ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Exploring the Chemical Composition, Antioxidant, and Antibacterial Properties of *Helix aspersa* Müller Flesh Crude Extract: A Comprehensive Investigation

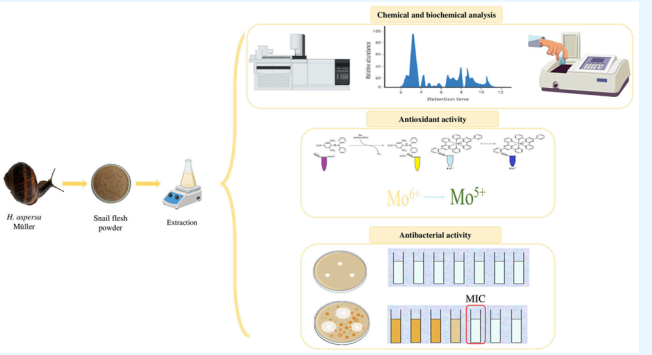
Marouane Aouji,* Malak Zirari, Hamada Imtara,* Amine Rkhaila, Bouchra Bouhaddioui, Ramzi A. Mothana, Omar M. Noman, Mahmoud Tarayrah, and Rachid Bengueddour

Cite This: *ACS Omega* 2024, 9, 34754−34764

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Snail flesh is a highly nutritious and easily digestible food commonly integrated into the human diet. In this study, snails belonging to the *Helix aspersa* Müller species were used to determine their chemical composition and evaluate the antioxidant and antibacterial activities of their flesh using successive maceration extractions with three solvents of different polarities. Biomolecules were analyzed spectrophotometrically, and their chemical compositions were determined by using gas chromatography coupled with mass spectroscopy. The antioxidant activity was assessed using three tests: DPPH, iron-reducing power test, and total antioxidant activity. The ethanol extract was found to be the most effective, with a high yield and high biomolecule content compared with other extracts. The extracts showed a significant amount of antioxidants, ranging from 3.14 to 7.04 mg AAE g^{-1} of dry matter, according to the total antioxidant activity assay. The DPPH scavenging capacity showed a reduction of the radical, with inhibitory concentrations ranging from 507.07 to 829.49 μg mL^{-1}. In contrast, the iron-reducing power ranged from 67.98 to 424.74 μg mL^{-1}. All of the strains studied responded favorably to the antimicrobial effects of *H. aspersa* extracts, with a zone of inhibition ranging from 8.48 to 15.53 mm. Additionally, at approximately 15 mg mL^{-1}, the ethanolic extract had the lowest minimum inhibitory concentration against *Pseudomonas aeruginosa*. *H. aspersa* Müller flesh is rich in biomolecules with antioxidant and antibacterial activities, which could justify its use as a natural product and in therapeutic applications in the food industry.



1. INTRODUCTION

Morocco has a diverse range of biological resources, including mollusks. Mollusks, including a diverse group of organisms, are the second-largest phylum within the animal world, with a remarkable number of species exceeding 50,000. These organisms fulfill a significant ecological function and provide substantial nutritional value.[1] Mollusks are a heterogeneous group with a great diversity of forms. Furthermore, they are a rich and varied source of bioactive compounds with diverse structures. These compounds are of great interest to the pharmaceutical and biomedical industries because of their beneficial properties.[2]

Mollusks contain many active compounds such as peptides, lipids, steroids, terpenes, nitrogenous chemicals, sterols, alkaloids, fatty acids, and their derivatives,[3−7] which can be used to develop dietary supplements, functional foods, nutraceuticals, and medicines.[5] In addition, highly effective mechanisms that are part of the innate immune system have been developed.[8] Mollusks are a good source of biologically active secondary metabolites.[9] The use of mollusks as a source of medicines has great potential for the development of new therapies for a wide range of diseases, such as ziconotide (Prialt), a peptide found in the marine cone snail, which was the first marine peptide approved by the FDA in 2004 as an analgesic,[10] the sea hare's Adcetris for the treatment of cancer.[3]

Natural substances that are important in medicine can be discovered by studying the physical and chemical traits of mollusks. These substances have potential for medical use, are relevant to the pharmaceutical industry, and have many applications. They also contain chemicals that are important in pharmacology.

Received: April 27, 2024
Revised: July 21, 2024
Accepted: July 23, 2024
Published: August 2, 2024

Mollusks have again become popular because they enjoy the taste and the flavor. The demand for snails has led to shortages, and snail farming has become an important source. Currently, the garden snail *Helix aspersa* Müller is the most popular worldwide[11] and is the preferred species for snail farming because it is well suited for commercial production. This is due to its ability to reproduce quickly, fast life cycle, taste, versatility, and ability to adapt to different regions and environments.[12]

Antioxidants are a class of substances capable of neutralizing free radicals, thereby preventing diseases related to oxidative stress. Among natural antioxidants are α-tocopherol, ascorbic acid, and phenolic compounds.[13] They play a crucial role in inhibiting and eliminating free radicals, thereby protecting the human body against infections and degenerative diseases. Antioxidants can delay or prevent oxidative damage and, therefore, can be useful as therapeutic products or food additives.

Public health is threatened by antibiotic resistance.[14] Therefore, it is critical to develop new medications to combat this resistance. The antibacterial, cytotoxic, anticancer, anti-inflammatory, antileukemic, antineoplastic, antioxidant, and antiviral properties of these bioactive substances have been previously studied.[15] Eating snails has several health benefits, including the prevention of certain diseases.[16,17]

According to Zhang et al.,[18] the type and polarity of the solvent used can affect the quality, quantity, extraction rate, and biological activity of an extract. Kang and Lee[19] also found that antioxidant activity depends on the type and polarity of the extraction solvent.

Bibliographic research has shown that it is important to study terrestrial gastropods; there have been few studies on the flesh of the snail of *H. aspersa* Müller. Our study focuses on the exploration of the chemical compounds and biological activities of the flesh extracts of *H. aspersa* Müller and fills in the gaps; in particular, we consider the data on the chemical composition of the flesh of *H. aspersa* Müller. We report here the study of the three extracts using gas chromatography coupled to mass spectrometry (GC−MS) and quantify the biomolecules to elucidate their compositions. Second, this study focuses specifically on antioxidant and antibacterial activities, thus providing valuable information for health and medicine.

