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Biochemical composition, functional properties, and biomedical applications of *Helix Aspersa* mucin

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Abstract
The mucins are glycoproteins of high-molecular weight that constitute the structural and functional basis of the mucus secretions in various animal taxa. The terrestrial gastropods *Helix aspersa* (syn. *Cornu aspersum*) secrete large quantities of mucus containing mucins alongside bioactive compounds that have been found to play a role in locomotor activity and hydration, defence, and reproduction. Mucus of *H. aspersa* has received significant academic interest over the past few years as a result of confirmed antimicrobial, antioxidant, anti-inflammatory, and wound-healing properties, and hence is extensively used in cosmetic and biomedical preparations. Nonetheless, various *H. aspersa* mucin have not been covered at all in terms of commercial demand as well as biochemical characterization and standardization, which remains to be examined in detail. This review aims to highlight existing information regarding the biochemical framework and functionality bioactivities, structural attributes and therapeutic application of *H. aspersa* mucin. It is all about the architecture of proteins and glycoproteins, the associated small bioactive compounds, antioxidant and antibacterial action mechanisms and other new uses in the field of wound healing, drug delivery, and biomaterials. The gaps in the literature available and discrepancies in methodology and future research requirement are also discussed in a bit to develop the trans-lateral development of mollusk-derived mucins in the biomedical and industrial disciplines.

Keywords: *Helix aspersa*, mucin, snail mucus, glycoproteins, antioxidant activity, antibacterial activity, wound healing, biomaterials

Introduction
Mucus is a viscoelastic biological secretion which coats epithelial surfaces across most animal species and provides the necessary lubrication, hydration and protection against physical, chemical and microbial hazards. Mucus consists of basic structural building blocks, i.e., mucins, which are large glycoproteins, characterized by extensive O-linked glycosylation of serine and threonine residues, to the extent that they resemble bottle-brush like macromolecules, which are capable of retaining water and form gels (Sheng and Hasnain, 2022) [1]. Its distinct physicochemical properties allow the mucins to provide the dynamic biological barriers along with the role in the wound healing process, cell signaling, and immune modulation (Herman *et al*., 2024) [2]. Despite the intensive research on mucins in vertebrate systems, especially with respect to respiratory, gastrointestinal, and oncological systems, limited research on mucins in invertebrate species is available. The terrestrial molluscs among the invertebrates are particularly known to be heavily reliant on the secretion of mucus in order to survive hence making it a valuable model to study the evolutionary conservation of mucins as well as the functional diversity of the mucins (Denny, 1989) [3].

Helix aspersa is a garden snail, which is one of the most widespread terrestrial gastropods and is highly farmed in the area of helicultures. Such a snails mucus is of great scientific and commercial interest because of its well-defined bioactivities and beneficial rheological characteristics that make it applicable in a wide range of topical and biomedical preparations (Laneri *et al*., 2019; Trapella *et al*., 2018) [4, 5]. Despite its extensive usage, there is a very clear lack of an integrated synthesis of the biochemical structure and physiological activity of *H. aspersa* mucin. The review attempts to fill this gap by summarizing the current evidence on its molecular features, biological functions as well as its uses.

Materials and Methods
1. Study Area
The terrestrial gastropods were bought from Enathi village (Ramanathapuram district, Tamil Nadu, India, 9.327392°N, 78.472595°E). It has a tropical climate and is a type of a coastal region with temperature ranges of 29.2°C to 37.8°C, a relative humidity of 75-79 %, and a precipitation rate of 827 mm per annum. These climatic parameters provide the best environment to gastropods living on land.

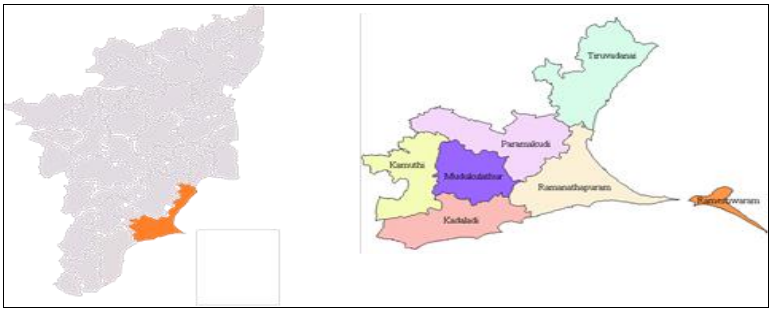


Fig 1: (A) shows the state of Tamil Nadu with Ramanathapuram district marked. (B) Shows the district of Ramanathapuram

Fig 2: Snails in Ramanathapuram district before collection

2. Mucus Extraction

The secretion of mucus was stimulated by applying the diluted lemon extract (1: 5 in sterile water) onto the surface of the foot with the help of sterile cotton swabs in a gentle manner. The secreted mucus was later transferred to sterile receptacles. The mucus was collected by additional scraping of the foot with sterile spatulas. The collected mucus was homogenised in the 0.2M phosphate buffer under cold condition and the resultant supernatant was centrifuged at 3000 rpm for 10 minutes followed by biochemical analyses.

