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# Antibacterial Activity of Terrestrial Snail Mucus: A Review


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***Abstract***

*Antibiotic resistance is one of the major health problems faced by various countries in the world. Due to the urgency of the antibiotic resistance problem, efforts to identify new lead compounds from new sources and analysis to determine their bioactivity are urgently needed for antibiotic development. One of the invertebrate organisms studied in relation to its potential to produce secondary metabolites that have antibacterial properties is the Gastropoda class which is included in the Mollusca phylum. Gastropoda consists of more than 80 thousand species and differs from other classes in the Mollusca phylum because of its torso body. Gastropoda has 3 sub-classes, namely: Prosobranchia, Opistobranchia, and Pulmonata. One of the land Gastropod species is snails, both those with shells (snails) and naked snails (slugs) which are included in the Pulmonata sub-class. Snail mucus is used as a traditional medicine and also as an innovative natural product to treat various health problems. Since Gastropods include a large number of species that live in various ecosystems, but research on the antibacterial potential of their secondary metabolites is still limited, this review aims to summarize research that explores the antibacterial effects of secondary metabolites in mucus produced by species in the class of Gastropods that live on land (terrestrial gastropoda) especially various species of terrestrial snails. The preparation of this review article uses a literature study method with a systematic approach. The articles used are international articles that discuss the antibacterial activity of secondary metabolites in mucus produced by snails. Articles were obtained from the PubMed database using the keywords snails AND antibacterial from the period 2000 to 2023, the period before 2000 was excluded due to the very minimal number of articles. The results obtained from the journal review that has been carried out are the presence of bioactive components such as peptides, glycopeptides, and other secondary metabolites found in the mucus of several species of land snails. These bioactive components have antibacterial activity against gram-positive and gram-negative bacteria.*



***Key Words****: antibacterial activity, land snails, mucus*

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## Introduction

Antibiotic resistance is one of the major health problems faced by various countries in the world. Antibiotic resistance is caused by changes in bacteria that make antibiotics less effective in treating infections. Predictive statistical model findings estimate that there were 4.95 million deaths due to antimicrobial resistance in 2019 worldwide, including 1.27 million deaths due to antibiotic resistance. The bacteria that are the main focus because they are associated with resistance that causes death are Eschericia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa (Murray et al., 2022). If not handled seriously, antimicrobial resistance is estimated to result in 700 thousand deaths per year and this number will increase to 10 million deaths by 2050 (O'Neill, 2016).

Due to the urgency of the antibiotic resistance problem, efforts to identify new lead compounds from new sources and analysis to determine their bioactivity are urgently needed for antibiotic development. Given that the field of antibiotic discovery and development is experiencing an innovation crisis where no new class of antibiotics has been discovered and approved by the United States Food and Drug Administration (FDA) since the late 1980s. One approach used to address this problem is to utilize natural products (NP). Of the 162 antibiotics approved by the FDA from 1981 to 2019, around 50% were obtained from natural materials sourced from microbes. In addition to microbial sources, natural materials from plants are also a promising source of antibiotics to explore. Natural materials from plants have advantages in the form of unique chemodiversity, wide distribution, ease of access, and a variety of antibacterial mechanisms (Porras et al., 2021).

Bacteria are widely distributed in the environment and bacteria can infect (e.g. Pseudomonas sp. infections) vertebrates, invertebrates and plants. To defend itself from bacterial infection, the organism has a strategy that can potentially be used as a source of antibiotics for human treatment (Pitt et al., 2019). One of the invertebrate organisms studied in relation to its potential to produce secondary metabolites that have antibacterial properties is the Gastropoda class which is included in the Mollusca phylum. The Mollusca phylum does not have adaptive immunity but they live in environments that have a diversity of microorganisms and are exposed to various types of pathogens. For its defense, Mollusca relies on innate immunity and the presence of secondary metabolites (Belouhova et al., 2022). The Mollusca phylum, when compared to other invertebrates, has a source of pharmacologically active compounds in hemolymph and mucus (Suárez et al., 2021).

