

Review

Giovanni Cilia¹ / Filippo Fratini^{1,2}

Antimicrobial properties of terrestrial snail and slug mucus

¹ Department of Veterinary Sciences, University of Pisa, Viale delle Piagge 2, Pisa, Italy, E-mail: filippo.fratini@unipi.it

² Interdepartmental Research Center "Nutraceuticals and Food for Health", University of Pisa, Via del Borghetto 80, Pisa, Italy, E-mail: filippo.fratini@unipi.it

Abstract:

Snail and slug mucus is a viscous-elastic substance secreted by specific glands with adhesive and lubricants properties that allows them to adhere tenaciously to many different surfaces. It has been used since ancient times for care and human health and it is still very important in traditional and folkloristic medicine. Recently, mucus from snail and slugs and its protein and components have been subjected to some investigations on their antibacterial, antiviral and antifungal activity due to extensive traditional uses and for a future application in medicine. Antimicrobial activities of crude mucus, and its components, against different microorganism have been reported, showing antimicrobial activities that lead their potential employment in several fields as natural additives. The purpose of this Review is to summarize the results of antimicrobial studies of snail and slug mucus and its compounds from the first scientific applications to the isolation of the single components in order to better understand its application and propose an employment in future studies as a natural antimicrobial agent.

Keywords: antibacterial activity, antifungal activity, antiviral activity, mucus, slug, terrestrial snails

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Introduction

Slugs and snails are terrestrial molluscs, belonging to the order *Pulmonata*, class *Gastropoda*, phylum *Mollusca*, characterized by a similar morphology.

The main difference between snails and slugs is the fact that the snails are provided of shells. Snails usually have a spiral-shaped shell which is wound around a spindle. This is the snail shell which they retract their soft bodies into when they detect danger [1]. The spiral direction is species-specific.

Slugs are snails-like animals without shell. Not having a shell to protect them, slugs have a very thick slime which makes them disgusting to predators [2].

Both slugs and snails are able to produce a viscous-elastic substance named slime or mucus, with adhesive and lubricant properties that allows them to adhere tenaciously to many different surfaces. The mucus has also other functions: hindering the molluscs dehydration and making slugs and snails unattractive to potential predators [1, 2].

Moreover, snail mucus has the ability to facilitate wound healing and to prevent its infections thanks to its many bioactive compounds [3, 4].

Over the few last years, numerous studies on mucus composition have clarified many aspects of its properties, although much remains to be investigated on its antibacterial activity.

Recently, several researches carried out on snail secretion composition have confirmed that the *Helix aspersa* mucus contains a great amount of natural substances with beneficial and therapeutic properties for human skin such as allantoin and glycolic acid [5].

This review aims to represent an organic collection of the results obtained from the few studies until today carried out to test the snail mucus antibacterial activity and its main components against various microorganisms.

Filippo Fratini is the corresponding author.

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Historic background

Although the use of many gastropods including snails for food has been demonstrated by numerous archeological discoveries, it is much more difficult to prove their use in therapy and medicine [6]. The brown garden snail, *Helix aspersa*, has been used in human medicine since ancient times; the earliest application of snail mucus secretions in medicine dates back to the Ancient Greeks. Hippocrates was reported to have used crushed snails to alleviate inflammatory skin conditions. Moreover, the father of Western medicine recommended snail mucus for the treatment of protozoa. Celse claimed that the crude snail with its shell had remarkable healing properties, while after boiling it also acquired emollient capacity. Pliny stated that snail preparations could be employed in every type of wound, such as burns, abscesses and nosebleeds. Galien recommended snails mucus against *hydrops foetalis* [7].

Dermatological preparation with snail mucus was employed during the eighteenth century to treat dermatological disorders and symptoms associated with tuberculosis and nephritis. In the nineteenth century there was renewed interest in the pharmaceutical and medical use of snails with more and more preparations. This interest in snails continued into the next century with the acquisition of new analytical data on mucus components. Recently, anecdotal reports of generic skin regeneration properties of the mucus from *Helix aspersa* have been explored; this has resulted in the commercial production of a topical preparation claimed to have “wound healing” as well as anti-ageing properties. These preparations were tested on burn patients and while they noted that a range of pathogenic bacteria were isolated from the wounds before treatment, this was not followed up with culture of post-treatment specimens [8].

