

Therapeutic enhancement of *Cornu aspersum* snail slime through medicinal plant diets

Abdelmajid El khayari^{1*}, Elhabib Rour¹, Sidi Mohammed Raoui², Aziz Bouymajane³, Zakya M'hamdi⁴, Mostapha Moutaouikil⁵, Hassan hajjaj⁵, Nabil Mzoudi¹, and Fouzia Rhazi Filali⁴

¹Research Team: Cell Signaling, Department of Biology, Faculty of Sciences, Moulay Ismail University, Meknes, Morocco

²Higher Institute of Nursing Professions and Health Techniques (ISPITS) of Meknes

³Faculty of Sciences and Techniques of Errachidia, Moulay Ismail University, Errachidia, Morocco

⁴Organic chemistry laboratory. Faculty of Science, Moulay-Ismaïl University, Meknes, Morocco

⁵Laboratory of Biotechnology and Bioresources Valorization, Faculty of Sciences, Moulay Ismail University, Meknes, Morocco

Abstract. Natural bioresources are increasingly explored as safer and more sustainable alternatives to synthetic pharmaceuticals, particularly in response to antimicrobial resistance and inflammatory side effects. This study examines the impact of medicinal plant-enriched diets on the bioactive composition and therapeutic potential of *Cornu aspersum* snail slime. Slime from plant-fed snails was compared with that from wild specimens using GC-MS/MS analysis, alongside evaluations of antibacterial, antioxidant, anti-inflammatory, and wound-healing activities through in vitro and in vivo assays. Slime derived from plant-fed snails exhibited significantly enhanced bioactivity, characterized by stronger antibacterial effects, increased antioxidant capacity, and improved anti-inflammatory and wound-healing responses. These findings demonstrate that dietary supplementation with medicinal plants is an effective strategy for enhancing the pharmacological value of *Cornu aspersum* snail slime.

* Abdelmajid El khayari: ab.elkhayari@edu.umi.ac.ma

1. Introduction

The accelerating rise of antibiotic-resistant infections constitutes a major global health challenge, prompting an urgent search for effective and sustainable alternatives to conventional antimicrobial agents [1]. Natural bioresources have emerged as promising candidates, with snail slime gaining particular attention due to its documented antimicrobial, antioxidant, anti-inflammatory, and wound-healing properties [2]. This viscoelastic secretion, produced by specialized epithelial cells, plays a vital role in snail survival by facilitating locomotion, protection, and defense against pathogens. Its therapeutic potential is attributed to a complex mixture of bioactive compounds, including allantoin, hyaluronic acid, proteins, and peptides, whose composition is modulated by biological, environmental, and nutritional factors [2].

Dietary enrichment with medicinal plants has recently been shown to enhance the bioactive quality of animal-derived products through the incorporation of plant secondary metabolites [3-5]. Medicinal species such as *Rosmarinus officinalis*, *Origanum compactum*, and *Thymus zygis* subsp. are well recognized for their antibacterial, antioxidant, and anti-inflammatory activities, making them suitable candidates for nutritional modulation in snail farming [3-5]. *Cornu aspersum*, a species widely exploited in food, pharmaceutical, and cosmetic industries, represents a valuable model for such investigations [6]. However, the bioactive composition and therapeutic efficacy of Moroccan *Cornu aspersum* slime, particularly under plant-enriched dietary conditions, remain largely unexplored. The present study addresses this gap by comparing the chemical composition and antibacterial, antioxidant, anti-inflammatory, and wound-healing activities of slime obtained from wild snails and from snails fed medicinal plant-supplemented diets.

2 Materials and methods

For four months, groups of *Cornu aspersum* snails (N = 360) were raised under controlled conditions, with each group (N = 4) receiving a different diet. Once the snails reached adulthood, their mucus was extracted and compared to that of wild snails. Gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) was employed to characterize snail slimes, as described by Sallam et al [11].

