus has potent antibacterial properties and it is capable of eradicating *S. aureus* even at low concentrations. The inhibitory diameters range from 18.67±1.53 to 25.16±1.04 mm and the MIC and MBC values range from 3.12 to 6.25 (v/v). Since the MBC/MIC ratio is always between 1 and 2, *Helix aspersa* mucus have a bactericidal rather than a bacteriostatic effect.

Time- kill assay

The time-kill curve in Figure 2 demonstrated that *Helix aspersa* mucus is a highly effective antibacterial agent against *S. aureus strains*, including reference strains and MDR isolates. Most strains exhibit a similar pattern of reduction in CFU, with significant declines observed within the first hours. Some isolates show slightly slower reduction rates compared to others but still achieve complete eradication by 24 hours. The progressive decrease in bacterial viability over time and the complete eradication of all strains by 24 hours strongly indicate that the mucus not only inhibits bacterial growth but actively kills the bacteria.

Antibiofilm effect

The antibiofilm effects of *Helix aspersa* mucus are illustarated in Figure 3. The results show a strong reduction of biofilm formation demonstrated by *Helix aspersa* mucus, with percentages ranging from 88.46±0.96% to 94.93±1.48% and percentages of biofilm elimination ranging from 79.69±1.36% to 91.38±1.80%.

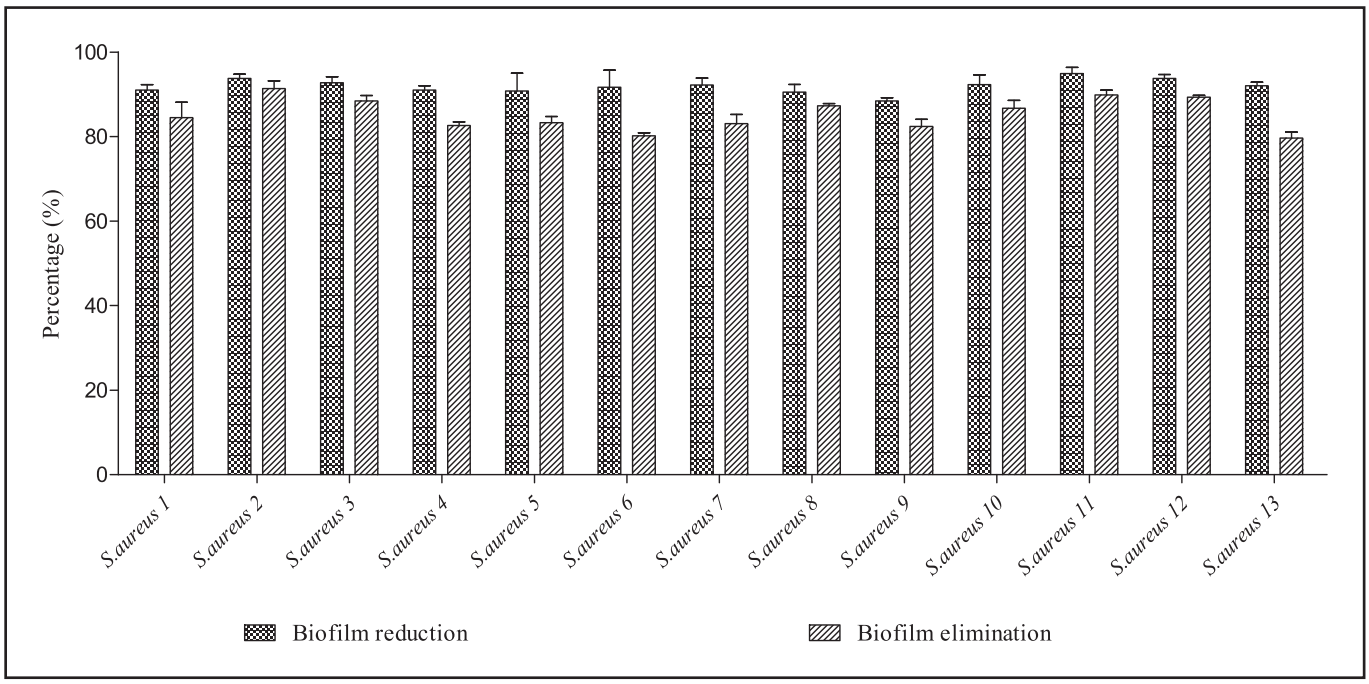

Figure 3. Antibiofilm effect of *Helix aspersa* mucus towards *S. aureus* strains.