Composition of snail mucus

Snail mucus composition varies according to species and according to its role, trail or adhesion function and for these functions, typically, consists of between 90% and 99.7 % water by weight [9]. The remaining part of mucus without water consists of a mixture of proteoglycans, glycosaminoglycans, glycoprotein enzymes, hyaluronic acid, copper peptides, antimicrobial peptides, and metal ions [10–13]. Atomic absorption spectrometry showed that glue from the slug *Arion subfuscus* contains substantial quantities of zinc, iron, copper and manganese. Experimentally it was shown that the addition of iron or copper to dissolved slug glue causes the proteins to precipitate rapidly but the addition of zinc had no effect, suggesting that some metal ions play an important role in gel formation [14].

The snail mucus principally contains allantoin, collagen, elastin and glycolic acid.

Allantoin, or 5-Ureidohydantoin, derives from the uric acid transformation by the enzyme uricase. It is known for its desquamating action, its promotion of cell proliferation and wound healing [15–17].

Glycolic acid, or alpha-Hydroxyacetic acid, has an excellent capability to penetrate skin and is capable to increase collagen synthesis [18–20].

Another study evidenced that snail mucus can be used, potentially, in regenerating and repairing bone and teeth, because it increased the expression of osteopontin and NF- κ B and induced the expression of some inflammatory genes in dental pulp cells [21].

Furthermore, glycoproteins and mucopolysaccharides, physiologically active bio-macromolecular structures are present in snail mucus [9, 22]. Mucin glycoproteins are the major macromolecular constituents of epithelial mucus and have long been implicated in health and disease [12, 23, 24]. The glycoproteins, such as achacin, are, probably, the components involved in antimicrobial activity of snail mucus. Indeed, achacin, other than inhibit bacteria growth, also appeared to attack the bacterial plasma membranes [25]. However, the achacin has the ability to catalyze oxidative deamination producing ketoacids, hydrogen peroxide and ammonia [26]. The antibacterial activity of achacin was found to be dependent on H₂O₂ production which is produced by the oxidative deamination reaction. These data illustrate that achacin may attack pathogens during other growth phases too by increasing the local concentration of H₂O₂ so as not to harm neighboring host cells [23, 25, 26].

Scientific evidence provides some credible basis for the possible use of mucus in wound management [27]. The mucus from *Cryptomphalus aspersa* (also known as *Helix aspersa* or the common garden snail) contains antioxidant superoxide dismutase (SOD) and Glutathione-S-Transferase activity (GST) activities. Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals or reactive oxygen species. SODs act as antioxidants and protect cellular components from being oxidized by reactive oxygen species [27]. Furthermore, the *Cryptomphalus aspersa* mucus stimulated fibroblast proliferation, extracellular matrix assembly and the regulation of metalloproteinase activities and concluded that these effects together provided an array of molecular mechanisms underlying the secretion's induced cellular regeneration, thereby supporting its possible use in repair of wounded tissues [27]. In a subsequent study it was also demonstrated that the mucus increased migration and increased the expression of cell-cell and cell-substrate adhesion molecules in mammalian fibroblast and keratinocyte cells [28].

It should be noted that some of these properties are analogous to claims made for some modern wound management materials.

Antimicrobial activity

The antibacterial activity of snail mucus was evaluated for the first time with a sample from *Achatina fulica* Férussac, the African giant snail [29]. In this study two different fractions of snail mucus were examined: the water-soluble fraction and the mucin fraction, contained proteins. Both fractions showed a positive antibacterial activity against Gram positive bacteria, such as *Bacillus subtilis* and *Staphylococcus aureus*, and Gram negative strains, like *Escherichia coli* and *Pseudomonas aeruginosa*. Furthermore, the mucin fraction resulted more effective against bacteria compared to water-soluble fraction [29].

