GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Effects of mucus of the snail *Archachatina marginata* on the bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *S. epidermidis*: The relative difference amongst the raw, diluted and filtered mucus

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GSC Biological and Pharmaceutical Sciences, 2025, 32(01), 360-370

Publication history: Received on 14 June 2025; revised on 21 July 2025; accepted on 24 July 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.32.1.0291

Abstract

The study aimed to assess the antibacterial activity of the raw, diluted and filtered mucus of the snail *Archachatina marginata* against the bacteria *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis*. The collected snails were manually stimulated and the secreted mucus obtained. Antimicrobial tests were then carried out using various fractions of this mucus. The method of antimicrobial-impregnated discs on the agar culture medium was used. Results showed that in the presence of raw mucus, the mean inhibition zone diameters ranged from 11.70 to 17.50mm. The largest was observed with *S. aureus* and the smallest with *P. aeruginosa.* When using diluted mucus, the mean values of the inhibition diameters of bacterial cells varied between 9.00 and 17.44 mm in the presence of the different doses of mucus. The largest diameter was recorded with *S. aureus* at the 80% mucus dose and the smallest with *E. coli* at the 20% mucus dose. With the filtered mucus, inhibition of *S. aureus* and *S. epidermidis* growth started with 20 µL of the mucus. Inhibition if *E. coli* growth was observed from a minimum volume of 20 µL of the mucus. However, no inhibition of *P. aeruginosa* growth was observed from 20 µL to 175 µL of filtrate and it particularly distinguished itself from the others. The MICs MBCs of the filtered mucus were greater than those of the raw mucus. It seems necessary to continue exploring the secretions of other molluscs against infectious bacteria, to search for therapeutic alternatives to classical antibiotics.

Keywords: Mollusc of *A. marginata;* Mucus; Raw fraction; Diluted and filtered fractions; Antibacterial activity

1. Introduction

Many infectious bacteria affecting humans are of various origins and various species. These bacteria include those of the genus *Escherichia*, *Staphylococcus*, and *Pseudomonas* [1]. *Pseudomonas aeruginosa* is a type of bacteria commonly found in the environment, like soil and water, but it can also cause infections in humans, particularly those with weakened immune systems or underlying health conditions. *P. aeruginosa* is able to adapt to the adverse environment in hosts by secreting a variety of virulence factors, which contribute to successful infection and causing disease [2, 3]. These infections can range from minor skin irritations to severe, potentially life-threatening illnesses [3].

E. coli can be categorized into various pathotypes depending on the presence of specific virulence factors, mechanisms of infection, tissue tropism, interactions with host cells and clinical symptoms [4]. These include: (i) an acute and

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prolonged diarrhea in infants associated to Enteropathogenic *E. coli* (EPEC), (ii) hemorrhagic colitis and hemolytic uremic syndrome associated to Enterohemorrhagic *E. coli* (EHEC), (iii) travelers' diarrhea associated to Enterotoxigenic *E. coli* (ETEC), (iv) acute and chronic diarrhea associated to Enteroaggregative *E. coli* (EAEC), (v) watery diarrhea in young children associated to Diffusely adherent E. coli (DAEC), (vi) dysentery and watery diarrhea associated to Enteroinvasive *E. coli* (EIEC), (vii) inflammatory bowel disease associated to Adherent-Invasive *E. coli* (AIEC), (viii) urinary tract infections (UTI) associated to Uropathogenic *E. coli* (UPEC),(ix) neonatal meningitis associated to Neonatal meningitis *E. coli* (NMEC), (x) bacteremia and sepsis associated to Septicemiaassociated *E. coli* (SEPEC), and (xi) severe respiratory and systemic infections in poultry associated to Avian pathogenic E. coli (APEC) [4, 5].

Staphylococci are pathogenic bacteria responsible for a broad spectrum of diseases with varying degrees of severity. They are one of the main causes of nosocomial infections (hospital-acquired infections) but can also be acquired outside hospitals. They are known to be a part of the natural skin flora, specifically colonizing external mucous membranes, but they are also often found in the environment (in untreated water, soil and contaminated objects). *S. aureus* is a highly adaptable and opportunistic pathogen that can colonize various anatomical sites, evade the host immune system, and cause a broad spectrum of infections. Its pathogenic potential is driven by a complex interplay of virulence factors that facilitate adherence, invasion, immune evasion, and tissue destruction [6, 7]. Its virulence factors, encompassing surface proteins, secreted toxins, and metabolic adaptations, enable *S. aureus* to colonize host tissues, evade immune defenses, and inflict cellular damage [7-9].