3. Biochemical Estimation

3.1 Total Protein Estimation

Protein content measurement was performed using Lowry method (Lowry *et al*., 1951) [6] where bovine serum albumin (BSA) was used as the standard. The values of absorbance were determined at 660 nm using a spectrophotometer.

3.2 Total Free Amino Acid

The measurement of total free amino acids was done through implementation of the ninhydrin technique as described by Sadasivam and Balasubramanian (1987) [7]. The readings of absorbance were recorded at 570 nm.

3.3 Antioxidant Activity (FRAP Assay)

The protocols followed in the ferric reducing antioxidant power (FRAP) assay were in accordance with the protocol developed by Pulido *et al*. (2000) [8]. The absorbance of the solution was measured at 593 nm, and the antioxidant capacity was measured in mM Fe (II) equivalents.

4. Antibacterial Assay

The agar disk diffusion methodology was applied to determine the antibacterial activity of the product as regards *Staphylococcus aureus* and *Klebsiella pneumoniae*. Muller-Hanto agar was used as a growth media and the inhibition zones were recorded as millimeters after incubating at 37°C within 24 hours of incubation.

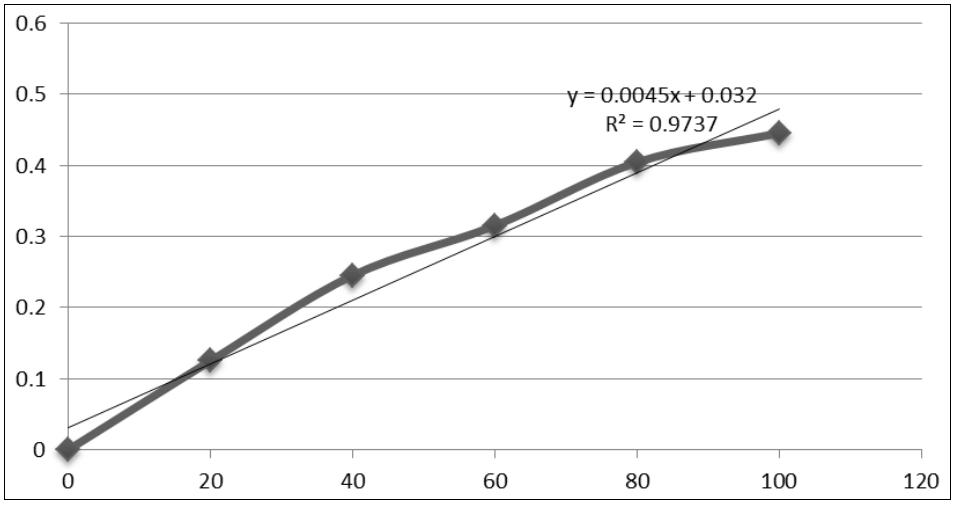
5. FTIR Analysis

Fourier Transform Infrared (FTIR) spectra was recorded by using Thermo Scientific NICOLET iS10 spectrometer in wavelength range of 4000-400 cm^{-1}. The determination of the functional groups was done using unique absorption peaks in the spectra.

Results

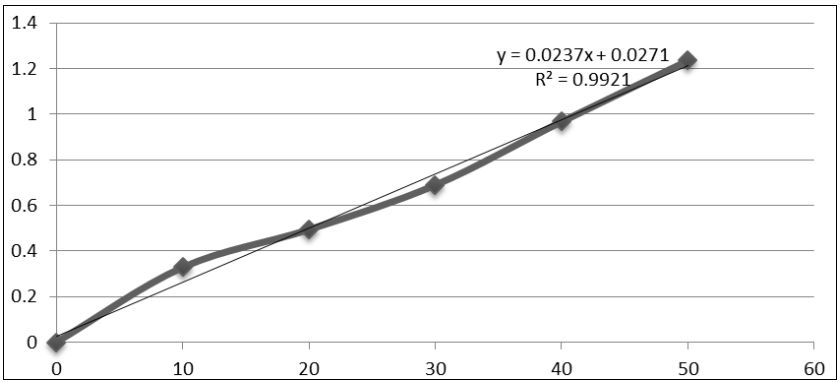
1. Total Protein Content

The concentration of the total protein in *H. aspersa* mucin was established at 68.33 +0.25mg/g.