Gastropoda consists of more than 80 thousand species and differs from other classes in the Mollusca phylum because of its torso body. Gastropoda has 3 subclasses, namely: Prosobranchia, Opistobranchia, and Pulmonata. They can live on land, freshwater, and sea (O'Brien & Pellett, 2022). One of the terrestrial Gastropod species is snails, both those with shells (snails) and naked snails (slugs) which are included in the Pulmonata subclass. Snail mucus is used as a traditional medicine and also an innovative natural product to treat various health problems (Belouhova et al., 2022). Snail mucus has an effect on wound healing and can prevent infection because it contains various bioactive components (Cilia & Fratini, 2018). Several previous studies have identified the antibacterial effects of secondary metabolites produced by several Gastropod species. Because Gastropods include a large number of species that live in various ecosystems, but research on the antibacterial potential of their secondary metabolites is still limited, this review aims to summarize research that explores the antibacterial effects of secondary metabolites in mucus produced by species in the gastropod class that live on land (terrestrial gastropods), especially various species of terrestrial snails.

## Research Methods

In compiling this review article, a literature study method with a systematic approach was used. Data in the form of research journals were collected from databases, analyzed, and summarized. The articles used were international articles that discussed the antibacterial activity of secondary metabolites in mucus produced by shelled land snails (snails). Articles were obtained from the PubMed database using the keywords "snails" AND "antibacterial" from the period 2000 to 2023, the period before 2000 was excluded due to the very minimal number of articles. The results obtained from the online search were 320 articles. Furthermore, these articles were entered into the citation manager, Zotero, then article selection was carried out, related to duplication and content. Articles were selected that were in accordance with the topic of discussion by reading the title and abstract so that 17 articles were obtained. The articles selected in PubMed were articles that discussed the antibacterial activity of secondary metabolites of terrestrial snail mucus. Articles that were in accordance with the discussion but did not contain complete content and were not open access were excluded from this article review. In addition, exclusion was also carried out on articles that predominantly involved in silico methodsand used synthetic peptide derivatives (snail-derived antimicrobial peptides) in testing their antibacterial activity.

## Results and Discussion

The literature obtained from the PubMed database from 2000 to 2023 was 320.

Screening of the title and abstract was carried out to select literature that was in accordance with the topic of discussion. Overall, this article review used 17 articles. The journals selected were all journals that were in accordance with the discussion in PubMed from 2000 to 2023.

Table 1. Literature Search Results

| **Component** | **Latin Name** | **Bacteria** | **Contents** | **Source** |
|---|---|---|---|---|
| *Mucus* | *Cornu aspersum* | - | Peptida Antimikrobial:<br>LGHDVH<br>AAGLAGAGNGGG<br>LLFSGGQFNG<br>LNLGLDAGGGDPGG<br>GAACNLEDGSCLGV<br>NLVGGLSGGGRGGAPGG<br>LGGLGGGGAGGGGLVGEPG<br>NLVGGLSGGGRGGAPGGGG<br>GLLGGGGGAGGGGLVGGLLNG<br>MGGLLGGVNGGGKGGGGPGAP<br>LFGGHQGGGLVGGLWRK<br>NGLFGGLGGGGHGGGGKGPGEGGG<br>LLLLMLGGGLVGGLLGGGGKGGG<br>PFLLGVGGLLGGSVGGGGGGGAPL<br>LPFLGLVGGLLGGSVGGGGGGGPAL<br>DVESLPVGGLGGGGGGAGGGGLVGGNLGGGAG | (Belouhova *et al.*, 2022) |
| *Mucus* | *Cornu aspersum* | *C. pefringens* NBIMCC 8615, *B. laterosporus* BT-271, *E. coli* NBIMCC 8785, *P. aureofaciens* AP9, *B. laterosporus strain* BT-271 | Peptida Antimikrobial:<br>DLTLNGLSPK<br>MPDGALLGGGD<br>DGPADNAQGAVG<br>SLEERDIQ<br>GGLLAAGAGGGAAV<br>LGLGNGGAGGGLVGG<br>LNLGLDAGGGDPGG<br>FNHKSLPKLEN<br>NLVGGLSGGGRGGAPGG<br>LGGLGGGGAGGGGLVGEPG<br>NLVGGSGGGGRGGANPLG | (Dolashki |