Staphylococcus epidermidis is a bacterium commonly found on human skin and is usually harmless, but it can cause infections, particularly in individuals with weakened immune systems or when it colonizes medical implants. *S. epidermidis* presents opposite roles. Its ability to form biofilms on medical devices when implanted and its antibiotic resistance places S. epidermidis as the second cause of nosocomial infections, although, the exact mechanisms by which its commensals strains in healthy skin interact with host cells and specific receptors remain poorly understood and is likely to be multifaceted [10-12].

Bacteria are becoming increasingly diverse and, above all, more resistant to antibiotics. This is causing significant public health problems [13-15]. This microbial resistance varies from one antibiotic to another, and from one individual to another. Antibiotic resistance could result from genetic modification during which random mutations in genes coding for enzymes can give rise to modified catalysts with increasingly broad resistance spectra [16-18]. It could also be linked to the existence of genes in bacterial genomes that could generate a resistance phenotype [17-19]. Some bacteria may therefore be naturally resistant to certain antibiotics. Others, however, develop resistance through genetic mutations induced by exposure to antibiotics. Antibiotic resistance has the potential to spread, given the ease of exchange of genetic material between bacterial species [20]. Alternatives to this microbial resistance to antibiotics have been explored for several decades. The use of these alternatives is also linked to the precariousness of socio-economic and hygiene conditions, but also to illiteracy and limited access to health care in rural areas in certain parts of the world. In some Asian and African countries, medicinal plants are used to treat various health problems [21]. These plants are used in the form of infusions, decoctions, or macerations, and the solvents are often water or alcohol. Others have suggested the mucus naturally secreted by certain mollusks.

Exploration of the properties of snail mucus has yielded encouraging results in various aspects of therapeutic research including oncology, drug delivery, cosmetics, antibacterial properties, among others [22, 23]. The snail *Archachatina marginata* is widespread in Africa, and little data are available on the activity of its mucus against several environmental bacteria of health importance. Few studies have been conducted on the potential variability of this antimicrobial activity as a function of mucus concentration, or as a function of its "raw" or "filtered" nature. Little is also known about the role of bacterial cell wall type on this activity. This study aims to evaluate the antibacterial effect of various concentrations on the one hand, and of the crude and filtered fraction on the other hand, of *Archachatina marginata* mucus on some Gram-positive and Gram-negative bacterial species of environmental and human health importance *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *S. epidermidis*.

2. Materials and method

2.1. Mollusk collection and mucus extraction

Thirty *Archachatina marginata* mollusks were collected in the suburbs of Yaounde (Cameroon, Central Africa), then identified. They were colleted during September 2023 (this period being included in the raining season in the region). Figure 1 shows a freshly harvested *A. marginata* specimen. It is an Eukaryota, of the Animalia Kingdom, Mollusca Phylum, Class of Gastropoda, Order of Stylommatophora, and of Achatinidae family [24, 25]. They were then kept in a plastic container containing moist soil and cabbage leaves (Brassicaoleracea) for food. In the laboratory, they were

housed individually in sterile glass boxes and fasted for three days. Mucus was extracted according to the modified protocol of Phrompanya et al. [26]. The snails were manually stimulated at their foot regions using sterile forceps and approximately 2 mL of mucus secretion per individual were collected and stored at 4°C.

Figure 1 Image of the mollusc *Archachatina marginata* used

2.2. Bacterial isolation and identification

The bacteria considered were *S. aureus* and *S. epidermidis* for Gram-positive bacteria, and *E. coli* and *P. aeruginosa* for Gram-negative bacteria. They were isolated from surface water by the membrane filter technique, using Millipore membranes with a porosity of 0.45 µm. The agar culture media used were Endo medium (Dico) for *E. coli*, CN agar (Biokar) for *P. aeruginosa*, and Mannitol Salt Agar (Bio-Rad) for *Staphylococcus*. The strains were then identified by standard biochemical methods [27, 28], and then stored in cold storage in glycerol. Each bacterium was activated on agar medium before use for antibacterial tests.

