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Antioxidant activity, biochemical composition and physicochemical properties of *Helix aspersa* Müller snail slime

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Abstract

Snails produce various kinds of slime, which serve a variety of purposes and are increasingly being used in medicinal and aesthetic employs. This study aimed to evaluate the chemical composition and biological activity of *H. aspersa* Müller snail slime. Slime was collected from one hundred snails of the species *H. aspersa* Müller for chemical analysis and evaluation of antioxidant activities. Results showed that the mucus contains approximately 98.24 ± 1.18% water, 2.96 ± 1.53% dry matter, 0.68 ± 0.02% acid, with a density of 1.02 ± 0.03 g.mL^{-1}, a slightly alkaline pH value (8.56), an average protein concentration of 4.96 ± 0.3 mg.mL^{-1}, a total sugar content of 2.69 ± 0.2 mg.mL^{-1}, reducing sugars of 0.31 ± 0.01 mg.mL^{-1}, and phenolic compounds of 0.38 ± 0.00 mg.mL^{-1}. Antioxidant activity evaluation results showed that *H. aspersa* Müller slime has a total antioxidant capacity of 0.34 ± 0.00 mg.mL^{-1} with an IC_{50} of 58.45 ± 3.62 µg.mL^{-1} against DPPH free radicals, as well as a strong iron-reducing capacity (IC_{50} of 22.71 ± 0.67 µg.mL^{-1}). Based on these findings, it appears that *H. aspersa* Müller slime contains molecular components and biological activities that may be linked to the animal's specialized tasks and have potential medicinal uses. This study demonstrates that *H. aspersa* Müller slime can be used as an antioxidant agent.

Keywords: *H. aspersa* Müller, slime, physicochemical composition, antioxidant activity

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1. Introduction

The mollusk class, which includes snails, contains approximately 80 000 species and is the second-largest phylum of invertebrates [1], with a global distribution [2] and comprising individuals living in terrestrial, freshwater, and marine habitats [3]. Research shows that mollusks have been used since ancient times [4]. Bioactive substances found in mollusks have been shown to have anticancer, antimicrobial, and antiviral effects [5].

Due to their high protein content, terrestrial snails have been used for centuries in a variety of culinary and medicinal applications [6]. Snails of the genus *Helix*, specifically *Cornu aspersum* aspersum (*H. aspersa* Müller) and *Cornu aspersum* maxima (*H. aspersa* maxima), are frequently used in scientific studies [7]. Snails, which are land mollusks with flexible bodies made up of a compressed head and foot, can secrete a viscous material called "slime or mucus" [8] that serves many purposes in the animal, including acting as a glue, moisturizing, nourishing, greasing, and protective [4,9]. Another benefit of the mucous is that it protects mollusks from exhaustion and makes them less appealing to predators [10]. As a further benefit, its antibacterial qualities help shield the animal from exhaustion and viral infections [11].

The mucopolysaccharides and glycoproteins that make up this mucous [12] are thought to play a role in its various medicinal actions [2]. These beneficial substances are capable of destroying cancer cells, reducing inflammation, eradicating germs, and eliminating tumor cells [13]. Their anti-aging qualities have led to their incorporation into a wide variety of hygiene products [14]. This secretion is revolutionizing the world of skincare [15]. The use of this substance in human health and makeup [16,17,18]. Snail mucus and its compounds have garnered a lot of attention for their potential applications in a variety of medical fields, including wound repair, the therapy of skin diseases, and the creation of mucoadhesive compositions [9,19].

Anti-inflammatory and antioxidant qualities in snail mucous have been related to its potential to alleviate experimental colitis [19]. Human cancer cells have been shown to be susceptible to the anti-tumor effects of *H. aspersa* maxima secretions [18]. Snail mucous has been shown to have multiple biochemical effects in various studies including antibacterial, antiviral, anti-tyrosinase, and anticancer properties [6,15,18]. Snail mucous is rich in a variety of substances, including allantoin, hyaluronic acid, peptides, and proteins [7,9,20]. Mucopolysaccharide content comprising minute quantities of glycolic acid and allantoin in *H. aspersa* Müller mucus confers antibacterial activity and several medicinal characteristics, including epidermal protection and wound healing [9,21].

Snail slime contains a variety of substances, such as proteins, glycoproteins, glycosaminoglycans, fatty acids, flavonoids, vitamins, glycolic acid, allantoin, minerals [22, 23,24], in addition to carbs [25]. Due to its unique make-up, numerous studies have pointed out its curative benefits in a variety of diseases [24,26]. Multiple investigations have shown that the mucous produced by *H. aspersa* has many natural compounds with medicinal effects on human epidermis [23]. Snail mucous has antimicrobial and wound-healing properties because of the abundance of medicinal chemicals it contains [27].

The objective of this study is to determine the chemical composition of *H. aspersa* Müller slime, the in vitro evaluation of antioxidant activity by determining the total antioxidant capacity, by the method of trapping the free radical DPPH.

2. Materials and methods

2.1. Snail harvesting and laboratory upkeep

A total of 100 healthy *H. aspersa* Müller species (Fig. 1) were collected and transported by land in canvas bags to the laboratory (temperature 25°C and humidity 70%). These individuals were placed in rectangular plastic boxes (24 x 32 x 12 cm) that contained a sponge, moist soil, and lettuce, carrots, and spinach as food. Each box contained fifty snails and was sprayed with water daily to maintain moisture while being thoroughly cleaned on a regular basis. Each snail was gently washed twice with tap water and once with distilled water to avoid contamination of the collected biological material. Snails weighing 10.22 ± 2.36g and with a shell size of 3.31 ± 0.37cm were selected for this study.

2.2. Preparation of snail mucus

Approximately 100 mL of mucous secretion was collected by manually stimulating the pedal glands using a small sterile metal rod with a tapered end. The mucus secretions were extracted in an aseptic manner, and observations of the physical characteristics of the mucus (color, viscosity, average volume per snail, texture, and thickness) were conducted. Euthanasia of the snails was not necessary, and the snails were handled in accordance with animal welfare principles for scientific experimentation.