Bioactive compounds such as proteins, peptides, and glycopeptides are found in the mucus of various Molluscs (Belouhova et al., 2022). Antimicrobial peptides (AMPs) or also known as host defense peptides, produced by various organisms, are bioactive compounds that provide protection from microbial infections. AMPs have various functions that are categorized as antiviral, antifungal, antiparasitic, antibiofilm, and antibacterial. Antibacterial peptides target bacterial membranes (through pore formation mechanisms or without pore formation mechanisms), DNA, RNA, and proteins. Peptides with short sequences can inhibit various microbes such as bacteria and fungi (Dolashki et al., 2020a).

Eremina deserterum is a snail species found in different locations in the Mediterranean region. In the study of El-Zawawy & Mona (2021), mucus extract from Eremina deserterum contained 3H-1,2,4-triazole-3-thione, 4,5-dihydro-4,5-diphenyl, phthalic acid, 7-bromoheptyl ethyl ester and methyl 1,2-benzisothiazole-3-acetate which showed antibacterial activity against multi-drug resistance (MDR) strains of P. aeruginosa, MDR E. coli, pandrug resistance (PDR) S. aureus, MDR A. niger, MDR R. solonifer, MDR Trichoderma harzianum, and PDR C. albicans. The substances contained in the mucus of H. aspersum and C. aspersum have antibacterial activity against P. aeruginosa. H. aspersum mucus contains AAMPs (Achacin-like proteins). The results of size exclusion chromatography (SEC) indicate that proteins with a molecular weight of 30-100kDa contribute to its antimicrobial activity. The results of electrophoresis show that proteins with a molecular weight of 30-40kDa and 50-60kDa are present in low concentrations. The strength of antibacterial activity in H. aspersum mucus is likely due to the interaction between the two proteins. In addition, other studies have found that C. aspersum mucus may have antibacterial activity because it contains a protein with a size of 17.5 kDa which is a sialic acid binding lectin. Some lectins have pathogen recognition receptors (PRR) that contribute to their antimicrobial activity. From the results of size exclusion chromatography, proteins with a molecular weight of 30-40kDa (exactly 37.5kDa) have strong antibacterial activity against P. aeruginosa. The protein was identified as aspernin which can be further explored for its antibacterial activity (Pitt et al., 2015, 2019). In the study of Vassilev et al. (2020) several metabolites in H. aspersa

Various compounds with antimicrobial effects, antioxidants, regenerative activities are found in the mucus of terrestrial snails (Belouhova et al., 2022; Dolashki et al., 2020a). In the mucus of Cornu aspersum, nine peptides with molecular weights of 1000 to 3000 Da were found. Metabolite analysis of snail mucus using nuclear magnetic resonance (NMR) techniques showed that metabolites that have antioxidant, antibacterial, and antimicrobial activities are metabolite fractions that have low molecular weight (MW) (MW <1kDa and MW <3kDa). The results of amino acid sequence analysis showed that peptides in C. aspersum mucus predominantly contain the amino acids glycine (G), leucine (L), proline (P), valine (V), phenylalanine (F), alanine (A), aspartic acid (D), asparagine (N), and tryptophan (W) which are identical to peptides with antimicrobial effects. These amino acids are important components of the innate immune system. C. aspersum mucus contains cationic and anionic peptides, but cationic peptides are dominant, which are characterized by an amphipathic structure and mostly hydrophobic surfaces. Cationic AMPs kill microbes by interacting with positively charged peptide residues with negatively charged components on the target cell membrane. This interaction will result in membrane permeabilization, depolarization, leakage, or lysis resulting in cell death. There are various models of mechanisms that explain the work of peptides, namely the toroidal pore model, the barrel stave model, the carpet model, etc. Some positively charged AMPs enter cells and bind to intracellular molecules that play an important role in cell survival. These peptides can interact with bacterial ribosomal proteins and induce cell death through interactions with intracellular DNA (Belouhova et al., 2022; Dolashki et al., 2020a).