2.3. Preparation of the bacterial inoculum

After reactivation of each bacterium, a colony was picked and streaked onto standard agar and incubated at 37°C for 24 hours. Cells from a pure culture were then introduced into a test tube containing 9 mL of sterile saline (0.85% NaCl). The homogenized bacterial suspension was vortexed and adjusted to a density of 0.5 Mac Farland (BaCl2 and 1% H2SO4). The concentration of bacteria in the stock suspension was approximately $1.5x10^8$ CFU/mL. Dilutions of 1/10 for Gram-positive bacteria and 1/100 for Gram-negative bacteria were carried out in sterile physiological water to successively obtain $1.5x10^7$ CFU/mL and $1.5x10^6$ CFU/mL in order to obtain an inoculum capable of giving a semi-confluent layer of colonies.

2.4. Mucus preparation and control antibiotics

From 10 mL of this raw mucus, dilutions were made with sterile distilled water in proportions of 0.2, 0.4, 0.6, and 0.8, i.e., dilutions of 20%, 40%, 60%, and 80%. For the filtered mucus extract, 9 mL was diluted in 9 mL of sterile distilled water. The mixture was then filtered through Millipore membranes with a porosity of 0.2 [26]. Gentamicin (80 mg/2mL) was used as the control antibiotic; 25 µL was collected and diluted in 975 µL of sterile distilled water to obtain a final concentration of 1 mg/mL.

2.5. Determination of the diameters of the bacterial inhibition zones

The tests were carried out in three series. The first series was carried out with filtered mucus. The volumes of this filtered mucus used varied from 20 to 175 µL. The second series was carried out with raw mucus. The third series was carried out with raw mucus diluted to different doses (80%, 60%, 40% and 20%).

The different Petri dishes containing the culture media were inoculated by swabbing. The inocula of the different strains previously prepared had the concentrations $1.5x10^7$ CFU/mL for Gram-positive bacteria and $1.5x10^6$ CFU/mL for Gram-negative bacteria.

The 6 mm diameter discs were made from Whatman No. 1 filter paper and then sterilized at 121°C for 15 minutes in an autoclave. These discs were then placed on the agar. In fact, 20 µL of the reference antibiotic were placed on the disc placed in the center of the agar and 20 µL, 40 µL, 60 µL, 80 µL of filtered mucus were placed on the other four discs for a first series. For the second series, volumes of 100 µL, 125 µL, 150 µL and 175 µL of the filtered mucus were deposited on the discs.

For raw mucus and its different dilutions, the antibacterial activity was analyzed according to the disk diffusion method on Muller Hilton agar medium [29]. The sterilized discs were soaked in raw mucus and its different doses 20%, 40%, 60%, 80%, then placed in a water bath at 37°C for 30 minutes. These mucus-impregnated discs were then left at room temperature for 5 minutes, then were placed on the surface of the culture media in Petri dishes containing the bacteria [30]. For each microorganism tested, the tests were carried out in triplicate. The above dishes were placed for 15 minutes (pre-diffusion) under the hood and then incubated at 37°C for 24 to 48 hours [31]. The diameters of the inhibition zones around the discs were then measured in millimeters using the caliper.

2.6. Determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC)

The antimicrobial susceptibility tests were carried out using the disk diffusion method according to the recommendations of the FMS-EUCAST [32]. In 96-well microplates, 100 µL of Muller Hilton broth was added to all the wells of the plate. Then, 100 µL of filtered mucus (40000 mg/mL), raw mucus (2000 mg/mL) and gentamicin (320 µg/mL) were collected and added to the 100 µL of the first wells of the column except those in rows D and H (blank). A series of 10 geometric dilutions of 2 was carried out from column 1 to column 11. Finally, 100 µL of a bacterial inoculum was introduced into the wells of the plate. The wells of column 12 containing the culture medium and the inoculum served as a negative control. The concentrations of mucus and gentamicin in the wells of a row varied respectively from 10,000 mg/mL to 9 mg/mL, from 500 mg/mL to 0.04 mg/mL and from 80 µg/mL to 0.078 µg/mL. The plates were then incubated at 37 °C for 24 hours. The lowest concentration at which no visible growth of the germ was observed (absence of turbidity) corresponds to the minimum inhibitory concentration (MIC) of the mucus.