2.3. Physicochemical analyses

2.3.1. Moisture content determination

The method used for moisture determination was based on the loss of sample mass until a constant mass was obtained at 105°C [28].

2.3.2. Dry matter determination

The determination of the percentage of dry matter involves placing the sample in an oven at a temperature of 70°C until a variation of at least 3 mg is obtained between two consecutive weighings performed separately with a 2-hour interval [29].

2.3.3. Acidity measurement

The pH measurement was determined using the method [30]. In addition, the determination of acid content was performed by titrating the acidity with a 20% sodium hydroxide solution in the presence of a colored indicator (Phenolphthalein) [28].

2.3.4. Density measurement

The perceived density of the ground samples was calculated using a modified version of the method defined by Pissia et al. [31]. A 25 mL graded glass container was used, and the sample was put into it before being measured again. By dividing the sample's mass by its volume, we were able to determine its perceived density, which we then represented as a value in $g.mL^{-1}$.

2.4. Biochemical analysis

2.4.1. Protein content

Blue pigment is created by the reaction of copper sulfate and Folin-ciocalteu reagent (phosphomolybdo-tungstic acid) with proteins in an alkaline solution. This test requires 500 µL of the extract to be combined with 2500 µL of a solution containing Na_2CO_3 (2%, w/v) in 0.1M NaOH, 1% $CuSO_4$, and $KNaC_4H_4O_6$ (2%, w/v). The mixture should sit for a minute at room temperature after being stirred. The next step is to add 250 µL of Folin's reagent while continuing to agitate the mixture. It takes 30 minutes of darkness to enable the color response to fully emerge. Absorbance is recorded at 750 nm, and values are reported as Bovine Serum Albumin (BSA) equivalents [32].

2.4.2. Total carbohydrate content

To determine the soluble sugar content, 500 µL of the extract is mixed with 500 µL of phenol (5%, w/v) and 2500 µL of concentrated sulfuric acid in a test tube. The mixture is then vortexed and heated in a water bath at 90°C for 10 minutes. After cooling for 5 minutes, the optical density is measured at 485 nm. The soluble sugar content is determined by comparing the results to a glucose reference (mg $GE.mL^{-1}$ of slime) [33].

2.4.3. Content of reducing sugars

To measure the amount of reducing sugars, Di-Nitro-Salicylic Acid is utilized as a reagent. Initially, 500 µL of the sample is mixed with 500 µL of DNS reagent and stirred. The mixture is then heated in boiling water for 20 minutes and allowed to cool. Afterward, 2000 µL of distilled water is added, and the spectrophotometer is used to measure

the optical density at 540 nm against the blank. The outcome is presented in mg GE.mL^{-1} of slime [34].

2.4.4. Total phenol content

The method utilized to determine the total phenol content is based on Zargoosh et al. [35] report with some adjustments. In summary, 200 µL of snail slime is mixed with 1000 µL of Folin-Ciocalteu (1/10, v/v). After 5 minutes of incubation, 800 µL of Na_2CO_3 (7.5%, w/v) is added. The mixture is then incubated at room temperature for 2 hours, and the optical density at 765 nm is measured. The outcome is presented as milligrams of gallic acid equivalent (mg GAE.mL^{-1} of slime).

2.5. Analysis by Infrared Spectroscopy (FTIR)

The purpose of analyzing the slime by infrared spectroscopy is to identify the presence of functional groups in the molecules. The infrared spectrum was obtained using Fourier Transform Infrared Spectroscopy (FTIR) Tensor II, over a range of frequencies between 4000 and 400 cm^{-1}. The method is based on measuring the wavelengths and absorption intensities of the material. The obtained infrared spectrum can provide qualitative information.

The wavelengths at which the sample absorbs are characteristic of the present chemical groups, and tables can be used to assign the absorbencies to the different chemical groups present.

2.6. Antioxidant activity

2.6.1. Total antioxidant capacity (TAC)

Antioxidant content was analyzed using an approach based on the technique reported by El Moussaoui et al. [36]. To conduct the test, 50 µL of the slime was combined with 2000 µL of solution (0.6 M sulphuric acid, 4 mM ammonium molybdenum, and 28 mM sodium phosphate) and heated to 95°C for 90 minutes. The absorption was read at 695 nm after having been cooled into the room temperature, with L-ascorbic acid acting as the standard antioxidant. In the end, the total capacity for antioxidants was calculated as the ascorbic acid equivalent per ml of slime (mg EAA.mL^{-1}).

2.6.2. Free radical scavenging activity (DPPH)

The absorption of DPPH radicals at characteristic wavelengths is followed by a decrease in optical density. From a series of tubes, 500 µL of ethanol solutions containing different concentrations of extracts were taken and 2500 µL of DPPH solution (0.2 mM) was added to all tubes. The contents were shaken well and allowed to react at room temperature for 30 minutes. The absorbance of the samples was recorded at 517 nm against the control. A negative control was made by mixing 500 µL of methanol and 2500 µL of DPPH solution, and the methanol was used as a blank in parallel with the test sample [37].

The percentage inhibition and calculated as follows:

$$\%\ \text{activity} = \frac{\text{Abs Cn} - \text{Abs Ech}}{\text{Abs Cn}} \times 100 \quad (1)$$

Where: Abs $_{Ech}$: Absorbance of the sample; Abs $_{Cn}$: Absorbance of negative control

The calculated values were transformed into a linear equation (Y = aX + b) with concentration in µg.mL^{-1} on the X-axis and the percentage reduction value on the Y-axis. The IC_{50} value was obtained from the calculation when the percentage reduction was 50%. The results are expressed as the 50% effective concentration (IC_{50}) in µg.mL^{-1} (Equation 2).

$$\text{IC50} = \frac{50-b}{a} \quad (2)$$

2.6.3. Ferric reducing antioxidant power assay (FRAP)

The formation of the ferrous form of the iron/ferricyanide complex was measured the formation of pearly Prussian blue at 700 nm [38]. 200 µL of extracts of varying concentrations, containing 250 µL of phosphate buffer (0.2 M, pH = 6.6) and 250 µL of potassium ferricyanide (1%, w/v), were allowed to react at 50°C for 20 minutes. Then, 2500 µL of TCA (10%, w/v) were added and the mixture was centrifuged (10 min, 3000 rpm). 2500 µL supernatant, 500 µL ferric chloride (0.1%, w/v) and 2500 µL distilled water were mixed. The absorbance was determined at 700 nm relative to negative control. The same procedure was followed for the standard solution of L-ascorbic acid. The reduction of activity can be calculated using the following equation.

$$\%\ \text{Reduction power} = \frac{(\text{Asample} - \text{Ablank})}{\text{Asample}} \times 100 \quad \textbf{(3)}$$

With, $\mathbf{A_{blank}}$ = Absorbance without sample and $\mathbf{A_{sample}}$ = Absorbance of the sample.