2. RESULTS AND DISCUSSION

2.1. Extraction Yield. The key factors in the extraction process are the solvent used and its polarity.[20] Figure 1 shows the extraction yields of *H. aspersa* Müller snail powder. It is worth noting that the recorded values vary significantly. The ethanol extract yield is 5.50%, and the chloroformic extract yield is 4.55%, these two yields are greater than the ethyl acetate yield (3.60%). The polarity of the extraction solvent enhances the efficiency.

2.2. Chemical Composition. The GC−MS analysis of different extracts from the flesh of *H. aspersa* Müller revealed 45 potential bioactive compounds, including fatty acids, sterols, and other metabolites, that could contribute to the medicinal quality of the animal, which were identified by correlating their retention time (RT) and mass spectra fragmentation patterns to those of known compounds described in the National Institute of Standards and Technology (NISTII) library (Table 1 and Figure 2).

Hexadecanoic acid, (*Z*,*Z*) 9,12-octadecadienoic acid, *cis*-5,8,11,14-eicosatetraenoic acid, heptadecanoic acid, clionasterol, cholest-4-en-3-one, and cholesterol were considered the most abundant. (*Z*,*Z*) 9,12-Octadecadienoic acid and hexadecanoic acid were the dominant ones in the chloroform extract. The predominant volatile compounds in the ethyl acetate extract were (*Z*,*Z*) 9,12-octadecadienoic acid and cholest-4-en-3-one (18.41 and 12.58%, respectively). The ethanol extract revealed the most diverse profile with the presence of 32 identified compounds, the highest content among which was established for (*Z*,*Z*) 9,12-octadecadienoic acid, at 66.83%.

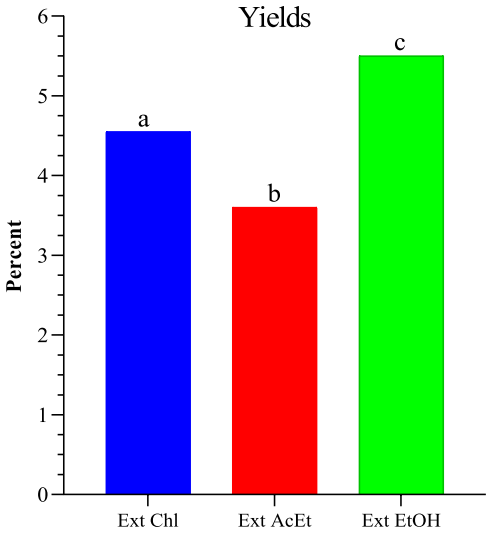


Figure 1. Histogram of the extraction yields. **Ex Chl**: chloroform extract, **Ex AcEt**: ethyl acetate extract, and **Ex EtOH**: ethanol extract. The significant difference ($p < 0.05$) between the different extracts is illustrated by the letters a, b, and c.

Among the saturated fatty acids, octadecanoic acid stands out, with a range of 2.02 to 8.92%, followed by hexadecanoic acid, with a range of 1.68 to 4.14%. Among the polyunsaturated fatty acids, (*Z*,*Z*) 9,12-octadecadienoic acid had the highest percentage, with a range of 18.41 to 66.83%, and *cis*-5,8,11,14-eicosatetraenoic acid ranged from 2.60 to 12.13%.

The bioactive substances discovered by GC−MS analysis are reported in Table 2 and demonstrate various important biological activities for this investigation. The stated biological activities are based on Dr. Jim Duke of the Agricultural Research Service/USDA's phytochemical and ethnobotanical databases.[21]

2.3. Biochemical Analyses. Initially, our objective was to quantify the bioactive components in the flesh, which may have biological properties.

Protein, polyphenols, total sugars, and reducing sugars are measured in milligram equivalents of bovine albumin serum, gallic acid, and glucose per gram of dry matter (mg of BSA, GA, and GE g^{-1} of DM). The linear regression equation of the standard calibration curves is used to calculate the results. Table 3 indicates that all extracts contain organic molecules whose concentrations increase with the polarity of the extraction solvent. The results revealed that solvent polarity had a significant impact on total biomolecule content.

The protein content of the ethanolic extract (3.74 ± 0.18 mg g^{-1} MS) is greater than that of the ethyl acetate and chloroform extracts (1.62 ± 0.09 and 1.34 ± 0.07 mg g^{-1} DM, respectively). These findings are compared to those of Gomot,[22] who discovered that protein levels varied depending on the type of snail examined.