Another antibacterial factor was isolated from the *Achatina fulica* mucus [30]. This unnamed antibacterial effect showed great inhibition activity against *Staphylococcus aureus*, followed, with high concentration, by *Bacillus subtilis* and *Pseudomonas aeruginosa* and, finally, by *Escherichia coli* [30].

An N-Acetylneuraminic Acid-Specific Lectin, called Achatinin, isolated from the African giant snail body-surface mucus showed an antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* only a concentration of 50 µg/ml [31].

As reported in Table 1, the mucus extracted from various species of land snail exhibited an inhibition action against Gram positive and Gram negative bacteria strains.

Hemocyanin β c-HaH	VRKNVDKLTKEDELYDLQRALRDVVA	<i>Helix aspersa</i> (Müller, 1774)	Garden snail	Worldwide	<i>E. coli</i> <i>P. aerugi- nosa</i> <i>St aureus</i> <i>Ent.</i> <i>faecium</i> <i>St epider- midis</i>	ATCC 20032	MM	7.5 $\mu\text{g/ml}$ 30 % [§] 175 % [§] 30 % [§] 275 % [§] 30 % [§]	[40]
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Ac.: *Acinetobacter*, B.: *Bacillus*, C.: *Candida*, E.: *Escherichia*, Ent.: *Enterococcus*, K.: *Klebsiella*, Lis.: *Listeria*; Prot.: *Proteus*, Ps.: *Pseudomonas*, Pn.: *Pantoea*, Serr.: *Serratia*, St.: *Staphylococcus*, Strep.: *Streptococcus*, Sal.: *Salmonella*, V. *Vibrio*; DDA: Disk Diffusion Assay, MM: Microdilution Method; ^awith 10 μg of snail slime; ^bDilution 1/1 of snail slime; ^cMIC 50; ^dMIC 90; ^eMethicillin-Resistant *St aureus*; ^fwith 100 μg of Achacin CRP; [§]Bacteria growth with a concentration of 6.5 μM of Hemocyanin β c-HaH.

The *Achatina fulica* mucus showed a great antibacterial activity against *Staphylococcus aureus* and *Streptococcus epidermidis* with high inhibition diameter, expressed in mm [33].

Archachatina marginata mucus was investigated in relationship with the different variety of this snail species, as reported in Table 1. The *Archachatina marginata* var. *saturalis* showed different antibacterial activity compared to *Archachatina marginata* var. *ovum* [34]. *Staphylococcus* spp. was inhibited mostly by var. *saturalis*, while *Pseudomonas* spp. and *Streptococcus* spp. resulted more susceptible to var. *ovum* [34], data showed in Table 1. Another interesting comparison was performed with *Archachatina marginata* normal skinned and albino skinned mucus samples [35]. The mucus collected from the normal skinned snail showed a more effective antibacterial activity against *Staphylococcus* spp. and *Pseudomonas* spp. than the albino skinned snail. Different trends were reported for *Escherichia coli* and *Salmonella* spp.; *Escherichia coli* resulted more susceptible with the albino skinned snail mucus, while *Salmonella* spp. with the normal skinned snail mucus [35], data reported in Table 1.

The *Helix aspersa* mucus showed efficient antibacterial activity against two different *Pseudomonas aeruginosa* strains [36]. The mucus from the same snail also resulted incisive against *Streptococcus pyogenes*, while no inhibition diameters were evaluated for other tested strains [36]. The *Helix aspersa* mucus purification was improved by Bortolotti et al. (2016). The mucus extracted showed the same antibacterial activity confirming the data previously evidenced by Pitt et al. (2015).

Achacin [23, 26, 37], Achatin CRP (C-reactive protein) [38] and Mytimacin-AF [39] and Hemocyanin β c-HaH [40] are antimicrobial peptide isolated from *Achatina fulica* and *Helix aspersa* mucus, respectively.

Achacin from African giant snail mucus inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* spp [26], while the MIC₅₀ value resulted effective against *Streptococcus agalactiae* and Methicillin-Resistant *Staphylococcus aureus* (MRSA) [37], as reported in Table 1.