The IC_{50} value can be calculated based on the equation as in the determination of the DPPH method.

2.7. Statistical analyses

The results of the performed tests are expressed as mean ± standard deviation in triplicates. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Duncan's test. Significance was accepted at $p \leq 0.05$ level.

3. Results and Discussions

Snail farming is a growing agricultural activity worldwide. In our work, we used manual extraction. Since extraction methods can affect the composition of mucus, we characterized the mucus, paying particular attention to its physical properties, quantification of biomolecules, and biological activities.

3.1. Physical properties of mucus

The physical properties of the mucus were observed (Table 3 and Fig. 2). The collected mucus was a thicker, slightly cloudy, pale yellow viscous liquid and odorless. Our results are compared to recent studies, Gugliandolo et al. [24], who found in *H. aspersa* Müller a clear, odorless and slightly amber liquid, Trapella et al. [9] reported a pale yellow, odorless liquid, as well as Okeniyi et al. [39], who found a colorless, elastic liquid, very smooth with high viscosity and low texture thickness in *A. fulica*, a slightly cloudy pale yellow viscous liquid in *A. achatina*, and a moderately viscous and thick, brown liquid with a thick texture and sticking globules in *A. marginata*. In this study, we found that the slime of *H. aspersa* Müller had an average moisture content of approximately 98.24 ± 1.18%, with an average dry matter content of 2.96 ± 1.53%. These results are comparable to those reported by Liudmyla et al. [40], who reported a median value of 98.43 ± 0.2%. Our results also indicate that the mucus had a density of 1.02 ± 0.03 g.mL^{-1}, an alkaline pH

of 8.56 ± 0.07, and an acidity of 0.68 ± 0.02%. Similar results were found by Newar and Ghatak [25] (pH close to 8.5), while Gugliandolo et al. [24] found a pH value of 4.8 and a density between 1 to 1.1 g.mL^{-1}, and Liudmyla et al. [40] showed an average value of 8.2. In contrast, Trapella et al. [9] reported a pH of 6.0-7.0 and a density of 1.02 g.mL^{-1}. Newar and Ghatak [25] suggest that the mucus contains numerous alkaline groups, such as amino groups, in its protein and carbohydrate components.

3.2. Biochemical composition

The slime of *H. aspersa* Müller was selected for analysis by quantifying bioactive molecules. The results obtained confirm that the slime of *H. aspersa* Müller contains 4.96 ± 0.3 mg.mL^{-1} of proteins, which are comparable to those found by Iguchi et al. [41] (3.99 mg.mL^{-1}), Di Filippo et al. [42] (1.80 mg.mL^{-1}), Trapella et al. [9] (100-250 mg.L^{-1}), and Gugliandolo et al. [24] (1.54 g.100g^{-1}). Our slime contains 2.69 ± 0.2 mg.mL^{-1} of total carbohydrates, a reducing sugar content of approximately 0.31 ± 0.01 mg.mL^{-1}, and an average content of phenolic compounds of approximately 0.38 ± 0.00 mg.mL^{-1}. Studies have shown a quantity of sugars ranging from 0.010 to 0.027 g.L^{-1} and 70-80 mg.L^{-1} of polyphenols [9], while Newar and Ghatak [25] showed 225 µg of proteins and 120 µg of sugars per sample. As snail mucus serves diverse functions in the life of snails, it is probable that the composition of snail mucus varies according to functional demands [43].

3.3. Infrared Spectroscopy (IRTF)

IR spectroscopy is employed in biological and clinical fields [9]. The IR spectrum obtained from *H. aspersa* Müller displays spectral bands characterizing functional groups in the mucus (Fig. 4). Starting from the highest wavenumber, the broad band centered around 3286 cm^{-1} can be attributed to the presence of a large amount of OH fragments (stretching vibrations of carboxylic acid -OH) occurring between 2900 and 3280 cm^{-1}. Contributions from polyphenols, mainly in the range of 1500-3500 cm^{-1}, cannot be excluded [44].

In the band region located at 1560-1640 cm^{-1}, the spectrum shows the presence of a band (around 1639 cm^{-1}) which, according to literature [45], is associated with stretching vibrations of the peptide bonds (CO-NH) in amide I [46]. According to Davies and Hawkins [47], this band indicates the presence of amide bonds in the central proteins of both glycosaminoglycans, proteoglycans, and lectins.

The bands around 1740 cm^{-1} and 1400 cm^{-1} correspond to distinct ionization states of carboxylate groups (C=O), which can be linked to acid derivatives of sugars and certain side chains of amino acids (COOH ↔ COO^- + H^+) according to [12,45]. This probably represents an increase in the number of ester bonds, which could amplify the intensity of the band at 1738 cm^{-1} (a strong band in the ester/acetyl band).

Between approximately 1050 and 1450 cm^{-1}, there is a range of bands associated with C-H and C-C groups, which can be associated with the carbon skeleton of central proteins (e.g. CH_2 and CH_3) as well as the ionization state of amino acid side chains and sugar side chains (COOH, COO^-) according to Skingsley et al. [12]. Indications of sulfated, acylated, and esterified structures are found in this region.