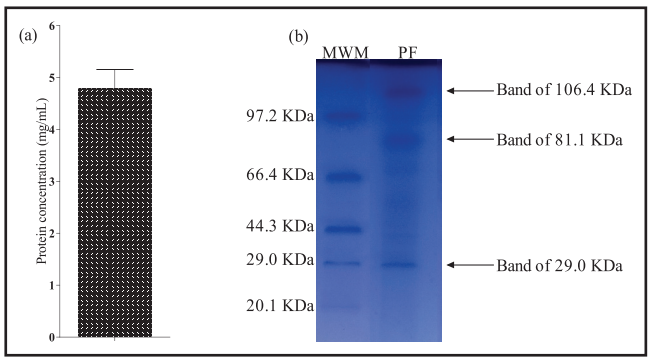

Figure 4. Protein profil of *Helix aspersa* mucus. (a): Protein concentration in the precipated fraction. (b): SDS electrophosis of protein. MWM: molecular weight markor, PF: precipated fraction.

Protein profile

The concentration and the electrophoresis of *Helix aspersa* proteins are represented in Figure 4. The results show that the concentration of proteins in the precipitated fraction is 4.47 mg/mL, corresponding to different protein sizes. Three bands of approximately 29.0 KDa, 80.1 KDa, and 106.4 kDa were obtained.

DISCUSSION

One of the main factors that influence wound healing whether it is following a wound, burn, or surgery is a bacterial infection. When a wound is infected with bacteria, it produces an inflammatory response, tissue damage, and accumulates fluid that interferes with the functioning of healing processes (Bouacha *et al*., 2022; Mahmud *et al*., 2022; Razdan *et al*., 2022). Various bacterial species are implicated in wound infection including *S. aureus,* which has been reported to be the most predominant isolate in infected wounds and an important cause of morbidity and mortality in recent years, especially in immunocompromised patients (Bouacha *et al*., 2022; Hassan *et al*., 2022).

Based on the results illustrated in Figure 1 and Table 2, *Helix aspersa* mucus exhibits an excellent antibacterial effect on all the strains. The results revealed that the mucus effectively prevents the growth of *S. aureus* colonies even at relatively low concentrations. The MIC values ranging from 3.12 to 6.25 (v/v) further support the efficacy of the mucus in inhibiting bacterial growth. Moreover, MBC values align closely with the MIC values, indicating that the mucus not only inhibits bacterial growth but also kills the bacteria at similar concentrations. In the antibacterial evaluation, the MBC/MIC ratio is a critical parameter in differentiating between bacteriostatic and bactericidal agents. With values below 4, the antibacterial agent has a bactericidal effect, meaning that it could kill the bacterial cells. However, an antibacterial agent with MBC/MIC ratio above 4 has a bacteriostatic effect, which means that it could only inhibit bacterial growth. In this context, *Helix aspersa* mucus with MBC/MIC ratio remains consistently between 1 and 2, suggesting that it has a bactericidal effect. These findings were further confirmed by the results of time kill curve in the Figure 2, which demonstrated that the *Helix aspersa* mucus exhibits a rapid kill rate, significantly reducing CFU within the first 6 hours for most strains. This is crucial in managing effectively the treatment of infected wounds. Moreover, the majority of strains show complete elimination by 24 hours, demonstrating the potent bactericidal properties of *Helix aspersa* mucus. The time-dependent nature of the killing effect suggests that the mucus proteins interact with bacteria in a manner that progressively inhibits and ultimately eradicates bacterial growth within a relatively short period. This is crucial for therapeutic applications, as it ensures that the bacteria are not merely inhibited but destroyed, reducing the risk of recurrence or resistance. Indeed, the antibacterial mechanism of snail mucus is multifactorial processes, involving a range of different compounds that act synergistically to inhibit bacterial growth and kill bacteria.