Antibacterial activity of mucus, *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Listeria monocytogenes* bacteria were used, and the disk diffusion method was used with Mueller Hinton agar (MHA) culture medium. We then determined the minimum inhibitory concentration and minimum bactericidal concentration of each saliva sample.

Antioxidant activity, 0.5 mL of each saliva sample was mixed with 1.5 mL of a methanolic solution containing DPPH (1,1-diphenyl-2-picrylhydrazyl), and the mixture was incubated in the dark at room temperature for 30 minutes. The absorbance was then measured at 517 nm using a spectrophotometer (UV-1601, Shimadzu). The results were quantified as percentage of antiradical activity (%) \pm standard deviation (SD), and the mean inhibitory concentration at 50% (IC₅₀ \pm SD) was calculated using the following formula:

$$I \% = (\text{Abs control} - \text{Abs sample}) * 100 / (\text{Abs control})$$

The anti-inflammatory activity and healing capacity, 30 mice were used. After anesthesia, standard 0.5 cm² square wounds were made on the backs of the mice. The healing efficacy of five different types of snail mucus was evaluated on five separate groups of mice, each consisting of five individuals. A sixth group, the control group, consisted of untreated mice to which no mucus was applied. The groups were organized as follows:

Group A: mice treated with snail slime fed exclusively on a flour-based diet. **Group B:** mice treated with snail slime fed on a flour-based diet enriched with rosemary (*Rosmarinus officinalis*). **Group C:** mice treated with snail slime fed a flour-based diet enriched with oregano (*Origanum compactum*). **Group D:** mice treated with snail slime from snails fed exclusively on a flour-based diet. **Group E:** mice treated with wild snail slime. **Control group:** untreated group consisting of mice that did not receive snail slime.

The induced wounds in the mice were treated daily through the topical application of snail mucus extracts. The treatment started 24 hours post-lesion induction and continued until complete wound closure. The evaluation of the mucus's bioactive efficacy in promoting wound healing was conducted by measuring the percentage of wound contraction.

Wound contraction percentage= (wound size at time T0 - wound size at time Ti) / Size of wound at time T0

After confirmation of euthanasia, the affected area on the animals' backs was surgically excised. The tissue samples taken were fixed in formalin and then embedded in paraffin using standard laboratory techniques. Serial sections 5 micrometers thick were made and stained with hematoxylin and eosin to analyze the inflammatory response.

Anti-inflammatory activity was determined using histological sections and classified as follows: - (no inflammatory reaction); + (inflammatory cells representing less than 10% of the number of cells observed in the wound area); ++ (inflammatory cells representing between 10% and 50% of the number of cells observed in the wound area); and +++ (inflammatory cells representing more than 50% of the number of cells observed in the wound area).

3 Results

3.1 GC-MS/MS analysis of *Cornu aspersum* snail slimes

The quantity diversity of chemical compounds identified in the slime of *Cornu aspersum* snail exhibited variations depending on the type of food provided to the snail, Table 1 show most abundant and differentially abundant compounds between the tested groups.

Table 1. Chemical composition of *Cornu aspersum* snail slime.

Name of compound	Molecular Weight	Retention Time (RT)	Area % per Group				
			A	B	C	D	E
1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	334	17.16	-	-	9.65	-	-
1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	334	17.16	-	-	9.65	-	-
.psi... psi. -Carotene,3,3',4,4'-tetrahydro-1,1',2,2'-tetrahydro-1,1'-dimethTrimethyloxy-2,2'-dioxo-	624	22.63	2.51	1.9	6.56	-	-
.psi... psi.-Carotene,1,1',2,2'-tetrahydro-1,1'-dimethoxy-	600	22.71	1.71	-	-	-	-
1,3-Dioxolane	284	23.03	-	-	-	14.11	5.46
3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	487	23.15	-	-	-	10.39	-
Salicylic acid, 2TMSderivative	282	24.37	-	-	-	17.76	12.6
9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	444	26.44	12.46	7.35	12.79	18.63	2.63
1,3-Dioxolane, 2-butyl-	130	26.45	-	-	0.76	-	-
.psi... psi. -Carotene,3,4-didehydro-1,1',2,2'-tetrahydro-1'-hydroxy-1-methoxy-	584	28.09	1.03	-	-	-	-
1,3-Dioxolane, 2-ethyl-	102	29.29	2.06	-	-	-	-
Prost-13-en-1-oic acid,9-(methoxyimino)-11,15-bis[(trimethylsilyloxy)-,trimethylsilyl ester, (8.xi.,12.xi.)-	599	29.72	-	2.2	24.64	-	8.94