The detection of faint and secondary signals at 1212 and 1366 cm^{-1} indicates the minor involvement of O-glycosidic bonds and carboxylate fragments linked to acid derivatives of sugars and amino acid chains [48].

Given the size and complexity of large protein molecules, IR spectroscopy does not provide detailed structural information. According to Davies and Hawkins [47], mollusk mucus is mainly composed of water (80-99%) in which protein-carbohydrate complexes are suspended. Lectins can bind glycosaminoglycans and glycoproteins to form two groups of mucopolysaccharides complexes.

The results of this investigation confirm the presence of numerous structural patterns that can be attributed to the kinds of molecules proposed by Skingsley et al. [12].

3.4. Antioxidant activity

In this study, we have demonstrated that the mucus of *H. aspersa* Müller snail exhibits antioxidant activity.

The total antioxidant capacity (TAC) was measured by establishing an ascorbic acid calibration curve and expressing the result in mg AAE.mL^{-1} of mucus. The total antioxidant capacity of the mucus was 0.34 ± 0.00 mg AAE.mL^{-1}.

To evaluate the in vitro anti-radical activity of *H. aspersa* Müller mucus, the DPPH biological assay was used, with ascorbic acid as the standard reference. The results of the DPPH free radical inhibition test showed that the percentage of free radical inhibition increases with increasing concentration. The results of the mucus' antioxidant activity are expressed in terms of the 50% inhibitory concentration (IC_{50}) of the DPPH radical.

The mucus had an IC_{50} of 58.45 ± 3.62 µg.mL^{-1} for its antioxidant activity against the DPPH radical, compared to ascorbic acid, which had an IC_{50} of 44.55 ± 0.61 µg.mL^{-1} (fig. 5). Noothuan et al. [23] demonstrated the antioxidant activity of *H. distincta* and *L. fulica* (25.5 and 129.9, respectively). In fact, the IC_{50} is inversely related to the antioxidant power of a compound, expressing the amount of antioxidant needed to reduce 50% of the free radical. The lower the concentration, the higher the antioxidant power [49]. The ability of the mucus to reduce iron was determined by measuring its capacity to transform Fe^{3+} to Fe^{2+} by donating an electron. The amount of Fe^{2+} was measured by monitoring the formation of Prussian blue at an absorbance of 700 nm.