https://doi.org/10.1021/acsomega.4c04042
ACS Omega 2024, 9, 34754−34764

Table 1. GC−MS Analysis of Crude Extracts of *H. aspersa* Müller Flesh

compounds	chemical class					
	Ex Chl		Ex AcEt		Ex EtOH	
	RT (min)	area %	RT (min)	area %	RT (min)	area %
tricosane	039.96	00.24				
docosane	046.13	00.24				
nonadecane	047.68	00.27				
cyclohexadecane	052.38	00.65			52.11	00.21
hexadecanoic acid	055.44	05.68	060.81	06.14	54.97	01.68
pentadecanoic acid, ethyl ester	066.24	01.73				
(*Z*,*Z*) 9,12-octadecadienoic acid	082.56	25.08	081.34	18.41	42.67	66.83
oxirane, [(hexadecyloxy)methyl	060.92	02.23				
octadecanoic acid	061.45	04.99	059.91	08.92	60.83	02.02
heptadecanoic acid	060.52	09.19	054.96	04.74	55.59	00.09
octadecane	062.39	00.05			66.03	00.16
cis-5,8,11,14-eicosatetraenoic acid	064.86	02.60	060.15	12.13	60.16	03.73
1,2-*O*-[2′-hydroxyoctadecyl]glycerol	065.79	02.10			70.07	00.03
hentriacontane	066.63	00.15				
(*E*)-butenedioic acid	065.93	01.08			68.74	00.09
cis-5,8,11,14,17-eicosapentaenoic acid	070.12	00.82				
(*Z*)-9-octadecenamide	071.32	01.04	075.05	02.70	75.12	00.62
elaidamide	074.68	00.81	041.82	03.62	80.79	00.10
cholesta-2,4-diene	103.45	01.53				
(3*β*)-cholest-5-en-3-ol	060.77	09.18	076.99	03.78	77.08	01.33
cholest-4-en-3-one	084.61	07.94	083.48	12.58	83.59	02.51
γ-sitostenone	085.78	01.46	082.00	00.89	86.86	00.06
clionasterol	076.08	08.74	103.44	03.85		
9,19-cyclolanost-24-en-3-ol	076.89	00.78				
2-pentacosanone			060.25	03.05		
cholesta-3,5-diene			069.64	03.40		
cholesta-4,6-dien-3-one			083.94	00.26		
methyl 9,10-octadecadienoate					58.41	00.02
13-octadecenoic acid					58.64	00.03
16-methylheptadecanoic acid					59.46	00.03
octanoic acid					60.27	01.15
methyl dehydroabietate					64.78	00.18
dehydroabietic acid					67.39	00.16
abietic acid					68.42	00.03
(3*Z*,13*E*)-2-methyloctadeca-3,13-dien-1-ol					65.81	00.08
8,11,14-eicosatrienoic acid					81.44	04.93
psi.,.psi,-carotene, 1,1′,2,2′-tétrahydro-1,1′-diméthoxy					78.33	00.06
dihydrobrassicasterol					79.12	00.05
vitamin E					81.74	00.01
cholesta-3,5-dien-7-one					82.84	00.07
(3*β*)-ergost-5-en-3-ol					83.27	00.09
β-sitosterol					84.78	00.05
ergosta-4,6,8(14),22-tetraen-3-one					85.03	00.02
4-campestene-3-one					85.40	00.06
docosanoic acid					94.46	01.89
fatty acids		51.17		50.34		81.36
alkanes		01.60		00.00		00.37
ketones		09.40		16.78		02.72
aAmides		01.85		06.32		00.72
sterols		10.27		07.25		00.14
fatty alcohols		12.06		03.78		01.44
total identified		88.58		84.47		84.15
not identified		11.42		15.53		15.85

The values of the total sugar analysis show a significant variance in the various extracts. The ethyl extract has the highest level (2.72 ± 0.11 mg g^{-1} DM), followed by the ethyl acetate extract. Total carbohydrate levels have been reported.[23]

In addition, we notice that the content of reducing sugars is higher in the ethanolic extract (2.07 ± 0.16 mg g^{-1} of DM), while ethyl acetate and chloroform extracts showed the lowest

https://doi.org/10.1021/acsomega.4c04042
ACS Omega 2024, 9, 34754−34764

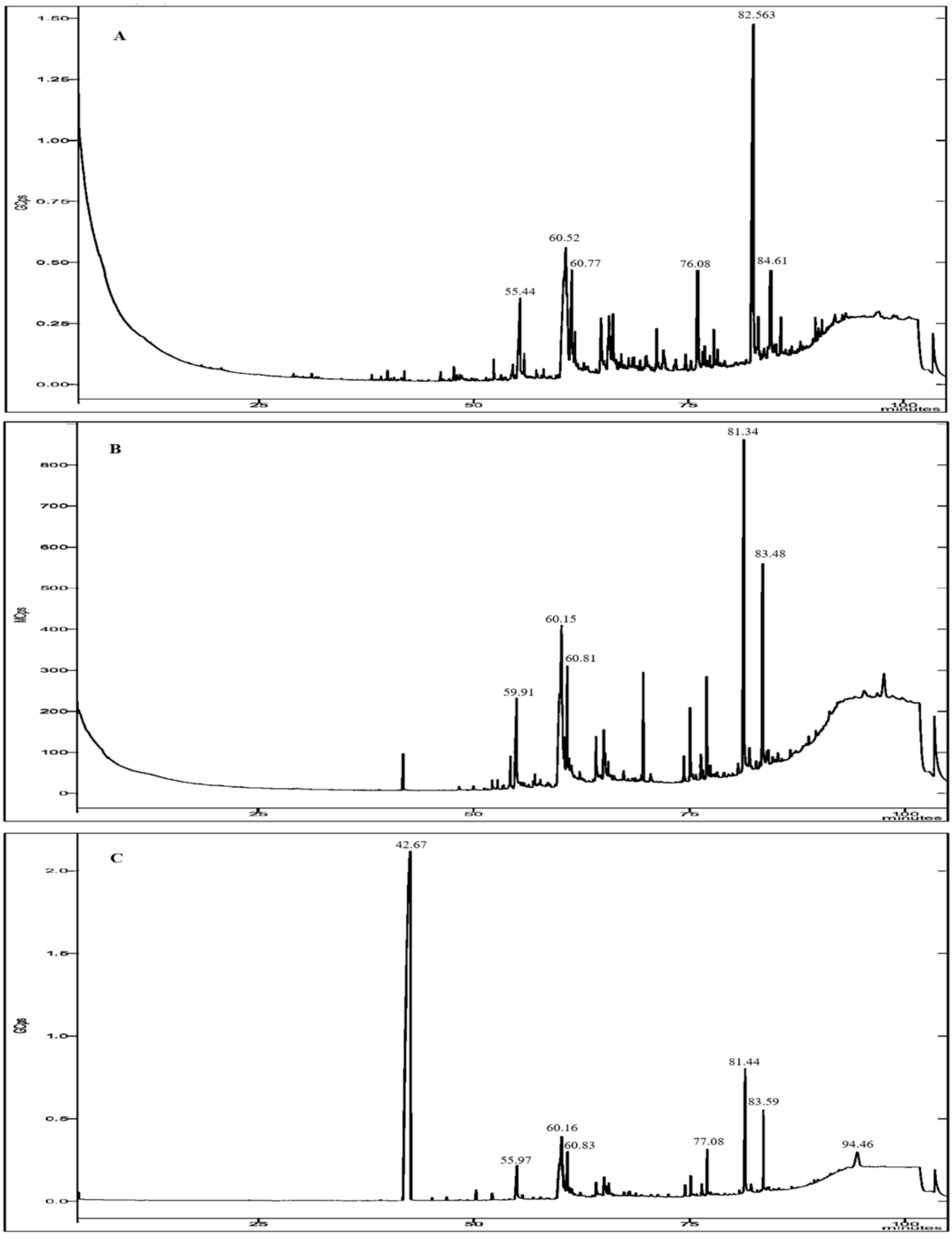


Figure 2. GC−MS chromatogram for the crude extract of the flesh of *H. aspersa* Müller. (A) Chloroform extract, (B) ethyl acetate extract, and (C) ethanolic extract.

levels. However, Gomot[22] reported total monosaccharide levels in *H. aspersa* maxima.