In the first group (Figure 1A) consisting of snails that were exclusively fed on flour, 16 chemical compounds were identified. The highest concentration was observed in compound 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis-, representing a 12.46% of total composition.

In the second group (Figure 1B) of snails fed with flour enriched with *Rosmarinus officinalis*, 20 different compounds were detected. Among these, the highest proportion was also the compound 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis accounting for 7.35% of the total.

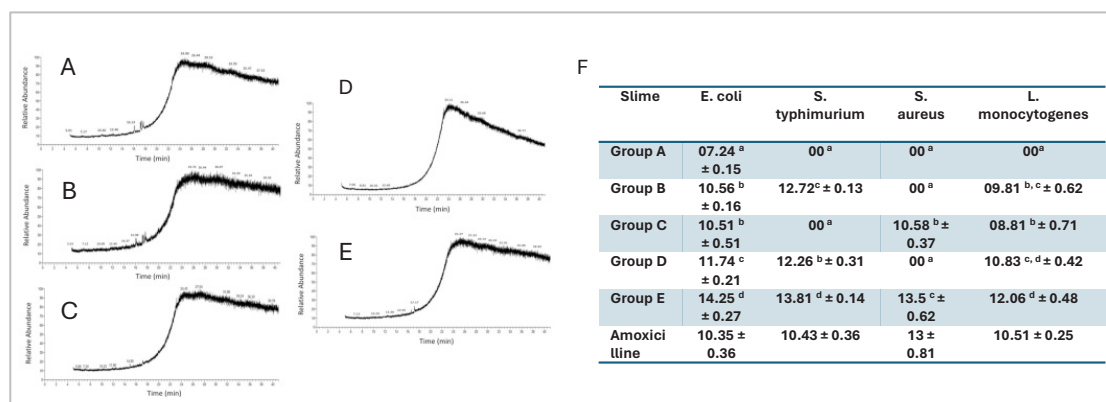


Fig. 1. GC-MS/MS analysis of *Cornu aspersum* snail slimes and Antibacterial activity against pathogenic bacteria. (A-E) Chromatograms obtained from different groups (A) Group A. (B)

Group B. (C) Group C, (D) Group D, (E) Group E. (F) analysis of inhibition zone of *Cornu aspersum* slimes against pathogenic bacteria (mm), Groups sharing the same letter are statistically not different.

In the third group (Figure 1C), where snails nourished with flour enriched with *Origanum compactum*, 18 unique compounds identified. The compound that exhibited the highest percentage was Prost-13-en-1-oic acid, 9-(methoxyimino)-11,15-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester, (8.xi.,12.xi.)-, accounting for 24.64% of the composition.

Group D, which consists of snails consuming flour mixed with *Thymus zygis subsp*, presented 21 different compounds in their slime (Figure 1D). The highest percentage was associated with 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis-, at a concentration of 18.63%.

Finally, the analysis of wild snail slime (Figure 1E) identified 22 distinct compounds, with the highest percentage recorded for Salicylic acid, 2TMS derivative (12.60%).