Recently, it has been emphasized that bacterial biofilm plays an important role in chronic wounds, particularly in the prolongation of the inflammatory phase of repair. They interfere with the healing process by forming a mechanical barrier that stops reepithelialization, causing persistent inflammation, and protection against antimicrobial agents (Las Heras *et al*., 2024). The polysaccharide matrix restricts antibiotic penetration and facilitates the exchange of resistance genes among bacteria (Nandhini *et al*. 2022; Mukherjee *et al*., 2024). It also helps MDR *S. aureus* evade the host immune system, persisting on medical devices

and tissues, and leading to chronic infections that are difficult to eradicate (Musini *et al*., 2021; Mlynarczyk-Bonikowska *et al*., 2022). According to the results in Figure 3, *Helix aspersa* mucus exhibits an excellent antibiofilm effect by inhibiting the formation of biofilms and eliminating the established ones. Although we have not found any study evaluating the antibiofilm effects of *Helix aspersa*. However, this phenomenon is probably caused by the disruption of quorum sensing systems, a regulatory mechanism, used to synchronize gene expression in response to environmental and population density fluctuations. Snail mucus could interfere with quorum sensing through several mechanisms, including binding to signaling receptors, blocking signal transduction, and preventing the synthesis of signal molecules. These potentially mitigate bacterial pathogenicity by inhibiting virulence factor expression and biofilm formation. The proteins and glycoproteins in the mucus can promote the proliferation and migration of skin cells, aiding in the healing process and helping to clear biofilms through natural wound-healing mechanisms. *Helix aspersa* mucus has anti-inflammatory properties, which reduce the inflammation associated with wound infections and create a less favorable environment for biofilm formation (Merah *et al*., 2022). Recently, studies have demonstrated that the application of *Helix aspersa* mucus to infected wounds significantly enhances wound healing (El-Zawawy & Mona, 2021; Aouji *et al*., 2023).

The therapeutic properties of snail mucus are attributed to the presence of various bioactive compounds, including glycoproteins, enzymes, peptides, and hyaluronic acid. One of the main compounds of snail mucus that has several therapeutic properties is its protein content. As illustrated in Figure 4, *Helix aspersa* mucus contains three proteins with different sizes (29, 81.1, and 106.4 KDa). These sizes correspond to a molecular weight range of achasin protein (around 10-120 kDa) found in some other species and reported previously by other authors (Nugrahananto *et al*., 2014; Pitt *et al*., 2015; Noothuan *et al*., 2021). However, (Bortolotti *et al*., 2016) have found only two proteins in the *Helix aspersa* mucus, one between 40 and 30 and another above 50 KDa. They also reported that the absence of the protein of 30 KDa decreases the antibacterial effect. This aligns with findings from another study (Dolashki *et al*., 2018), suggesting that the smaller protein of 30 KDa contributes ignificantly to the mucus's antibacterial properties. Furthermore, (Suárez *et al*., 2021) have reported that mucus proteins ranging from 20 to 80 KDa from *Achatina fulica* are a source of antimicrobial, antibiofilm and, anti-virulence bioactive molecules against *S. aureus* strains. Other authors reported that from several bands, only those with molecular weights ranging from 11.45 to 83.67 KDa (Nugrahananto *et al*., 2014) and from 71 to 73 KDa (Berniyanti *et al*., 2007) correspond to proteins with antibacterial effects. Therefore, the antibacterial activity in *H. aspersa* mucus seems to be attributed to one or more proteins with molecular weights ranging from 30 to 110 KDa, which act synergistically to disrupt bacterial growth. These proteins could work together to enhance their antibacterial properties, creating a more potent defense against bacterial infections. By interacting with various bacterial cell structures and functions, they inhibit essential processes such as cell wall synthesis, protein synthesis, and DNA replication.

CONCLUSION

In this study, it has been demonstrated that *Helix aspersa* mucus has strong antibacterial and antibiofilm effects towards *S. aureus* strains. Analysis of the protein profile revealed the presence of three distinct proteins of varying sizes, which likely contribute to its therapeutic properties. The antibacterial and antibiofilm effects of *Helix aspersa* mucus make it a promising natural alternative to synthetic antibiotics, with significant potential for applications in treating infected wounds.

Conflict of interests

The authors declare that they have no conflict of interest.

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