The ethanol extract (1.26 ± 0.08, 0.32 ± 0.04, and 0.16 ± 0.03 mg GAE g^{-1} of DM) had the most polyphenols, followed by the ethyl acetate and chloroform extracts. The results show that the total polyphenol content increases with the polarity of the extracting solvent, and the difference between the three extracts is statistically significant ($p < 0.05$). Ghedadba et al.[24] stated that the high content of phenolic compounds is related to the high solubility of phenols in polar solvents. A recent study reported 0.132 mg mL^{-1} of polyphenols.[25]

The results show that the average amount of flavonoids in the extracts of *H. aspersa* Müller made with chloroform, ethyl acetate, and ethanol is 0.04 ± 0.00, 0.06 ± 0.00, and 0.13 ± 0.00 mg QE g^{-1} of DM, respectively. The difference in flavonoid content between the three extracts is statistically significant ($p < 0.05$).

2.4. Determination of Antioxidant Activity. Various methods were employed to assess antioxidant activity designed to have varying levels of activity.

2.4.1. Total Antioxidant Capacity (TAC). A standard ascorbic acid curve was established to evaluate the total antioxidant capacity. The results reveal that the three extracts showed

https://doi.org/10.1021/acsomega.4c04042
ACS Omega 2024, 9, 34754−34764

Table 2. Activities of Some Identified Compounds in the Extract of *H. aspersa* Müller

compounds name	Activities
hexadecanoic acid	antioxidant, flavour, antifibrinolytic, hypocholesterolemic, antiandrogenic, lubricant, hemolytic, 5-α reductase inhibitor, nematicide, antialopecic, antiandrogenic nematicide, antiandrogenic, hemolytic
methyl 11-octadecenoate	allelopathic, pesticide
9-octadecenoic acid	anti-inflammatory, antialopecic, α-reductase inhibitor lubricant, antitumor, dermatitigenic, immunostimulant, antileucotriene-d_4, lipoxygenase inhibitor, allergenic, flavor, hypocholesterolemic, insectifuge, percutaneo-stimulant, perfumery, and propecic
pentadecanoic acid	antioxidant
docosanoic acid	hair moisturizer
bonadecane	antimutagenic
vitamin E	cancer preventive
Oxirane	precursor of progesterone, antimicrobial
octadecanoic acid	antioxidant, hypoglycemic, and thyroid-inhibiting properties
(Z,Z) 9,12-octadecadienoic acid	anti-inflammatory, nematicide, insectifuge, hypocholesterolemic, cancer preventive, heptaoprotective, antistaminic, antiacne, antiarthritic, 5-α reductase inhibitor, anticoronary, anticancer

Table 3. Concentration of Biomolecules in *H. aspersa* Müller Crude Extracts

	concentration (mg g^{-1} DM)				
	protein	polyphenols	total sugar	reducing sugar	total flavonoids
Ex Chl	1.34 ± 0.07^{a}	0.16 ± 0.03^{a}	1.16 ± 0.10^{a}	0.99 ± 0.10^{a}	0.04 ± 0.00^{a}
Ex AcEt	1.62 ± 0.09^{a}	0.32 ± 0.04^{a}	1.73 ± 0.24^{a}	1.39 ± 0.12^{ab}	0.06 ± 0.00^{a}
Ex EtOH	3.74 ± 0.18^{b}	1.26 ± 0.08^{b}	2.72 ± 0.11^{b}	2.07 ± 0.16^{b}	0.13 ± 0.00^{b}

different antioxidant activities, as shown in Figure 3. The ethanol extract had the highest TAC at approximately 7.04 ± 0.14 mg g^{-1} DM, followed by the chloroform extract at approximately 4.66 ± 0.14 mg g^{-1} DM, and finally, the ethyl acetate extract had a TAC of 3.14 ± 0.03 mg g^{-1} DM. The difference in TAC between the three extracts of *H. aspersa* Müller was statistically significant ($p < 0.05$). These results suggest that the flesh of *H. aspersa* Müller could serve as a natural source of antioxidants. Similar results were reported by Gogas et al.[26] in *H. aspersa* Müller (0.587 ± 0.715) fed with different protein sources under intensive farming conditions.

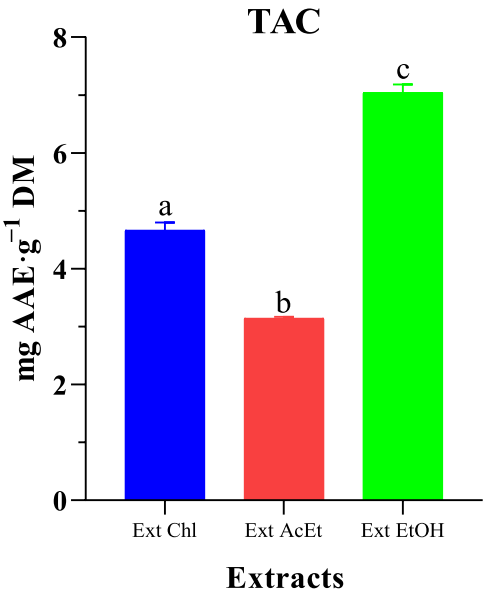


Figure 3. TAC of *H. aspersa* Müller crude extracts. **Ex Chl**: chloroform extract, **Ex AcEt**: ethyl acetate extract, and **Ex EtOH**: ethanol extract. The significant difference ($p < 0.05$) is illustrated by the letters a, b, and c.

In order to assess antioxidant capability, several researchers employ the phosphomolybdate method, which is one of the most reliable techniques.[27] The extracts' TAC differed widely among the several solvents, demonstrating that each solvent with a certain polarity may isolate particular components with a particular antioxidant ability.

2.4.2. Determination of Free Radical Scavenging Activity (DPPH). The DPPH free radical method has been widely used for different natural and synthetic antioxidant compounds and potential free radical scavenging activity.[27] First, the percentage of DPPH free radical inhibition was identified at different concentrations (200 to 1000 μg mL^{-1}) of each extract.