3.2 Antibacterial activity of *Cornu aspersum* snail slimes

Interestingly, as showed in Figure 1F, it is evident that the *Cornu aspersum* snail slime in all samples displayed antibacterial activity against *Escherichia coli*. The most substantial inhibition zone observed in Group E, which consisted of wild snails, with an impressive area of 14.25 mm.

Group A demonstrated antibacterial activity only against *Escherichia coli* and showed no effect on the other bacterial strains.. In Group B, antibacterial activity against *Escherichia coli*, *Salmonella typhimurium* and *Listeria monocytogenes*, but *Staphylococcus aureus* was not affected. Group C exhibited antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes*, while it had no effect on *Salmonella typhimurium*. Remarkably, Group D showed significant antibacterial activity against three different bacteria: *Escherichia coli*, *Salmonella typhimurium* and *Listeria monocytogenes*. However, no effect was observed against *Staphylococcus aureus*. Group E displayed antibacterial activity against all the tested strains.

It is important to note that the slime with antibacterial effects against the tested bacteria also displayed a bactericidal action, as outlined in Table 3.

Table 2. Minimum inhibitory concentration and minimum bactericidal concentration of *Cornu aspersum* slimes.

		Group A	Group B	Group C	Group D	Group E
<i>Escherichia coli</i>	MIC (µg/ml)	190	150	120	140	230
	MBC (µg/ml)	400	400	400	300	300
	MBC/MIC	2.1	2.6	3.33	2.14	1.3
	Effect	Bactericidal	Bactericidal	Bactericidal	Bactericidal	Bactericidal
<i>Salmonella typhimurium</i>	MIC (µg/ml)	-	140	-	120	130
	MBC (µg/ml)	-	500	-	400	400
	MBC/MIC	-	3.57	-	3.33	3.07
	Effect	-	Bactericidal	-	Bactericidal	Bactericidal
<i>Staphylococcus aureus</i>	MIC (µg/ml)	-	-	120	-	130
	MBC (µg/ml)	-	-	300	-	400
	MBC/MIC	-	-	2.5	-	3.07
	Effect	-	-	Bactericidal	-	Bactericidal
<i>Listeria monocytogenes</i>	MIC (µg/ml)	-	130	120	110	130
	MBC (µg/ml)	-	400	400	400	300
	MBC/MIC	-	3.07	3.33	3.63	2.3
	Effect	-	Bactericidal	Bactericidal	Bactericidal	Bactericidal

1.2 Antioxidant activity of *Cornu aspersum* slimes

The antioxidant activity of *Cornu aspersum* slimes was determined using a DPPH assay. Based on IC₅₀ values, Group E exhibited the highest radical scavenging activity, followed by Group B, Group D, Group C and Group A (Table 3) (Figure 2).

Table 3. IC₅₀ concentration of *Cornu aspersum* snail slimes. Means with different superscripts are significantly different (p < 0.05)

Snail slime	Group A	Group B	Group C	Group D	Group E	Ascorbic acid
IC ₅₀ (mg/mL)	4.83 ^a ± 0.04	4.52 ^c ± 0.02	4.81 ^a ± 0.06	4.67 ^b ± 0.03	3.62 ^d ± 0.08	0.929 ^e ± 0.002

1.3 Wound healing properties of *Cryptomphalus aspersus* snail slime

The progression of wound healing in mice was meticulously monitored throughout the experiment following the application of various samples of snail slimes from *Cryptomphalus aspersus*. Notably, all wounds in treated groups healed completely within 21 days, while the control group required 28 days for full recovery. By the fourteenth day, a significant acceleration in wound healing was observed in the treated groups compared to the control.

Specifically, wounds in the group Group C, as well as Group E, exhibited noticeable healing improvements during the first week of treatment compared to other groups, as illustrated in the Figure 2.