Figure 1: *H. aspersa* Müller

Figure 2: Appearance of slime *H. aspersa* Müller

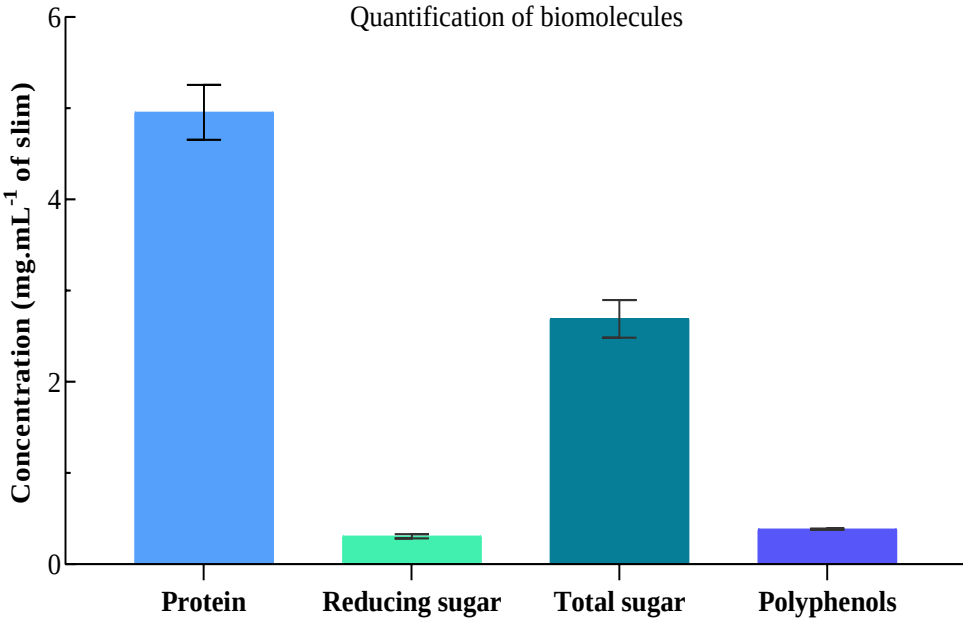

Figure 3: Histogram of biomolecules in the slime of *H. aspersa* Müller

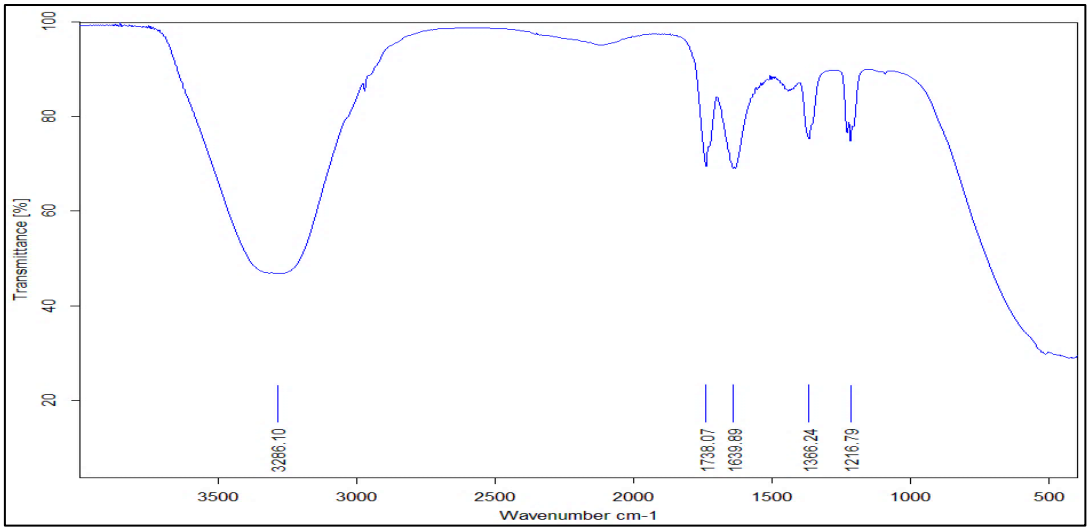

Figure 4: Spectral analysis of *H. aspersa* Müller

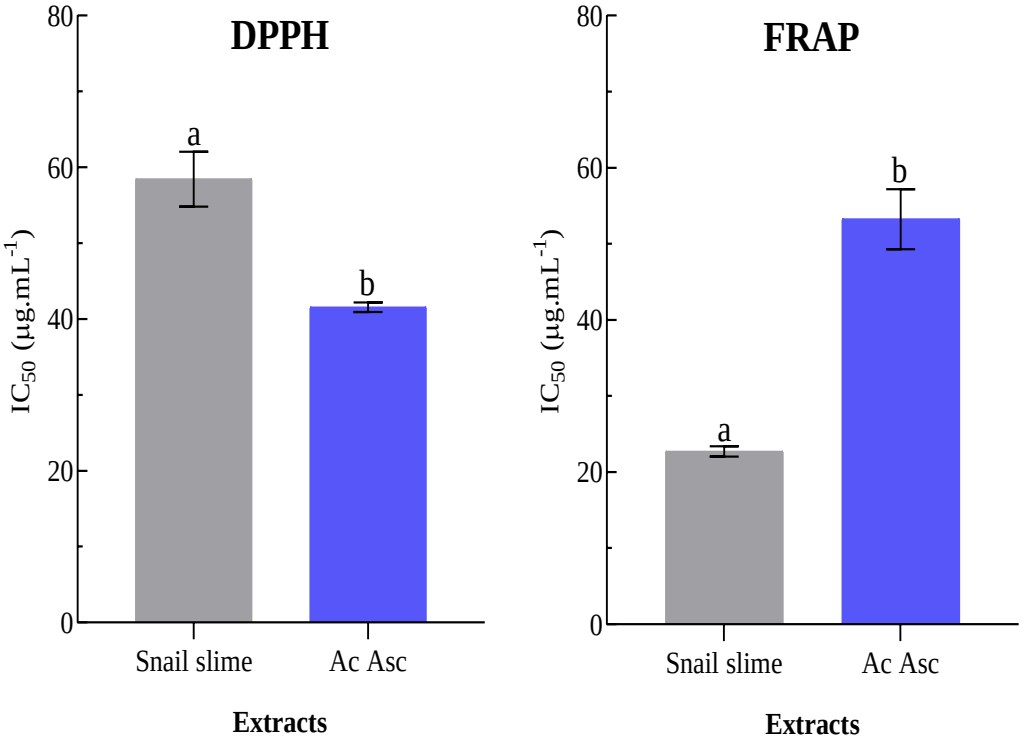

Figure 5: Results of DPPH and FRAP tests of the slime of *H. aspersa* Müller and ascorbic acid
The significant difference ($p \leq 0.05$) is illustrated by the letters **a** and **b**

Table 3: Physical characteristics of slime *H. aspersa* Müller

Mucus parameter	Physical characteristics of mucus
Color	Yellow and slightly cloudy
Texture / Thickness	Very thick sticky balls
Viscosity	High
VM / snail (mL)	2.23 ± 0.40

In this study, the mucus of *H. aspersa* Müller showed good iron reduction activity with an IC_{50} value of 22.71 ± 0.67 µg.mL^{-1}. However, the reducing power increases with the concentration of mucus. The reducing activity was compared to ascorbic acid as a standard antioxidant (IC_{50} of 53.25 ± 3.94 µg.mL^{-1}), which showed a significant difference ($p \leq 0.05$) compared to the tested extract. Results have been found in different organs of different snail species [50,51,52,53].

The reduction capacity of the samples could be due to di and mono hydroxyl substitutions in the aromatic cycle, which possess powerful hydrogen donation activities [51]. Additionally, Brieva et al. [18] discovered that the mucus of *H. aspersa* contains antioxidant activities of superoxide dismutase and glutathione-s-transferase. Therefore, the richness of our sample in biomolecules is the basis of their antioxidant activity. The identified compounds have been found to possess anti-inflammatory properties and inhibit angiogenesis, a process crucial for tumor growth. As a result, they help in restoring the immune system [54]. In addition to their antioxidant properties, these compounds exhibit a range of biological activities, including anticoagulant, antiallergic, anti-inflammatory, and vasodilatory activities [55]. These activities could also be explained by the presence of hydroxyl groups in phenolic compounds that can trap free radicals [49].

Furthermore, these antioxidant properties have been reported and confirmed in the mucus of *H. aspersa* Müller due to the presence of allantoin, whose antioxidant properties have been demonstrated [56]. The main function of antioxidants depends on their ability to reduce oxidative damage [57]. Hatuikulipi et al. [19] confirmed that the mucus of *H. aspersa* was found to have anti-inflammatory and antioxidant properties, which helped in reducing colon inflammation. Various bioactive compounds and antioxidant properties were identified in extracts from different parts of several snail species [58,59,60]. Due to their antioxidant properties specifically, their ability to donate a hydrogen atom-reductones are crucial to iron reduction capability [61]. Similarly, the beneficial substances in *H. aspersa* Müller mucus can combine with radicals, giving electrons to transform them to more stable molecules and stopping the free radical chain reaction.

4. Conclusions

Physicochemical tests performed on snail slime allow determination of its biochemical composition. Overall, the results are not in contradiction with literature data, proving the abundance of biomolecules in *H. aspersa* Müller slime and its high biological value. Infrared spectroscopy analysis of *H. aspersa* Müller slime reveals information about existing functional groups. The in vitro antioxidant activity of the slime has been evaluated by total antioxidant capacity, DPPH radical scavenging test and iron reduction test. These methods can highlight the antioxidant activity of *H. aspersa* Müller mucus. This species could therefore be considered as a source of natural bioactive molecules and antioxidants. All these in vitro results serve as a starting point for identifying naturally occurring substances with biological activity. Further tests will be necessary to find formulations that use the antioxidant properties of this slime in the pharmaceutical and cosmetic industries. These results allow evaluation of the composition of *H. aspersa* Müller slime as a good source of bioactive molecules. Therefore, it can be a good indicator of antioxidant activity. These therapeutic effects justify the relevance of using snail slime.