Table 4 shows the results of the free radical inhibition test performed on the crude extracts of the *H. aspersa* Müller flesh. All three extracts showed dose-dependent inhibition. The free radical scavenging capacity of the crude extracts of *H. aspersa* Müller flesh was determined by using IC_{50} values. The ethanol extract exhibited higher scavenging activity than the ethyl acetate and chloroform extracts. The antioxidant activity of the crude ethanol extract was 89.36 ± 0.43%, while the ethyl acetate extract showed an activity of 71.51 ± 0.29% and the chloroform extract showed an activity of 60.12 ± 0.72% at the highest concentration.

The IC_{50} values were 507.1 ± 1.2, 706.8 ± 8.03, and 829.5 ± 13.04 μg mL^{-1}, respectively, for ethanol, ethyl acetate, and chloroform extracts. The antioxidant activity of all three extracts was lower than that of L-ascorbic acid, with an IC_{50} value of 41.55 ± 0.00 μg mL^{-1}. The hydroxyl radical scavenging activity of L-ascorbic acid was 89.46% at the highest concentration (200 μg mL^{-1}). IC_{50} values were significantly different in all extracts ($p < 0.05$), indicating that each solvent extracts a specific type of metabolite that could carry different levels of DPPH free radical scavenging activity.

Compared to the previous results, in their study, Borquaye et al.[28] reported that the ethyl acetate extract of *Galatea paradoxa* had an IC_{50} of 1259.0 μg mL^{-1}, while the methanolic extract displayed 370.0 μg mL^{-1}. Similarly, the ethyl acetate extract of

https://doi.org/10.1021/acsomega.4c04042
ACS Omega 2024, 9, 34754−34764

Table 4. Comparison between the Effects of Antibiotics on Pathogenic Bacteria In Vitro[a]

antibiotics	*P. aeruginosa*		*E. coli* (ATCC 35218)		*L. monocytogenes* (ATCC 7644)		*S. aureus* (ATCC 29213)	
	ZI (mm)	Pr	ZI (mm)	Pr	ZI (mm)	Pr	ZI (mm)	Pr
AMP	00.00 ± 0.00^{a}	R	00.00 ± 0.00^{a}	R	00.00 ± 0.00^{a}	R	00.00 ± 0.00^{a}	R
COF	09.60 ± 0.12^{b}	S	17.00 ± 0.00^{c}	S	10.00 ± 0.09^{b}	S	00.00 ± 0.00^{a}	R
CUS	10.00 ± 1.02^{b}	S	00.00 ± 0.00^{a}	R	28.00 ± 0.16^{d}	ES	00.00 ± 0.00^{a}	R
ETM	19.00 ± 0.24^{d}	S	00.00 ± 0.00^{a}	R	25.00 ± 0.05^{cd}	ES	20.00 ± 0.00^{bc}	S
NM	19.67 ± 0.10^{d}	S	13.50 ± 0.00^{b}	S	30.00 ± 0.37^{d}	ES	18.00 ± 0.00^{b}	S
PRP	00.00 ± 0.00^{a}	R	00.00 ± 0.00^{a}	R	17.00 ± 0.20^{bc}	S	00.00 ± 0.00^{a}	R
TC	00.00 ± 0.00^{a}	R	12.00 ± 0.00^{b}	S	00.00 ± 0.00^{a}	R	15.00 ± 0.00^{b}	S

[a]ZI: zone of inhibition; Pr: profile; R: resistant; S: sensitive; ES: extremely sensitive; ER: extremely resistant. Means in the same column with the same letter do not differ significantly from each other at the 5% level of significance according to the Duncan's test.

Table 5. Average Diameter of Pathogen Inhibition Zones (ZI) Generated by *H. aspersa* Müller Flesh Extracts[a]

	ZI (mm)			
	P. aeruginosa	*E. coli* (ATCC 35218)	*L. monocytogenes* (ATCC 7644)	*S. aureus* (ATCC 29213)
Ext EtOH	15.26 ± 0.21^{a}	15.53 ± 0.21^{a}	12.30 ± 0.52^{a}	13.30 ± 0.23^{a}
Ext AcEt	10.47 ± 0.31^{b}	10.92 ± 0.13^{b}	09.98 ± 0.16^{b}	09.53 ± 0.15^{b}
Ext Chl	08.07 ± 0.15^{c}	09.77 ± 0.25^{c}	09.60 ± 0.22^{b}	08.48 ± 0.38^{c}

[a]**Ex Chl**: chloroform extract, **Ex AcEt**: ethyl acetate extract and **Ex EtOH**: ethanol extract. Means in the same column with the same letter do not differ significantly from each other at the 5% significance level according to Duncan's test.

Littorina littorea showed an IC_{50} of 1065.0 μg mL^{-1}, while the methanolic extract had a concentration of 780.0 μg mL^{-1}. On the other hand, the methanolic extract of *B. spinosa* had an IC_{50} value of 39.43% at 10,000 μg mL^{-1}.[29]

2.4.3. Determination of Reducing Power (FRAP). Table 4 shows what happened when extracts of *H. aspersa* Müller flesh were used in the FRAP radical absorbance inhibition test. The IC_{50} values showed a statistically significant difference ($p < 0.05$) between ascorbic acid's antioxidant activity and that of the three extracts. All tested extracts inhibited the FRAP absorbance, and this inhibition was dose-dependent for all three extracts. The ethanol extract showed significantly higher antioxidant activity (67.98 ± 4.31 μg mL^{-1}) compared to the ethyl acetate (267.69 ± 19.83 μg mL^{-1}) and chloroform (424.74 ± 14.33 μg mL^{-1}) extracts but had lower antioxidant activity than ascorbic acid (53.24 ± 3.94 μg mL^{-1}). This indicates that *H. aspersa* Müller extracts have important antioxidant characteristics. Similar results have been reported for marine species.[30]

Subavathy and Janet[31] reported a maximum activity at 500 μg mL^{-1} in *Turbo brunneus, Babylonia spirata,* and *Cypraea annulus* of 73.35, 87.5, and 95.36%, respectively, and a minimum activity at 100 μg mL^{-1}, of 52.07, 57.51, and 67.21%, respectively, in *T. brunneus, B. spirata,* and *C. annulus.*

The different amounts of biomolecules and their antioxidant capacity in terms of DPPH, FRAP, and total antioxidant capacity may be due to the polarity index of the molecules and their association with the polarity index of the solvent.