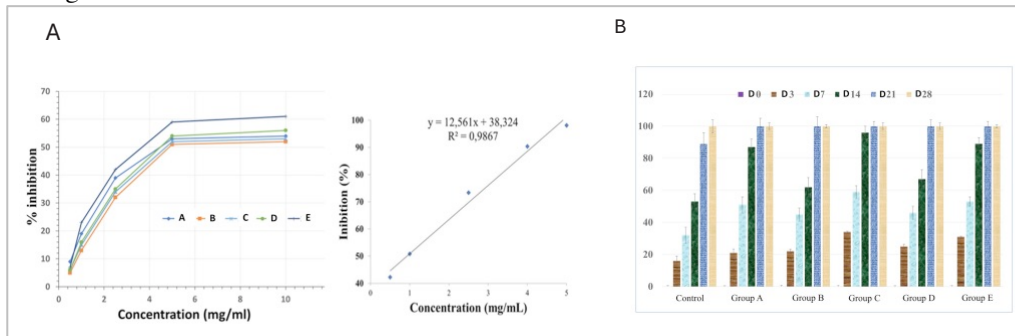


Fig. 2. Antioxidant and wound healing activity of *Cornu aspersum* snail slimes. (A) Free radical scavenging activity of *Cornu aspersum* snail slimes and calibration curve of ascorbic acid (B) Percentage contraction of wounds treated with *Cornu aspersum* snail slime

1.4 Anti-inflammatory effect of *Cryptomphalus aspersus* snail slime

Three days post-injury, significant inflammation was observed in all samples, with inflammatory cells comprising more than 50% of the affected area. This was characterized by prominent neutrophil infiltration along with widespread lymphocyte presence (figure 4) (table 5).

By the seventh day, persistent neutrophil infiltration indicated ongoing wound inflammation. This inflammation remained intense in the control group and in the group treated with slimes from snails fed with *Rosmarinus officinalis*-enriched feed (Group B), while it was moderate in the group treated with slimes from snails fed with plain flour (Group A). In contrast, the other groups displayed only mild inflammation (figure 4) (table 5).

At the 14-day mark, inflammation remained pronounced in the control group and slightly reduced in the group treated with *Rosmarinus officinalis*-enriched snail slimes (Group B). However, no inflammation was evident in the other treated groups (figure 4) (table 5). By 21 days, inflammatory cells persisted in the control group, constituting 10–50% of the skin cells, but no inflammatory cells were detected in the groups treated with various snail slimes samples. Finally, at 28 days, all groups, including the control, showed no signs of cellular inflammation (figure 4) (table 5).

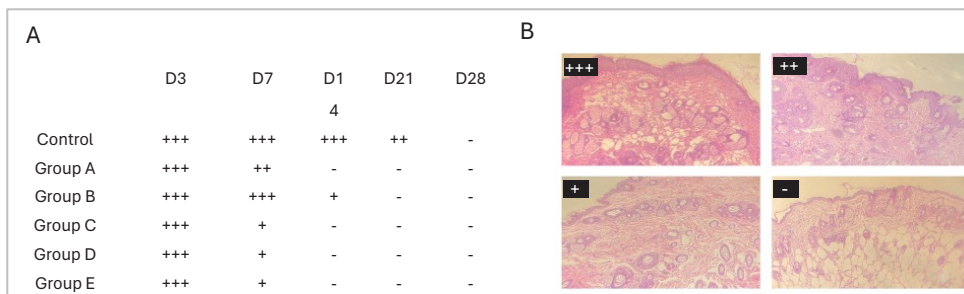


Fig. 3. Anti-inflammatory activity of *Cornu aspersum* snail slimes. (A) The intensity of the inflammatory response across different groups (B) Haematoxylin-eosin staining images of mouse skin

tissue after treatment with *Cornu aspersum* snail slime showing the evolution of inflammatory intensity. - (No inflammatory reaction); + (Less than 10% inflammatory cells; ++ (Inflammatory cells representing between 10 and 50%; and +++ (Inflammatory cells representing more than 50%).