Conflict of interest

There were no declared potential conflicts of interest pertaining to this paper.

References

[1] M.V. Vinarski, O.V. Aksenova, I. N. Bolotov. (2020). Taxonomic assessment of genetically-delineated species of radicine snails (Mollusca, Gastropoda, Lymnaeidae). Zoosystematics and Evolution. 96(2):577-608.

[2] H. Abd El Azeem, G. Osman, S. El-sabbagh, S. Sheir. (2020). Antibacterial activity of some terrestrial gastropods from Egypt against Staphylococcus aureus and Escherichia coli. Egyptian Journal of Zoology. 74:1-12.

[3] T. Neubauer, M. Harzhauser, E. Georgopoulou, A. Kroh, O. Mandic. (2015). Tectonics, climate, and the rise and demise of continental aquatic species richness hotspots. Proceedings of the National Academy of Sciences 112: 11478-11483.

[4] S. Greistorfer, W. Klepal, N. Cyran, A. Gugumuck, L. Rudoll, J. Suppan, J. von Byern. (2017). Snail mucus-glandular origin and composition in Helix pomatia. Zoology. 122: 126-38.

[5] J. Rajaganapathy, S.P. Thyagarajam, J.K. Edward. (2000). Study on cephalopod's ink for anti-retroviral activity. Indian Journal of Experimental Biology. 38, 519–20.

[6] S. Ulagesan, H.J. Kim. (2018). Antibacterial and antifungal activities of proteins extracted from seven different snails. Applied Science. 8: 1362.

[7] M. Dankowiakowska, B. Domagalska. (2018). Land snails as a source of raw materials with potential use in cosmetology and dermatology. Polish Journal of Cosmetology. 21: 114.

[8] C. Etim, G. Aleruchi, Obande. (2016). Antibacterial properties of snail mucus on bacteria isolated from patients with wound infection. British Microbiology Research Journal. 11: 1-9.

[9] C, Trapella, R. Rizzo, S. Gallo, A. Alogna, D. Bortolotti, F. Casciano, G. Zauli, P. Secchiero, R. Voltan. (2018). HelixComplex snail mucus exhibits pro-survival, proliferative and pro-migration effects on mammalian fibroblasts. Scientific Reports. 8: 17665-74. https://doi.org/10.1038/s41598-018-35816-3

[10] E.M. Hämäläinen, S. Järvinen. (2012). Snails: biology, ecology, and conservation. Hauppauge, NY: Nova Science Publisher's, 2012.

[11] N. Nantarat, Y. Tragoolpua, P. Gunama. (2019). Antibacterial activity of the Mucus Extract from the Giant African Snail (Lissachatina fulica) and Golden Apple Snail (Pomacea canaliculata) against pathogenic bacteria causing skin diseases. Tropical Natural History. 19: 103-112.

[12] D.R. Skingsley, A.J. White, A. Weston. (2000). Analysis of Pulmonate Mucus by Infrared Spectroscopy. Journal of Molluscan Studies. 66: 363-372. 10.1093/mollus/66.3.363.

[13] T. Anand, J. Edward. (2002). Antimicrobial activity in the tissue extracts of five species of cowries Cypraea spp. (Mollusca: Gastropoda) and an ascidian Didemnum psammathodes (Tunicata: Didemnidae). Indian Journal of Marine Science. 31: 239-42.

[14] P. Dolashka, A. Dolashki, L. Velkova, S. Stevanovic, L. Molin, P. Traldi, R. Velikova, W. Voelter. (2015) Bioactive compounds isolated from garden snails. Journal of Bioscience and Biotechnology. 147–55.

[15] C. Ellijimi, M.B. Hammouda, H. Othman, W. Moslah, J. Jebali, H.B. Mabrouk, M. Morjen, M. Haoues, J. Luis, N. Marrakchi, K. Essafi-Benkhadir, N. Srairi-Abid. (2018). Helix aspersa maxima mucus exhibits antimelanogenic and antitumoral effects against melanoma cells. Biomedicine & Pharmacotherapy 101:871-880.

[16] M.T. Truchuelo, M. Vitale. (2019). A cosmetic treatment based on the secretion of Cryptomphalus aspersa 40% improves the clinical results after the use of nonablative fractional laser in skin aging. Journal of Cosmetology and Dermatology. 19:622-628.

[17] A. Brieva, N. Philips, R. Tejedor, A. Guerrero, J.P. Pivel, J.L. Alonso-Lebrero, S. Gonzalez. (2008). Molecular basis for the regenerative properties of a

secretion of the mollusk Cryptomphalus aspersa. Skin Pharmacology and Physiology. 21:15-22.

[18] S.G. Fabi, J.L. Cohen, J.D. Peterson, M.G. Kiripolsky, M.P. Goldman. (2013). The effects of filtrate of the secretion of the Cryptomphalus aspersa on photoaged skin. Journal of Drugs and Dermatology. 12: 453-7.

[19] T. Hatuikulipi, D. Naimi, M. Kouachi, L. Bouchetob. (2016). Preventive effect of Helix aspersa slime against experimentally chemo-induced colitis in rat. Pharmacy letter. 8:200-206.

[20] M.A. El Mubarak, F.N. Lamari, C. Kontoyannis. (2013). Simultaneous determination of allantoin and glycolic acid in snail mucus and cosmetic creams with high performance liquid chromatography and ultraviolet detection. Journal of Chromatography. 1322: 49-53.