2.5. Antibacterial Activity. *2.5.1. Disc Diffusion.* In a previous study by Aouji et al.,[32] the sensitivity of various bacterial strains to specific antibiotics was investigated. The results indicated that *Escherichia coli* was sensitive to cefotaxime sodium, netilmicin, and tetracycline, whereas *Staphylococcus aureus* was sensitive to erythromycin, netilmicin, and tetracycline. Additionally, *Pseudomonas aeruginosa* displayed resistance to ampicillin, piperacillin, and tetracycline, whereas *L. monocytogenes* exhibited resistance to ampicillin and tetracycline (Table 4).

Furthermore, the flesh extracts of *H. aspersa* Müller demonstrated antimicrobial effects against all bacterial strains examined (Table 5). Notably, the choice of solvent used for the extraction appeared to influence the inhibition of bacterial growth. Additionally, the findings indicated that the antimicrobial activity of *H. aspersa* Müller tended to increase with the increasing polarity of the solvent used for extraction.

The ethanol extract exhibited the highest inhibitory diameters, measuring 15.53, 15.26, 12.30, and 13.30 mm against *E. coli, P. aeruginosa, S. aureus,* and *L. monocytogenes,* respectively. In contrast, the chloroform extract exhibited the lowest inhibitory diameters, with inhibition zones of 8.07 and 8.48 mm against *P. aeruginosa* and *S. aureus,* respectively. The statistical analysis indicated that there were significant differences in the inhibitory effects of the three extracts on the bacterial strains that were tested ($p \geq 0.05$). Nevertheless, while examining *L. monocytogenes,* there was no notable disparity seen between the ethyl acetate and chloroform extracts ($p < 0.05$). In conclusion, the results of this study indicate that extracts obtained from the *H. aspersa* Müller flesh have antibacterial activities against pathogenic microorganisms (Table 6).

Anand and Edward[15] performed a comparative study on the antibacterial properties of ethanolic extracts obtained from the gastropod species *B. spirata* and *T. brunneus.* The researchers observed an increase in the antibacterial efficacy against *E. coli, K. pneumoniae, Pseudomonas vulgaris,* and *Salmonella typhi.*

Table 6. Minimal Inhibitory Concentration of Various Extracts of *H. aspersa* Müller Flesh against Bacteria[a]

	CMI (mg mL^{-1})			
	P. aeruginosa	*E. coli* (ATCC 35218)	*L. monocytogenes* (ATCC 7644)	*S. aureus* (ATCC 29213)
Ext Chl	30	45	40	30
Ext AcEt	20	30	25	35
Ext EtOH	15	20	25	20

[a]**Ex Chl**: chloroform extract, **Ex AcEt**: ethyl acetate extract, and **Ex EtOH**: ethanol extract.

https://doi.org/10.1021/acsomega.4c04042
ACS Omega 2024, 9, 34754−34764

Table 7. Pearson Correlation Coefficients between Different Parameters of the Chloroform

	Pr	Rs	Ts	Pp	Ft	DPPH	FRAP	TAC	*P. aeruginosa*	*E. coli*	*L. monocytogenes*
Rs	−0.368										
Ts	0.828	0.217									
Pp	−0.626	0.956	−0.080								
Ft	0.202	−0.985	−0.382	−0.891							
DPPH	−0.707	−0.397	−0.982	−0.109	0.549						
FRAP	0.886	0.106	0.994	−0.192	−0.276	−0.955					
TAC	−0.873	0.775	−0.449	0.927	−0.654	0.273	−0.547				
P. aeruginosa	−0.747	−0.343	−0.991	−0.051	0.500	0.998	−0.970	0.328			
E. coli	0.949	−0.642	0.609	−0.839	0.500	−0.449	0.695	−0.982	−0.500		
L. monocytogenes	0.949	−0.642	0.609	−0.839	0.500	−0.449	0.695	−0.982	−0.500	1.000	
S. aureus	0.747	0.343	0.991	0.051	−0.500	−0.998	0.970	−0.328	−1.000	0.500	0.500

Table 8. Pearson Correlation Coefficients between Different Parameters of the Ethyl Acetate Extract

	Pr	Rs	Ts	Pp	Ft	DPPH	FRAP	TAC	*P. aeruginosa*	*E. coli*	*L. monocytogenes*
Rs	0.815										
Ts	0.955	0.607									
Pp	−0.986	−0.900	−0.893								
Ft	0.367	−0.240	0.626	−0.208							
DPPH	−0.996	−0.759	−0.978	0.967	−0.449						
FRAP	0.982	0.910	0.882	−1.000	0.185	−0.961					
TAC	0.759	0.996	0.532	−0.856	−0.327	−0.697	0.868				
P. aeruginosa	−0.367	0.240	−0.626	0.208	−1.000	0.449	−0.185	0.327			
E. coli	−0.783	−0.277	−0.932	0.669	−0.866	0.836	−0.652	−0.189	0.866		
L. monocytogenes	−0.367	0.240	−0.626	0.208	−1.000	0.449	−0.185	0.327	1.000	0.866	
S. aureus	0.989	0.721	0.988	−0.951	0.500	−0.998	0.944	0.655	−0.500	−0.866	−0.500

2.5.2. Determination of Minimum Inhibitory Concentration by the Broth Dilution Method. The minimum inhibitory concentration (MIC) refers to the lowest concentration of an antibacterial agent that is necessary to effectively eradicate certain bacteria.[33] In general, the findings of the study indicate the clear presence of antibacterial activity in the three extracts derived from *H. aspersa* Müller flesh. The MIC values of the extracts obtained from the *H. aspersa* Müller flesh were determined.

Our results revealed a significant level of antibacterial efficacy against the examined bacterial strains. The MIC values for the tested extracts varied when they were assessed against the target microorganisms. Specifically, the ethanol extract displayed an MIC of 15 mg mL^{-1} against *P. aeruginosa*, whereas the chloroform extract had an MIC of 45 mg mL^{-1} against *E. coli*.