The MBC was determined by subculture. For this, 100 µL of the contents of the wells at concentrations greater than or equal to the MIC were inoculated onto agar medium in Petri dishes and incubated for 24 hours at 37 °C. The MBC considered is the smallest concentration of mucus or gentamicin that left 0.01% of cells surviving from the initial inoculum [33]. Calculation of the MBC/MIC ratio made it possible to determine the bactericidal effect when it is less than 4, or the bacteriostatic effect when it is greater than or equal to 4 [33]. All tests were carried out in triplicate.

2.7. Data analysis

The relationships between the analyzed parameters were evaluated by Spearman's r correlation test. Comparisons of the means of the inhibition diameters were performed using the Kruskal Wallis H test and the Mann Whitney U test, using SPSS version 16.0 software.

3. Results

3.1. Diameters of the bacterial inhibition zones

3.1.1. With raw mucus

Overall, inhibition diameters varied between mucus doses and bacterial species. The most sensitive bacterial species in the presence of the different doses of mucus and gentamicin was the *S. aureus* strain with the largest inhibition diameters. The most resistant strain was *P. aeruginosa*.

The mean inhibition zone diameters ranged from 14.76 ± 2.35 mm to 25.83 ± 2.45 mm in the presence of gentamicin. The highest value (25.83 mm) was recorded with *S. aureus* and the lowest (14.76 mm) with *S. epidermidis*. In the presence of raw mucus, the mean inhibition zone diameters ranged from 11.70±2,07 to 17.50±3.87 mm. The largest value (17.50 mm) was observed with *S. aureus* and the smallest (11.70 mm) with *P. aeruginosa* (Figure 2).

3.1.2. With diluted mucus

With diluted mucus, the inhibition diameters varied depending on the degree of mucus dilution, as well as depending on the bacterial species present (Figure 2). Overall, the mean values of the inhibition diameters of bacterial cells varied between 9.00 ± 1.70 and 17.44 ± 4.41 mm in the presence of the different doses of mucus. The largest diameter (17.44 mm) was recorded with *S. aureus* at the 80% dose and the smallest (9.00 mm) with *E. coli* at the 20% dose (Figure 2).