Discussion

The snail slime ingredients have garnered significant scientific attention among scholars. Snail sucus has been found to be applicable in various fields such as medicine, pharmaceuticals, cosmetics, and other sectors. In the present study, technology plays a prominent role as GC-MS/MS technology was used to shed light the chemical profiles of slime derived from wild *Cornu aspersum* snails. These snails were obtained from Moroccan environments, and they were farmed *Cornu aspersum* snails fed diets enriched with medicinal plants. The current study's findings imply that the diet plays a critical role in enhancing the bioactive compound profile of snails.

Moreover, the antibacterial efficacy of *Cornu aspersum* snail slime was pronounced across all experimental groups, particularly against *Escherichia coli*. In this regard, a complete inhibition was observed with a notable inhibition zone of 14.25 mm. This supasses since surpasses the 11.63 mm inhibition which makes the findings of this research inconsistent with previous research [8], underscoring the advanced antibacterial potential of snail slime. The heightened activity in snails fed with medicinal plants and wild snails consuming diverse herbivorous diets can be attributed to bioactive compounds such as 1,3-Dioxolane [9], 3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone [10], Salicylic acid, 2TMS derivative [9,11], and 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester [12]. Accordingly, the results of this research accentuate the synergistic ability of medicinal plants in improving snail slime's antibacterial.

Antioxidant assessments indicated significant activity across all snail slime samples, with enhanced capacities in groups nourished with medicinal plants like *Rosmarinus officinalis*, *Origanum compactum*, and *Thymus zygis subsp.* The presence of compounds such as psi., .psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy [13], 1,3-Dioxolane [9], and 1,2-Cinnolinedicarboxylic acid,1,2,3,5,6,7,8,8a-octahydro-4-trimethylsilyloxy-, diethyl ester [14] reinforces the potential of dietary interventions in boosting antioxidant properties.

Moreover, *Cryptomphalus aspersus* slimes, according to vivi studies, have been found to be effieicent in speeding up wound healing and modulating inflammatory responses. These Medicinal plant-enriched diets further amplified these effects. Furthermore, the findings of this study corroborated with other research findings [15], implying that histological analyses outlined reduced neutrophil infiltration, emphasizing the slime's role in mitigating excessive inflammation and fostering tissue regeneration.

Conclusion

This study underscores the synergistic benefits of *Cornu aspersum* snail slime combined with medicinal plant diets in enhancing antibacterial, antioxidant, anti-inflammatory, and wound-healing properties. These findings illuminate promising pathways for the development of innovative therapeutic agents targeting microbial infections, oxidative stress, and wound healing, presenting significant potential for advancing health and wellness solutions.

Conflict of interest

The authors declare no conflict of interest.

Adherence to Ethical Standards in Animal Research

All animal experiments conducted for this research were performed in strict compliance with the applicable legal and institutional guidelines. The study protocols were reviewed and approved by the institutional ethics committee, ensuring that all procedures were carried out responsibly and ethically. The welfare of the animals was prioritized, with measures taken to minimize discomfort and adhere to the highest standards of care.

Funding

This research has been conducted in the absence of any funding.

Data Availability:

Full list of chemical compounds from the GS-MS analysis is available from the corresponding author upon request