The MIC values recorded for the ethanol extract against the four bacterial strains ranged from 15 to 25 mg mL^{-1}. *P. aeruginosa* displayed the lowest MIC at 15 mg mL^{-1}, followed by *S. aureus*, *E. coli*, and *L. monocytogenes* at concentrations of 20, 20, and 25 mg mL^{-1}, respectively. The ethyl acetate extract exhibited MIC values of 20, 30, 25, and 35 mg mL^{-1} against *P. aeruginosa*, *E. coli*, *L. monocytogenes*, and *S. aureus*, respectively. In contrast, the chloroform extract displayed the lowest efficacy, with MIC values ranging from 30 to 45 mg mL^{-1}. Specifically, the MIC was determined to be 30 mg mL^{-1} against both *S. aureus* and *P. aeruginosa*, while *L. monocytogenes* exhibited a MIC of 40 mg mL^{-1}, and *E. coli* demonstrated a MIC of 45 mg mL^{-1}.

Discrepancies in MIC values can be attributed to variations in the chemical composition of the extracts. The ethanol extract, characterized by a high concentration of polar compounds such as phenols and flavonoids, known for their potent antibacterial properties, displayed more significant antimicrobial effects. Conversely, the chloroform extract contained a higher proportion of nonpolar molecules, resulting in relatively weaker antibacterial activity.

Rota et al.[34] have elucidated that the antibacterial action of phenolic compounds arises from the presence of numerous hydroxyl groups, which have the capacity to interact with bacterial cell membranes, inducing alterations in their structure and composition. Helal et al.[35] proposed that phenolic compounds can modulate the bacterial cell membrane structure, diminish lipid content, and ultimately hinder bacterial growth. Prashar et al.[36] suggested that phenolic substances can inflict damage on or permeate bacterial cell lipid structures through membrane saturation.

Furthermore, Ulagesan and Kim[37] demonstrated the antibacterial activity of crude protein derived from six distinct snail species against specific bacterial and fungal strains. Our findings indicate that extracts sourced from the *H. aspersa* Müller flesh have the potential to serve as a novel reservoir of antibacterial agents.

2.6. Correlation between Different Parameters. Table 7 presents the Pearson correlation coefficients between the different parameters of the chloroform extract, where some parameters are positively correlated and others are negatively correlated. Starting with the positively correlated parameters, namely, Pr with Ts, *E. coli*, *L. monocytogenes*, *S. aureus*, and FRAP (r = 0.828, 0.949, 0.949, 0.747, and 0.886, respectively), Rs with Ts, Pp, FRAP, and TAC (r = 0.217, 0.956, 0.106, and 0.775, respectively), Ts with FRAP (r = 0.994), Pp with TAC (r = 0.927), Ft with DPPH (r = 0.549), and finally DPPH with TAC (r = 0.273). Those that are negatively correlated are TAC with FRAP (r = −0.547), FRAP with DPPH (r = −0.955), DPPH with Pr and Rs, Ts and Pp (r = −0.707, −0.397, −0.982, and −0.109, respectively), Pp with Pr and Ts (r = −0.626 and −0.080, respectively), Pr with Rs (r = −0.368), and finally FRAP

https://doi.org/10.1021/acsomega.4c04042
ACS Omega 2024, 9, 34754−34764

Table 9. Pearson Correlation Coefficients between Different Parameters of the Ethanolic Extract

	Pr	Rs	Ts	Pp	Ft	DPPH	FRAP	TAC	*P. aeruginosa*	*E. coli*	*L. monocytogenes*
Rs	−0.334										
Ts	−0.645	0.936									
Pp	0.216	0.848	0.606								
Ft	−0.781	−0.327	0.027	−0.779							
DPPH	0.681	−0.918	−0.999	−0.567	−0.075						
FRAP	−0.935	0.646	0.874	0.143	0.509	−0.896					
TAC	1.000	−0.359	−0.665	0.191	−0.764	0.700	−0.944				
P. aeruginosa	−0.182	−0.866	−0.634	−0.999	0.756	0.596	−0.178	−0.156			
E. coli	−0.942	0.000	0.353	−0.530	0.945	−0.397	0.763	−0.933	0.500		
L. monocytogenes	−0.182	−0.866	−0.634	−0.999	0.756	0.596	−0.178	−0.156	1.000	0.500	
S. aureus	0.182	0.866	0.634	0.999	−0.756	−0.596	0.178	0.156	−1.000	−0.500	−1.000

with TAC (r = −0.824), *P. aeruginosa* with *S. aureus* (r = −1.000).

According to the results presented in Table 8, it is evident that a number of factors studied in this research have shown a positive correlation between them. FRAP shows a positive correlation with Pr, Rs, Ts, and Ft (r = 0.982, 0.910, 0.882, and 0.185, respectively), while TAC shows a positive correlation with Pr, Rs, Ts, and FRAP (r = 0.759, 0.996, 0.532, and 0.868, respectively) and *S. aureus* with Ts and FRAP (r = 0.988 and 0.944). Furthermore, a negative correlation has been observed between DPPH and Pr, Rs, Ts, and Ft (r = −0.996, −0.759, −0.978, and −0.449, respectively) and Pr with Pp (r = −0.986).

The coefficients of Pearson correlation between the various examined characteristics showed that numerous factors correlated favorably (Table 9), including Ft and FRAP (r = 0.509), Pp with TAC and *S. aureus* (r = 0.191 and 0.999), and Pr with Pp, DPPH, and TAC (r = 0.216, 0.681, and 1.000, respectively). FRAP showed a positive correlation with Rs, Ts, Pp, and Ft (r = 0.646, 0.874, 0.143, and 0.509, respectively). On the other hand, Ts is strongly negatively correlated with DPPH (r = −0.999), the latter is negatively correlated with FRAP, Rs, Pp, and Ft (r = −0.896, −0.918, −0.567, and −0.075, respectively), and *L. monocytogenes* with Pp (r = −0.999).