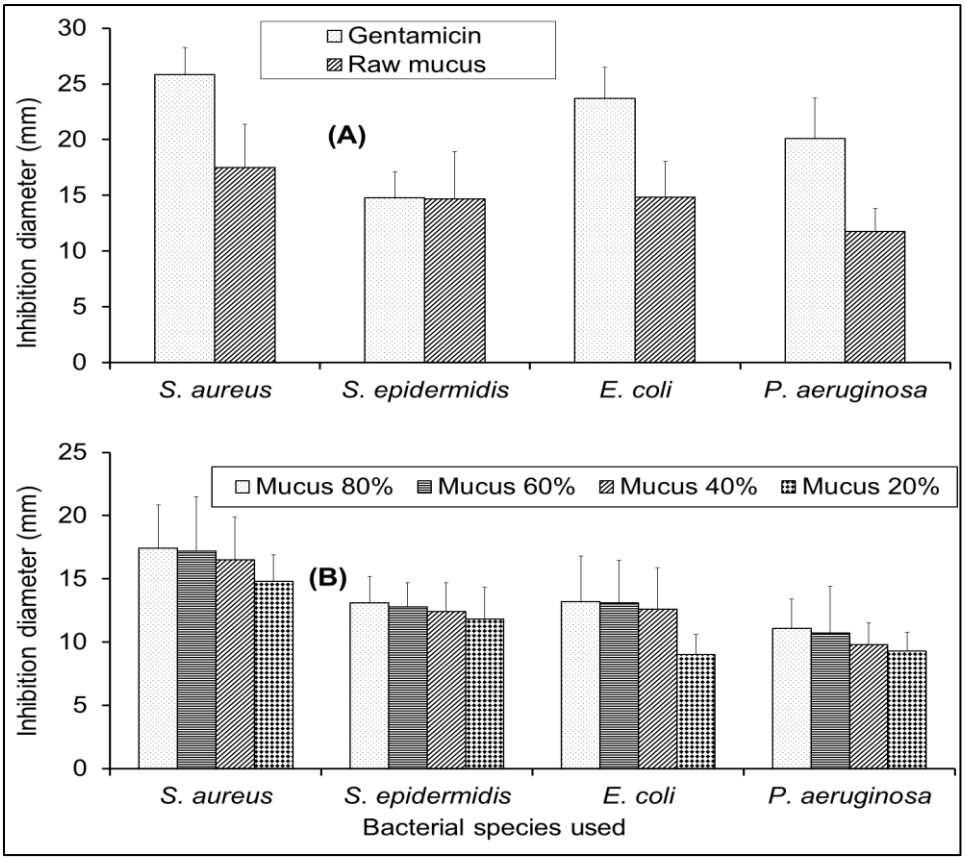

Figure 2 Variation of the cells inhibition diameters when using Gentamicin and the raw mucus (A), and when using diluted raw mucus (B)

3.1.3. With filtered mucus

The filtered mucus inhibited cell's growth of *S. aureus* and *S. epidermidis* strains starting at 20 µL of the mucus. Between 20 µL and 80 µL of filtrate, no growth inhibition was observed for *E. coli* (Figure 3). With 100 to 175 µL of filtered mucus, inhibition of *E. coli* growth was observed. However, no inhibition of *P. aeruginosa* growth was observed from 20 µL to 175 µL of filtrate (Figure 3).

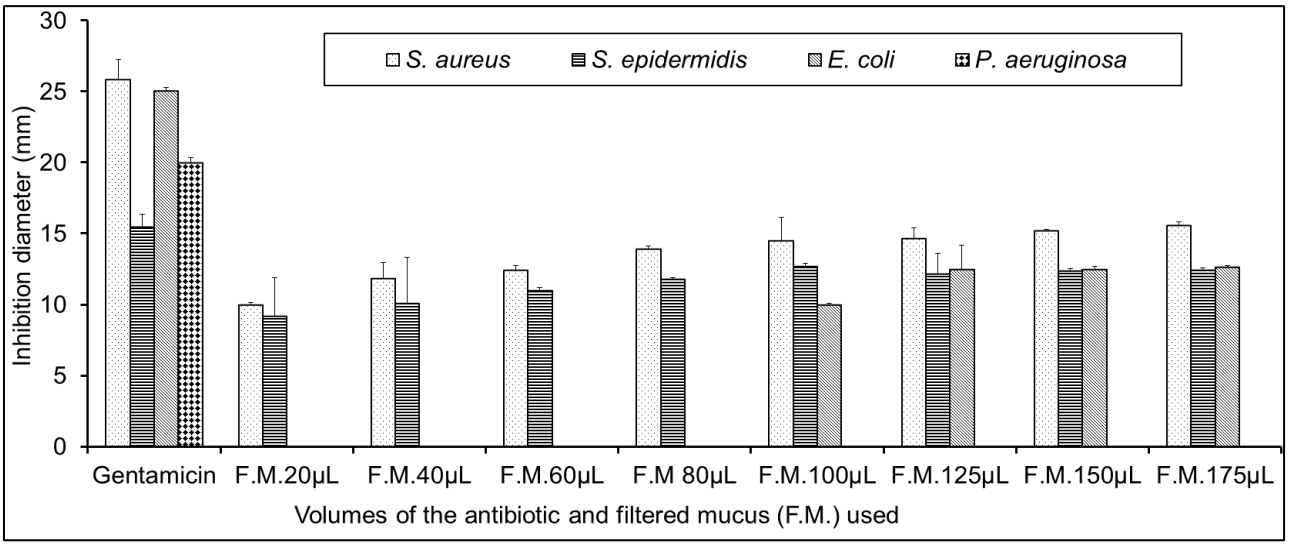

Figure 3 Variation of the cells inhibition diameters when using Gentamicin and various volume of filtered mucus

The mean values of the inhibition diameters fluctuated between 15.50 ± 0.86 mm and 25.83 ± 1.44 mm in the presence of gentamicin. At the presence of the filtered mucus, the inhibition diameters varied from 10.03±01 mm (with 20µL) to 15.6±0.2 (with 175µL) at the presence of *S. aureus*, from 9.2±2.7 mm (with 20µL) to 12.7±0.2mm (with 100µL) at the presence of *S. epidermidis*, and from 0mm (with 20-80µL) to 12.65±0.1 mm (with 175 µL at the presence of *E. coli* (Figure 3).