References

1. Anand U, Jacobo-Herrera N, Altemimi A, Lakhssassi N (2019). A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. *Metabolites*. 9 (11): 1–13. doi.org/10.3390/metabo9110258.
2. Chinaka NC, Chuku LC, George G, Oraezu C, Umahi G, Orinya OF (2021). Snail Slime: Evaluation of Anti-Inflammatory, Phytochemical and Antioxidant Properties. *Journal of Complementary and Alternative Medical Research*. 13 (1): 8–13. <https://doi.org/10.9734/jocamr/2021/v13i130214>.
3. Bouhdid S, SkaliS N, Idaomar M, Zhiri A, Baudoux D, Amensour M, Abrini J (2008). Antibacterial and Antioxidant Activities of Origanum Compactum Essential Oil. *African Journal of Biotechnology*. 7 (10): 1563–70.
4. Rota MC, Antonio H, Rosa MM, Jose AS, María JJ(2008). Antimicrobial Activity and Chemical Composition of Thymus Vulgaris, Thymus Zygis and Thymus Hyemalis Essential Oils. *Food Control*. 19 (7): 681–87. <https://doi.org/10.1016/j.foodcont.2007.07.007>.
5. Nieto G, Gaspar R, Julián C (2018). Antioxidant and Antimicrobial Properties of Rosemary (*Rosmarinus Officinalis*, L.): A Review. *Medicines*. 5 (3): 98. <https://doi.org/10.3390/medicines5030098>.
6. Cilia G, Fratini F (2018). Antimicrobial properties of terrestrial snail and slug slime. *Journal of Complementary and Integrative Medicine*. 15(3), 2017-0168. <https://doi.org/10.1515/jcim-2017-0168>
7. Sallam AA, El-Massry SA, Nasr IN (2009). Chemical Analysis of Slime from Certain Land Snails under Egyptian Conditions. *Archives of Phytopathology and Plant Protection*. 42 (9): 874–81. <https://doi.org/10.1080/03235400701494448>.
8. Pitt S J, Graham MA, Dedi CG, Taylor-Harris PM, Gunn A (2015). Antimicrobial properties of slime from the brown garden snail *Helix aspersa*. *British journal of biomedical science*. 72(4), 174-181. <https://doi.org/10.1080/09674845.2015.11665749>

9. Nobre PC, Borges EL, Silva CM, Casaril AM, Martinez DM, Lenardão EJ, Perin G (2014). Organochalcogen compounds from glycerol: Synthesis of new antioxidants. *Bioorganic and Medicinal Chemistry*. 22(21), 6242–6249. <https://doi.org/10.1016/j.bmc.2014.08.018>
10. Harun O, Aiza NAA, Nor SMA, Nurfarah FMK, Siti ZMS (2020). Antimicrobial Efficacy, Antioxidant Profile and Nine Alternative Active Constituents from Petroleum Ether and Ethyl Acetate Extract of *Entada Spiralis*.” *Malaysian Journal of Analytical Sciences*. 24 (5): 707–18.
11. El-Esawi MA, Hosam O, Elansary NA, El-Shanhorey AME, Abdel-Hamid HMA, Mohamed. S E (2017). Salicylic Acid-Regulated Antioxidant Mechanisms and Gene Expression Enhance Rosemary Performance under Saline Conditions. *Frontiers in Physiology*. 8 : 1–14. <https://doi.org/10.3389/fphys.2017.00716>.
12. Govindappa M, Prathap S, Vinay V (2014). Channabasava, R. Chemical composition of methanol extract of endophytic fungi, *Alternaria* sp. of *Tebebuia argentea* and their antimicrobial and antioxidant activity. *Int J Biol Pharm Res*. 5(11), 861-869.
13. Khan S, Kaur H, Jhamta R (2019). Evaluation of antioxidant potential and phytochemical characterization using GC-MS analysis of bioactive compounds of *Achillea filipendulina* (L.) leaves. *Journal of Pharmacognosy and Phytochemistry*. 8(3), 258-265
14. Salem MZM, Hosam O, Elansary AAE, Aleš Z (2016). In Vitro Bioactivity and Antimicrobial Activity of *Picea abies* and *Larix decidua* wood and bark extracts. *BioResources*. 11 (4): 9421–37.
15. Ahmad TB, Liu L, Kotiw M, Benkendorff K (2018). Review of anti-inflammatory, immune-modulatory and wound healing properties of molluscs. In *Journal of Ethnopharmacology*. Vol. 210, <https://doi.org/10.1016/j.jep.2017.08.008>