Graph 1: Protein Test

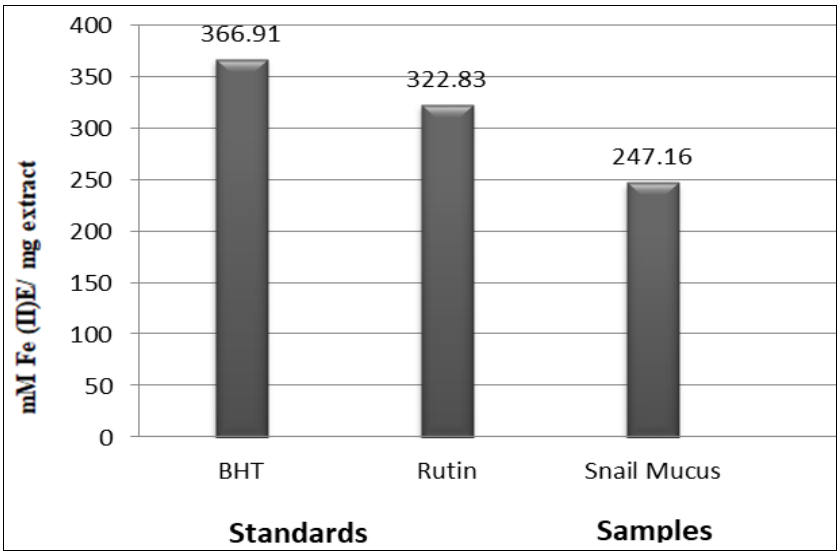
2. Total Free Amino Acids: The total concentration of the free amino acids was found to be 16.37 ± 0.05 mg/g indicating that there was a lot of availability of the amino nitrogen.



Graph 2: Amino acid Test

3. Antioxidant Activity

Ferric Reducing Antioxidant Power (FRAP) was measured and found to have a value of 247.16±1.13 mM equivalent of Fe(II)/mg as compared to the Rutin (322.83 ± 4.46) and BHT (366.91 ± 3.50).



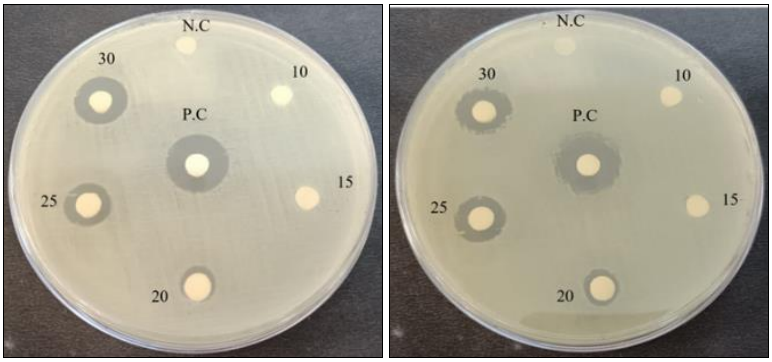
Graph 3: Antioxidant Assay

4. Antibacterial Activity

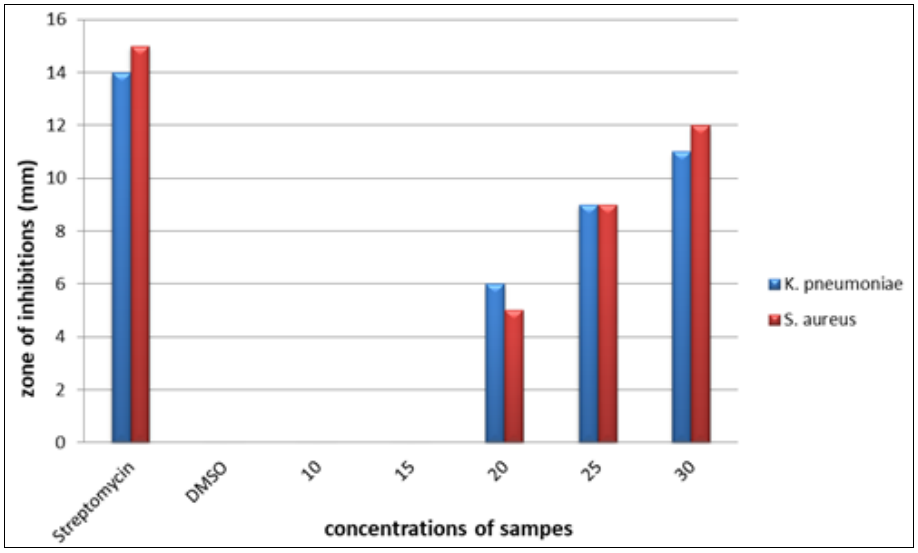
The mucin extract had concentration-dependent antibacterial effectiveness against the two microorganisms under test.