3.2. Comparison amongst the inhibition diameters

Data comparisons were made using the Kruskal Wallis H test. A comparison was first carried out amongst the inhibition diameters of the bacterial strains in the presence of the different doses of mucus. No significant difference was observed ($P \geq 0.05$). Another comparison was carried out amongst the inhibition diameters of the bacterial strains obtained in the presence of the different doses of mucus and those obtained in the presence of Gentamicin. Significant differences emerged ($P \leq 0.05$). A third comparison was carried out amongst the inhibition diameters of bacterial strains in the presence of different filtered mucus volumes. A significant difference was observed ($P \leq 0.05$). A comparison of the mean values of the inhibition diameters taken two by two using the Man Withney U test showed that this significant difference ($P \leq 0.05$) was between the inhibition diameters of *S. aureus* and those of *P. aeruginosa*, between the inhibition diameters of *S. epidermidis* and those of *P. aeruginosa*, and between the inhibition diameters of *E. coli* and those of *P. aeruginosa*.

3.3. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the mucus and Gentamicin

The MICs of *A. marginata* mucus ranged from 1250 to 2500 mg/mL with the filtered mucus. The lowest value was recorded in the presence of *S. aureus*. With the raw mucus, the MICs ranged from 62.5 mg/mL to 250 mg/mL, with the lowest value recorded in the presence of *S. aureus*. The MBCs values were 10000 mg/mL with the raw mucus, and 500 mg/mL with the filtered mucus (Figure 4).

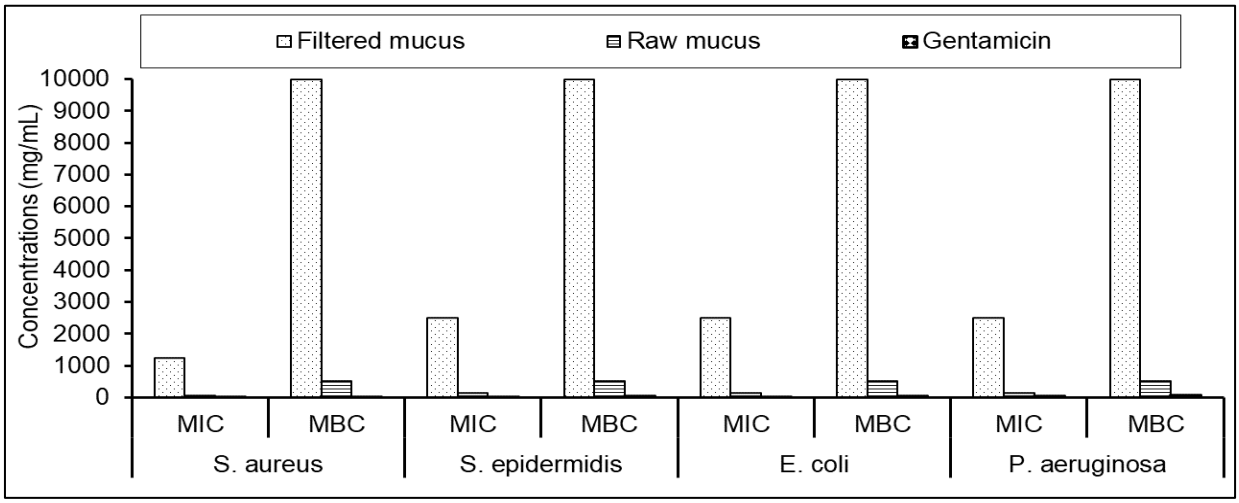

Figure 4 Variation of the MIC and MBC values with respect to the bacterial species and the mucus used

The CMB/MIC ratio showed that the raw mucus of *A. marginata* and its filtrate have a bacteriostatic action on the four bacterial species considered. Considering the values of the inhibition diameters and the values of the CMB/MIC ratio, it is noted that the different doses of the mucus and its filtrate have a lower activity compared to that of gentamicin (Table 1).