eptides with amino acid sequences LGHDVH, LLFSGGQFNG LGLGNGGAGGGLVGG,

GLLGGG GGAGGGGLVGGLLNG, MGGLLGGVNGGGKGGGGPGAP, LFGGHQ GGGLVGGLWRK, NGLFGGLGGGGHGGGGKGPGEGGG, LLLLML GGGLVGGLLGGGGKGGG (Table 1) are predicted to have antibacterial effects. In addition, peptides with amino acid sequences LGHDVH and NGLFGGLGGGGHGGGGKGPGEGGG have antiviral activity. Peptides with amino acid sequences GLLGGGGGAGGGGLVGGLLNG, LFGGHQGGGLVGGLWRK, NGLFGG LGGGGHGGGGKGPGEGGG, LLLLMLGGGLVGGLLGGGGKGGG are predicted to have antibacterial and antifungal activities. Peptides with the highest potential antibacterial and antifungal activity have high glycine and leucine residues and one or two proline residues (Belouhova et al., 2022; Dolashki et al., 2020a).

Several peptides identified in Cornu aspersum in the study by Dolashki et al. (2020b) belong to the group of anionic antimicrobial peptides (AAMPs) which have been increasingly identified in invertebrates, vertebrates, and plants over the past few decades. In general, AAMPs show antibacterial activity, but some are multifunctional, also showing antifungal, anticancer, neuropeptide activity, and have potential as conventional antibiotics. Membrane interactions are key to the antimicrobial activity of AAMPs. Some of the mechanisms of action of these peptides follow the toroidal pore formation and carpet models.

Achacin is a glycoprotein found in the mucus of Achatina fulica Férussac and Lissachatina fulica. The results of the sequence analysis show that achacin belongs to the amine oxidase family (flavin enzymes). This enzyme family consists of various amine oxidases including L-amino acid oxidase (LAO). LAO catalyzes the oxidative deamination reaction of L-amino acids which will then produce α-ketoacids, hydrogen peroxide (H2O2), and ammonia (NH3). Some LAOs have antibacterial effects. Achacin recognizes and binds to bacteria that are in the growth phase and the presence of H2O2 will have a lethal effect on bacteria (Ehara et al., 2002; Noothuan et al., 2021). Another study identified the presence of AMP mytimacin-AF in A. fulica mucus. Mytimacin-AF is a cationic peptide rich in cysteine and belongs to the mytimacin family. Mytimacin-AF consists of 80 amino acids including 10 cysteines. Mytimacin showed strong antimicrobial activity against Staphylococcus aureus ATCC 25923, Bacillus pyocyaneus CMCCB 10104, Bacillus dysenteriae, with minimum inhibitory concentration (MIC) values of 1.9 μg/ml, 3.75 μg/ml, 3.75 μg/ml, respectively (Zhong et al., 2013).

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mucus identified through nuclear magnetic resonance (NMR) namely 3-hydroxybutyrate, 4-methyl-2-oxovaleric acid, acetic acid, alanine, allantoin, betaine, choline, cytosine, ethanol, formic acid, glucose, glycine, glycerol, isobutyric acid, isovaleric acid, lactic acid, phenylalanine, L-tartaric acid, succinic acid, sucrose, valine, TSPA. In addition, there are several peptides identified through tandem mass spectrometry with a molecular weight of less than 1kDa. These peptides are LGHDVH, LFSNQLFN, DQDSHPYSGP, LGLGNGGAGGGLVGG, NNTVCGV.

## Conclusion

Several bioactive components such as peptides, glycopeptides, and other secondary metabolites were found in the mucus of several species of land snails. These bioactive components have antibacterial activity against gram-positive and gram-negative bacteria. Further research such as isolation and identification of the structure of bioactive components produced by land snail mucus through techniques in structural biology is very necessary to determine the biological activity and working mechanism of these bioactive components. In addition, metabolomic analysis can also be used to determine metabolic pathways in bacteria that are affected by bioactive molecules from land snail mucus.

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