The C-reactive protein Achatin from *Achatina fulica* mucus showed effective antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus epidermidis* and *Pantoea ananatis* with MIC values, with 100 μ g of protein, ranging from 11 to 12 mm, while the most resistant bacteria strain resulted in *Bacillus subtilis* (18 mm) followed by *Salmonella thyphimurium* (15 mm) [38].

Two unknown and undefined compounds isolated on TLC (Thin Layer Chromatography) from *Achatina fulica* mucus showed an inhibition to *Escherichia coli* and *Vibrio cholerae* growth [41]. Unfortunately, these antibacterial compounds have not been identified, but it is interesting to continue this research line.

Staphylococcus aureus resulted in the most sensitive bacteria strain subject to antibacterial activity of Mytimacin-AF, a protein from African giant snail mucus, followed by *Bacillus pyocyaneus* and *Bacillus dysenteriae* [39]. *Bacillus megatherium* and *Klebsiella pneumoniae* showed high resistance to peptide Mytimacin-AF [39], data reported in Table 1.

Hemocyanin β c-HaH from *Helix aspersa* mucus inhibited the growth of *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, while *Pseudomonas aeruginosa* and *Enterococcus faecium* have grown unconditionally with the presence of the antimicrobial peptide [40].

Two glycosylated peptides with mass of 4021.04 and 6403.73 Da, isolated from Fraction B of *Helix aspersa* and *Helix lucorum* mucus showed inhibition of *Propionibacterium acnes*, *Escherichia coli* and *Helicobacter pylori* growth [42].

Unfortunately, lectin isolated from the *Achatina fulica* mucus did not show an inhibition activity against *Staphylococcus aureus* and *Escherichia coli* even though an hemagglutinating activity was evidenced from the same investigation [43].

Mytimacin-AF showed also an antifungal activity against *Candida albicans* with a MIC value of 7.5 μ g/ml [39], while the *Helix aspersa* mucus did not show inhibition action against the same yeast [36].

The fraction 39 and fraction 50 from *Phyllocaulis boraceiensis*, tropical leatherleaf slug, mucus was analyzed using Fourier Transform Infrared Spectrometry (FT-IR) and tested against Measles virus (MV), a single-stranded, negative-sense, enveloped RNA virus of the genus *Morbillivirus* [44]. The antiviral action could be correlated to polyunsaturated fatty acids in fraction 39, in detail the most active fatty acids against MV were hydroxy-tritriacontapentaenoic acid and hydroxy-pentatriacontapentaenoic acid. Furthermore, the fraction 50 showed a lower antiviral activity against tested virus [44].

Conclusions

From available literature snail and slug mucus and its derivate components, such as achacin, achatina CRP and mytimacin-AF, showed a high activity against Gram positive and Gram negative bacteria, virus and yeast. Moreover, several studies could be carried out on its antimicrobial activity against other microorganisms, especially against multidrug resistant bacteria, such as MRSA (methicillin-resistant *Staphylococcus aureus*). This is particularly important since one of the major public health problems is currently represented right from the

onset by an increasing number of antibiotic resistant bacteria. The indiscriminate use of antibiotics has led to the selection of resistant clones many for which an adequate therapy is often not provided. Multidrug resistant bacteria management requires increasing attention towards the antibacterial molecules or products used. For this reason, researches in recent years has been directed towards the discovery of new antimicrobial substances, particularly natural substances such as plant extracts, essential oils and antimicrobial peptides isolated from many different animals. Based on the results obtained by several studies on the antimicrobial properties of snail and slug mucus, it seems clear that this natural product could be a potential subject of further investigations. The new findings regarding its active components, their inner mechanisms of action and the possibility of isolation and purification of the pure substances, represent a starting point for the formulation of new products for therapeutic and pharmacological uses as an alternative to conventional antibiotics. Natural peptides, as like those extracted from the snail and slug mucus, could be considered as potential alternatives in therapy.

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