Table 1 Activity of the mucus tested on each of bacterial species considered

Antimicrobial used	**Bacterial species used and antibacterial activity noted**			
	S. aureus	*S. epidermidis*	*E. coli*	*P. aeruginosa*
Raw mucus	Bacteriostatic	Bacteriostatic	Bacteriostatic	Bacteriostatic
Filtered mucus	Bacteriostatic	Bacteriostatic	Bacteriostatic	Bacteriostatic
Gentamicin (80 mg/2mL)	Bactericidal	Bactericidal	Bactericidal	Bactericidal

4. Discussion

Some antibacterial activity of *A. marginata* mucus was observed against the 4 bacterial strains used, both Gram-negative and Gram-positive (Figures 2-4**)**. This indicates the presence of antibacterial substances. Although the biochemical analysis of this mucus is not yet completed, it is indicated that mucus of most *Archachatina* species contains two proteins, achacin and mytimacin, with broad-spectrum antibacterial activity against the many Gram-negative bacteria and Gram-positive bacteria [22]. The compound achacin has L-amino acid oxidase activity and its antibacterial activity has been indicated to be dependent on cytotoxic H_2O_2 production which is produced by the oxidative deamination [34, 35]. Some authors indicated that the high molecular weight lectin has been sometimes found in the mucus of some snail*s*. It is secreted from the same collar tissue where achacin is secreted and is believed to accelerate the antibacterial activity of achacin by increasing the local concentration of hydrogen oxides in the mucus [30, 34, 36].

The relative difference has been noted between the inhibition diameter of mucus amongst the bacteria. This was also observed between the activity amplitude of filtered and unfiltered mucus. It is possible that filtration retains certain macromolecules and decreases their concentration in the filtrate, or decreases the concentrations of some metallic elements bound to peptides in this filtrate, which would act as catalysts [23, 31, 37]. This would probably be the origin of the observed differences.

Some works have revealed that *Staphylococcus* sp can sometimes be more susceptible to the *A. marginata* mucus secretion compared to other bacterial species [30]. In addition, achacin is known to inhibit the formation of the peptidoglycan layer and cytoplasmic membrane of bacterial cells [38, 39]. Gram positive bacteria are known to possess thick-layer peptidoglycan which is highly prone to the activity of achacin, this may account for the high inhibitory effect of snail slime against Gram positive bacteria compared to Gram negative bacteria. Gram negative bacteria, on the other hand, possess lipopolysaccharide that protects them from harmful substances such as antibiotics [38, 39].

The MICs and MBCs values registered undergoes variations. Rashad et al. [40] also noted that these two microbial parameters of the snail mucus often vary from one bacterial species to another, and from a given strains to another for thesame bacterial species. This would be related to the general cells characteristics as well as the mucus chemicals. Some authors indicated that mucins antibacterial potential varies with respect of the chemicals composition as wel as with the bacterial strains, and can seriously decrease with time [31, 41]. The shorter the storage time, the higher the antimicrobial effect and no zone of inhibition can be observed after 24 hours of storage, mainly with aqueous extract [31]. Other authors noted that mucus of *A. marginata* has no effect against many bacteria including *E. coli*, *Salmonella typhi* and *Bacillus subtilis* after 2 hours storage, and against *S. aureus* after 24 hours storage. However, it remains active against *P. aeruginosa* after 24 hours storage [31]. The acidic extract of this mucus also appears to have a relatively high activity even after 48 hours of storage [31].

The composition of snail mucus is complex and highly variable. It is indicated that water extract of snail mucin contained 84 % protein, 2.91 % fats and 1.2 % carbohydrate, with high contains in iron, calcium, potassium, copper, zinc and phosphorus, with a pH ranged from 9.7 to 9.9, and hydrophile-lipophile balance varying from 10 to 11.7 [42]. Some of the most significant constituents include allantoin, ureidohydantoin, lectin, collagen, elastin, glycolic acid, complex mix of proteoglycans, glycosaminoglycans, glycoprotein enzymes, hyaluronic acid, copper peptides, antimicrobial peptides, and metal ions, each with unique roles contributing to its therapeutic properties [23, 37, 43]. Trace amounts of sialic acid are also known to be present, mediating cellular interactions and masking antigens from human macrophages [23, 44]. It is suggested that each mucus may also be able to vary its formulation [31, 43].