Table 1: Zone of Inhibition of 2 bacterium

Sample Concentration (10mg/ml)	Zone of inhibition (*Staphylococcus aureus*)	Zone of inhibition (*Klebsiella pneumoniae*)
Negative control (DMSO)	-	-
10 µl	-	-
15 µl	-	-
20 µl	6	5
25 µl	9	9
30 µl	11	12
Positive control (Streptomycin) 1mg/ml	14	15

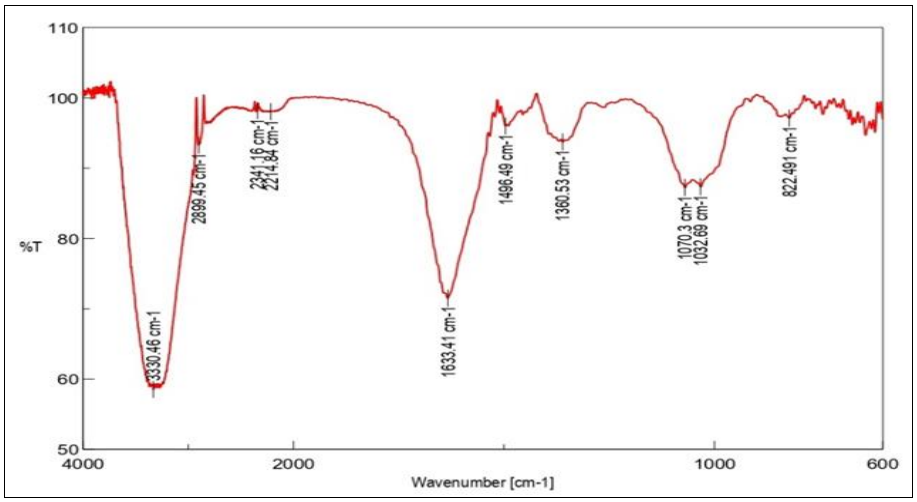


Klebsiella pneumonia *Staphylococcus aureus*



Graph 3: FRAP Assay

5. **FTIR Analysis:** The presence of different functional groups like amines, alkanes, alkenes, phenols, alcohols and carbohydrates was confirmed by FTIR spectral analysis.



Graph 4: FTIR Assay

S. No	Characteristic absorption (cm^{-1})	Intensity	Assingment	Functional group
1	3030.46	Stretching Vibration, Medium	=C-H &=CH2 (usually sharp)	Alkenes
2	2899.45	Stretching, Vibration, Strong	CH3, CH2, CH 2 or 3 bands	Alkanes
3	2214.84, 2341.16	O=C=O stretching	antisymmetric C−O	carbon dioxide
4	1633.41	Bending Vibration, medium-strong	NH_2 Scissorsing (1°-amines)	Amines
5	1496.49	Bending Vibration, Medium	CH2 and CH3 deformation	Alkanes
6	1032.69	Stretching Vibration, Strong	Usually broad C-O	Phenols & Alcohols
7	822.49	Bending, Vibration, medium	= C-H & = CH_2 Out of plane bending	Alkenes

Discussion

H. aspersa mucin has a high level of protein and amino acid which enhances its use as a structural and functional biopolymer. The antioxidant and antibacterial effects are observed in accordance with the presence of glycoproteins, peptides, phenolics, and trace minerals that have been reported in the previous studies (Pitt *et al*., 2015; Gugliandolo *et al*., 2021) [9, 10]. FTIR outcome serves as the additional evidence of biochemical complexity of snail mucus, which is supported by the molecular evidences of the multifunctional bioactivity of the snail mucus.

Research Gaps and Future Perspectives

Although there is increased interest, studies on *H. aspersa* mucin have a number of challenges. The studies are prone to differences in extraction methods, analysis routines and assays condition, which restrict comparability. Extensive proteomic, glycomic, and metabolomic profiling has not been done yet, and the effect of the environmental variability on the bioactivity is not thoroughly comprehended.

Future studies would need to focus more on standardized methodology, molecular level characterization and clinical

validation in order to accommodate regulatory approval and scale-up into industry. The knowledge of the structure-function relationships will help maximize snail-derived mucins as therapeutic biomaterials.

Conclusion

The current research shows that the *Helix aspersa* mucin is a biochemically enriched secretion which has elevated concentration of proteins, amino acids, varying functional groups and large antioxidant and antibacterial properties. Biochemical estimation, when combined with functional assays, is a great experiment in supporting its use in biomedical and cosmetic. Such results stress that standard extraction and characterization guides should be used to maximize the use of snail mucin as a natural therapeutic biomaterial.

Acknowledgments

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