[21] V. Gentili, D. Bortolotti, M. Benedusi, A. Alogna, A. Fantinati, A. Guiotto, G. Turrin, C. Cervelati, C. Trapella, R. Rizzo, G. Valacchi. (2020) HelixComplex snail mucus as a potential technology against O3 induced skin damage. PLoS ONE. 15: e0229613. https://doi.org/10.1371/journal.pone.0229613

[22] A. Onzo, R. Pascale, M.A. Acquavia, P. Cosma, J. Gubitosa, C. Gaeta. (2021). Untargeted Analysis of Pure Snail Slime and Snail Slime-Induced Au Nanoparticles Metabolome with MALDI FT-ICR MS. Journal of Mass Spectrometry. 56: e4722. https://doi.org/10.1002/jms.4722

[23] N. Noothuan, K. Apitanyasai, S. Panha, A. Tassanakajon. (2021). Snail mucus from the mantle and foot of two land snails, Lissachatina fulica and Hemiplecta distincta, exhibits different protein profiles and biological activity. BMC Research Notes. 14: 138. https://doi.org/10.1186/s13104-021-05557-0

[24] E. Gugliandolo, M. Cordaro, R. Fusco, A.F. Peritore, R. Siracusa, T. Genovese et al. (2021) Protective Effect of Snail Secretion Filtrate against Ethanol-Induced Gastric Ulcer in Mice. Sci. Rep 11: 3638.

[25] J. Newar, A. Ghatak. (2015) Studies on the Adhesive Property of Snail Adhesive Mucus. Langmuir 31: 12155-12160. https://doi.org/10.1021/acs.langmuir.5b03498

[26] P.C. Mane, S.A.R. Sayyed, D.D. Kadam, D.M. Shinde, A. Fatehmulla, A.M. Aldhafiri et al. (2021). Terrestrial Snail-Mucus Mediated Green Synthesis of Silver Nanoparticles and in Vitro Investigations on Their Antimicrobial and Anticancer Activities. Sci. Rep. 11: 13068.

[27] M. Adikwu, T.C. Enebeke. (2008). Evaluation of snail mucin dispensed in brachystegia gum gel in wound healing agent. Anim Res Int. 4: 685-97.

[28] A. Benahmed-Djilali, T. Chemoul, S. Kal, M. Nabiev, C. Besombes. (2017). Propriétés d'une pommade antibactérienne formulée à base de saponines extraites des feuilles de noyer. Phytothérapie. https://doi.org/10.1007/s10298-017-1145-9

[29] AOAC (2010). Official Method of Analysis, Association of Official Analytical Chemists, (18éme éd) Washington DC

[30] G. Brunetti, A. Traversa, F. De Mastro, C. Cocozza. (2019). Short term effects of synergistic inorganic and organic fertilization on soil properties and yield and quality of plum tomato. Scientia Horticulturae. 252: 342-347, https://doi.org/10.1016/j.scienta.2019.04.002.

[31] M.A. Pissia, A. Matsakidou, A. Paraskevopoulou, V. Kiosseoglou. (2022). Structural characteristics and physicochemical properties of freeze-dried snail meat. LWT. 155:112980, https://doi.org/10.1016/j.lwt.2021.112980.

[32] B. Deepachandi, S. Weerasinghe, T.P. Andrahennadi, N.D. Karunaweera, N. Wickramarachchi, P. Soysa, Y. Siriwardana. (2020). Quantification of soluble or insoluble fractions of Leishmania parasite proteins in microvolume applications: a simplification to standard lowry assay. International Journal of Analytical Chemistry. 2020. https://doi.org/10.1155/2020/6129132

[33] S.S. Nielsen. (2010). Phenol-Sulfuric Acid Method for Total Carbohydrates. In: Nielsen, S.S. (eds) Food Analysis Laboratory Manual. Food Science Texts Series. Springer, Boston, MA. https://doi.org/10.1007/978-1-4419-1463-7_6

[34] A. Negrulescu, V. Patrulea, M.M. Mincea, C. Ionascu, B.A. Vlad-Oros, V. Ostafe. (2012). Adapting the reducing sugars method with dinitrosalicylic acid to microtiter plates and microwave heating. Journal of the Brazilian Chemical Society. 23:2176-2182.

[35] Z. Zargoosh, M. Ghavam, G. Bacchetta, A. Tavili. (2019). Effects of ecological factors on the antioxidant potential and total phenol content of Scrophularia striata Boiss. Scientific Reports. 9:16021.https://doi.org/10.1038/s41598-019-52605-8

[36] A. El Moussaoui, E.Z. Jawhari, A.M. Almehdi, H. Elmsellem, K.F. Benbrahim, Bousta, D., Bari A. (2019). Antibacterial, antifungal and antioxidant activity of total polyphenols of Withania frutescens. L. Bioorganic Chemistry. 93: 103337.

[37] B. Huang, H. Ke, J. He, X. Ban, H. Zeng. Wang Y. (2011). Extracts of Halenia elliptica exhibit antioxidant properties in vitro and in vivo. Food and Chemical Toxicology. 49:185–190.

[38] M. Oyaizu. (1986). Studies on product of browning reaction prepared from glucose amine Japanese. Journal of Nutrition. 44: 307.

[39] F.A. Okeniyi, O.M. Oghenochuko, S.O. Olawoye, R.A. Animashahun, A.G. Adeyonu, O.B. Akpor. (2022). Antimicrobial potentials of mucus mucin from different species of giant African land snails on some typed culture pathogenic bacteria. Asian Journal of Agriculture and Biology. 4:202107294. DOI: https://doi.org/10.35495/ajab.2021.07.294

[40] K. Liudmyla, C. Olena, S. Nadiia. (2022). Chemical properties of Helix aspersa mucus as a component of cosmetics and pharmaceutical products, Materials Today Proceedings. 62(15): 7650-7653. https://doi.org/10.1016/j.matpr.2022.02.217.

[41] S.M.M. Iguchi, A. Takashi, J.J. Matsumoto, (1982). Antibacterial activity of snail mucus mucin. Comparative Biochemistry & Physiology. 72: 571-4.