The mucus of *Archachatina* also contains many functional proteins involved in various metabolic pathways, such as those of glycans and carbohydrates. Some of them have molecular weights varying from 83.67 to 9.7 kDa [45]. These proteins also act as bicarbonate transporter, pores ion channels, Na^+/sulfate/carboxylate cotransporter, facilitative glucose transporter, Na^+/Ca^{2+} exchanger, multifunctional anion exchanger, hydrolyases, Ca^{2+} transporter, oxidoreductases and Na^+-dependent ascorbic acid transporter [46].

P. aeruginosa was observed to be distinct from other bacteria tested at the presence of mucus (Figures 2-3). It is citrate, catalase and oxidase positive bactériaspecies. *Spagnolo et al.* [47] *noted that P. aeruginosa* is able to selectively inhibit various antibiotics from penetrating its outer membrane and has high resistance to several antimicrobial agents. According to *Mah et al.* [48], one locus identified as being an important genetic determinant of the resistance in this bacteria is *ndvB*, which encodes periplasmic glucans that may interact with antimicrobial agents and cause them to become sequestered into the periplasm. It has been suggested that an intrinsic resistance to *P. aeruginosa* can reduce or inactivate the effectiveness of antibiotics through their own products or functional properties, *via* the possession of efflux pumps that exclude antibiotics from the bacterial cells, and low outer membrane permeability [49, 50]. Acquired

resistance is another approach by which *P. aeruginosa* acquires antimicrobial resistance, including horizontal gene transfer which plays a pivotal role in the acquisition of antibiotic resistance genes, and accumulation of genetic mutations which consist in a complex phenomenon involving various mechanisms that enable the bacterium to withstand the effects of antimicrobial drugs. These mutations affect critical aspects of bacterial biology, resulting in several resistance mechanisms, for example enhancing β-lactamase production [49, 50].

The exact mechanisms by which snail mucus exert their antibacterial effects can vary. It is indicated that they exhibits antibacterial activity primarily due to the presence of antimicrobial proteins and other bioactive compounds like mucin and glycoproteins. These peptides can directly interact with bacterial cell membranes, creating pores or disrupting their structure, leading to cell lysis and death. They can also interfere with bacterial cell wall synthesis, preventing the bacteria from building a protective barrier [23]. Some may disrupt the bacterial cell membrane, causing leakage of cellular contents. Others might interfere with essential bacterial processes like DNA replication or protein synthesis, or can inhibit bacterial growth and interfere with their communication systems [23, 40]. In addition to direct antibacterial effects, snail mucus can also create a protective barrier on the human skin, preventing bacterial translocation and inappropriate immune stimulation [23, 40]. The promoting angiogenesis and anticancer properties of the snail mucus have also been reported [51].

5. Conclusion

This study aims to evaluate the antibacterial effect of the crude and filtered mucus of the snail *A. marginata* against 2 Gram-positive (*S. aureus* and *S. epidermidis)* and 2 Gram-negative (*E. coli*, *P. aeruginosa)* bacteria. It has been noted that with the raw mucus, inhibition diameters varied between mucus doses and bacterial species. With diluted mucus, the inhibition diameters varied depending on the degree of mucus dilution, as well as depending on the bacterial species present. The filtered mucus, lowest volumes dit not affected *E. coli* growth, with *S. aureus* and *S. epidermidis* excepted. However, no inhibition of *P. aeruginosa* growth was observed for the mucus volumes considered. The CMB/MIC ratio showed that the raw mucus of *A. marginata* and its filtrate have a bacteriostatic action on the four bacterial species considered. It seems necessary to continue exploring the secretions of other molluscs against infectious bacteria, to search for therapeutic alternatives to classical antibiotics.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Author's contributions

All authors participated in the redaction of this document.

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