[42] M.F. Di Filippo, V. Di Matteo, L.S. Dolci, B. Albertini, B. Ballarin, M.C. Cassani, N. Passerini, G.A. Gentilomi, F. Bonvicini, S. Panzavolta. (2022). Effectiveness of Snail Slime in the Green Synthesis of Silver Nanoparticles. Nanomaterials. 12:3447. https://doi.org/10.3390/nano12193447

[43] M. Denny. (1983). Molecular biomechanics of molluscan mucous secretions. In: Hochachka PW, editor. Metabolic biochemistry and molecular biomechanics. Cambridge: Academic Press. 431-465.

[44] J. Gubitosa, V. Rizzi, A. Lopedota, P. Fini, A. Laurenzana, G. Fibbi, F. Fanelli, A. Petrella V. Laquintana, N. Denora, R. Comparelli P. Cosma. (2018). One pot environmental friendly synthesis of gold nanoparticles using Punica Granatum Juice: a novel antioxidant agent for future dermatological and cosmetic applications. Journal of Colloid and Interface Science. 521: 50–61, 10.1016/j.jcis.2018.02.069.

[45] S.E. Glassford, B. Byrne, S.G. Kazarian. (2013). Recent applications of ATR FTIR spectroscopy and imaging to proteins. Biochemical Biophysical Journal. Proteins Proteomics. 1834:2849-2858. DOI: 10.1016/j.bbapap.2013.07.015.

[46] D.M. Hashim, Y.B.C. Man, R. Norakasha, M. Shuhaimi, Y. Salmah, Z.A. Syahariza. (2010). Potential Use of Fourier Transform Infrared Spectroscopy for Differentiation of Bovine and Porcine Gelatins. Food Chemistry. 118:856-860.

[47] M.S. Davies, S.J. Hawkins. (1998). Mucus from marine molluscs. Advances in marine biology. 34:2-71.

[48] J. Gubitosa, V. Rizzi, P. Fini, A. Laurenzana, G. Fibbi, C. Veiga-Villauriz et al. (2020). Biomolecules from Snail Mucus (Helix Aspersa) Conjugated Gold Nanoparticles, Exhibiting Potential Wound Healing and Anti-Inflammatory Activity. Soft Matter. 16: 10876-10888.

[49] S. Morel, S. Arnould, M. Vitou, F. Boudard, C. Guzman, P. Poucheret, F. Fons, S. Rapior. (2018). Antiproliferative and antioxidant activities of wild Boletales mushrooms from France. International Journal of Medical Mushroom. 20(1):13-29. 10.1615/.2018025329

[50] P. Subavathy, S.M.B. Janet. (2020). Molecular characterization of protein and antioxidant capacity of Turbo brunneus R. Cypraea annulus L. and Babylonia spirata L. Int J Pharm Sci & Res. 11(7):3285-93. 10.13040/IJPSR.0975-8232.11(7).3285-93.

[51] A. Pachaiyappan, A. Muthuvel, G. Sadhasivam, V.J.V. Sankar, N. Sridhar, M. Kumar. (2014). In vitro antioxidant activity of different gastropods, bivalves and echinoderm by solvent extraction method. International Journal of Pharmaceutical Science and Research. 5(6), 2539.

[52] R.A. Nazeer, S.Y. Naqash. (2013). In vitro antioxidant activity of two molluscs, Loligo duvauceli Orbigny and Donax cuneatus Linnaeus, by solvent extraction methods. Mediterranean Journal of Nutrition and Metabolism. 6: 17–21. DOI 10.1007/s12349-011-0088-1

[53] P. Sivaperumal, K. Kamala, E. Natarajan, E. Dilipan. (2013). Antimicrobial peptide from crab haemolymph of Ocypoda macrocera (Milne Edwards 1852) with reference to antioxidant: A case study. International Journal Pharmaceutical Science. 5 (2): 719-727.

[54] S. Tabrez, M. Priyadarshini, M. Urooj, S. Shakil, G.M. Ashraf, M.S. Khan et al. (2013). Cancer Chemoprevention by Polyphenols and Their Potential Application as Nanomedicine. Journal of Environmental Science and Health. 31: 67-98. 10.1080/10590501.2013.763577

[55] I. Gulcin, Z. Huyut, M. Elmastas, H.Y. Aboul-Enein. (2010). Radical scavenging and antioxidant activity of tannic acid. Arabian Journal of Chemistry 3:43-53. https://doi.org/10.1016/j.arabjc.2009.12.008

[56] N. Kostadinova, Y. Voynikoy, A. Dolashki, E. Krumova, R. Abrashev, D. Kowalewski, S. Stevanovic, L. Velkova, R. Velikova, P. Dolashka. (2018) Antioxidative screening of fractions from the mucus of garden snail Cornu aspersum. Bulgarian Chemical Communications. 50 (2): 176-183.

[57] B. Nazima, V. Manoharan, S. Prabu. (2015). Oxidative stress induced by cadmium in the plasma, erythrocytes and lymphocytes of rats: Attenuation

by grape seed proanthocyanidins. Human and Environmental Toxicology. 35(4): 428-447.

[58] P. Petsantad, P. Sangtanoo, P. Srimongkol, T. Saisavoey, O. Reamtong, N. Chaitanawisuti, A. Karnchanatat. (2020). The antioxidant potential of peptides obtained from the spotted babylon snail (Babylonia areolata) in treating human colon adenocarcinoma (Caco-2) cells. RSC Advances 10(43): 25746–25757.

[59] S. Purwaningsih, E. Handharyani, W.A.R. Ananda. (2019). Hepatoprotective activity of mangrove snail (Telescopium sp.) extract in sprague dawley rats induced by paracetamol. IOP Conf Series. Earth and Environmental Science. 241: 012022.

[60] B. Lawal, O.K. Shittu, P.C. Ossai, A.N. Asmau, A.M. Ibrahim. (2015). Evaluation of antioxidant activity of giant African snail (Achachatina maginata) haemolymph in CCl4-induced hepatotoxixity in albino rats. Br J Pharm Res. 6:141-54.

[61] Y. Zou, Y. Lu, D. Wei. (2004). Antioxidant activity of a flavonoid-rich extract of Hypericum perforatum L. in vitro. Journal of Agricultural and Food Chemistry. 52: 5032–5039.