3. CONCLUSIONS

This study investigated the biomolecule content and the antioxidant and antibacterial potential of raw extracts from the flesh of *H. aspersa* Müller. These extracts have a high abundance of molecules and significant antioxidant activity. The extracts also showed antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, and *L. monocytogenes*. The GC−MS analysis identified 45 compounds, including octadecanoic and hexadecanoic acids, which may contribute to the observed activities. The results show that the extraction process and the polarity of the solvent significantly influence the yields of bioactive compounds and the biological activities of the *H. aspersa* Müller flesh. The ethanol extract contains the highest concentrations of proteins, sugars, polyphenols, and flavonoids. This enriched profile contributed to the superior biological properties observed compared with ethyl acetate and chloroform extracts. The ethanol extract presented a significant advantage in terms of total antioxidant capacity, DPPH radical scavenging activity, and iron reduction capacity. In addition, it has shown the most powerful antibacterial efficacy, with the largest areas of inhibition against the bacterial strains tested. The study highlights the importance of solvent selection to maximize extraction of valuable bioactive compounds.

These results underline the potential of the flesh of *H. aspersa* Müller as a rich source of natural antioxidants and antibacterial agents. However, further research is needed to elucidate the specific compounds responsible for the antioxidant and antimicrobial effects. Future studies should focus on the isolation, purification, and identification of key bioactive molecules. In addition, in vitro and in vivo studies are necessary to confirm the efficacy and safety of these extracts for potential applications in the food and pharmaceutical industries.

4. MATERIALS AND METHODS

4.1. Snail Collection and Preparation of the Flesh Powder. Uninfected *H. aspersa* Müller snails were collected in the spring from the Moulay Bousselham region of Morocco (34° 52′ 43″ N, 6° 17′ 36″ O). These were immediately transferred to the laboratory, where healthy individuals were housed in rectangular plastic boxes (24 × 32 × 12 cm) with a sponge, moistened soil, and food (lettuce, carrot, and spinach). Boxes containing 50 snails were sprayed with water daily to keep them moist. It is important to note that the snails were treated as part of a scientific experiment that followed animal care guidelines.

150 snails were cleaned under running water and then removed from their shells, and the visceral masses were removed. The feet (with the head) were recovered and cleaned of mud with a NaCl solution (1.2%) and were washed with distilled water and dried on absorbent paper. The samples were dried in an oven (60 °C for 48 h) and then ground and sieved for recovery.[38]

4.2. Extracts of Molecules. A sequential maceration approach was used with three organic solvents, chosen based on increasing polarity: chloroform (boiling point: 61.2 °C), ethyl acetate (boiling point: 77.1 °C), and ethanol (boiling point: 78.3 °C) (starting with chloroform). At a rate of 10 g of flesh in 100 mL of solvent, extraction was performed at room temperature with constant agitation and light protection for 48 h (repeated twice).

To obtain a concentrated filtrate, traces of the solvent were removed using a rotating evaporator (HEI-VAP Core, HEIDOLPH) after filtering through a filter paper. The crystals were then crushed again. The dried marc (residue) was then extracted again under the same conditions but with different solvents (ethyl acetate and ethanol).

Three raw organic extracts were obtained from this extraction series: chloroform extract (**Ex Chl**), ethyl acetate extract (**Ex AcEt**), and ethanol extract (**Ex EtOH**). They were recovered in sterile glass vials, securely sealed, and refrigerated at 4 °C until use.

https://doi.org/10.1021/acsomega.4c04042
ACS Omega 2024, 9, 34754−34764

Using the following formula, the extraction yield (EY) was calculated in relation to the dry matter weight

$$\text{EY}\ (\%) = \frac{\text{mass of the obtained extract}}{\text{mass of initial powder}} \times 100$$

4.3. GC−MS Analysis of the Chemical Content of Snail Powder Extracts. The *H. aspersa* Müller flesh extracts were analyzed by gas chromatography−mass spectrometry under the following conditions: the injector port temperature was 250 °C. The starting oven temperature was 40 °C, and the temperature was gradually increased at 8 °C min^{-1} for 18 min until it reached 260 °C. The BR-5 ns FS capillary column (30 m × 0.25 mm ID × 0.25 m) was utilized. In the undivided mode, the injection volume of helium was 1.0 mL min^{-1}. The whole analysis took 105 min. The mass spectrometry detector (MSD) was set to electronic impact ionization mode, with an ionizing energy of 70 eV and an m/z scan range of 50 to 500. The temperature of the ion source was 230 °C, and it then quadrupled to 150 °C. The electron multiplier voltage (EM voltage) was kept at 1100 V above the self-regulatory level, with a 3 min solvent delay.[38]

4.4. Quantification of Bioactive Contents. Protein and carbohydrate contents were determined using the Deepachandi method[39] and the Nielsen method,[40] respectively, with the reagents Folin−Ciocalteu, sulfuric acid, and phenol.

In contrast, reducing sugars are quantified using the Negrulescu method,[41] which uses dinitro-salicylic acid as a reagent. The Zargoosh et al.[42] methods were modified to determine phenolic compounds; the reduction of the Folin−Ciocalteu reagent during polyphenol oxidation is the basis for these analytical methods. The researchers modified the methods described in the paper by Zirari et al.[43] to measure the amount of flavonoid compounds in their samples.

4.5. Antioxidant Activity. The antioxidant activity of crude extracts from *H. aspersa* Müller was measured by looking at total antioxidant activity, DPPH radical scavenging activity, and total reducing power, using the protocols described in our previous study.[44]

4.6. Antibacterial Assays. *4.6.1. Bacterial Strains.* We determined the antibacterial properties of the crude extract of *H. aspersa* Müller flesh against the bacterium *P. aeruginosa*, which was obtained from the Plant, Animal and Agro-Industry Productions Laboratory (PAAP lab) at Ibn Tofail University. We also used other reference strains of bacteria in our study, including *L. monocytogenes* (ATCC 7644), *S. aureus* (ATCC 29213), and *E. coli* (ATCC 35218). To prepare the bacteria for testing, 1 mL of the stored bacterial suspension was mixed with 2 mL of nutrient broth. This broth provides the bacteria with the nutrients they need to grow and multiply.

4.6.2. Antibiotics. The antibiotics used were ampicillin (AMP) (10 μg), cefuroxime sodium (CUS) (30 μg), cefotaxime sodium (COF) (30 μg), erythromycin (ETM) (25 μg), netilmicin (NM) (30 μg), piperacillin (PRP) (100 μg), and tetracycline (TC) (30 μg).

4.6.3. Disc Diffusion Method. The Kirby−Bauer disk diffusion technique was used in this investigation to assess the antibacterial activity of *H. aspersa* Müller flesh extracts.[45] Whatman N°3 paper disks (6 mm) were sanitized, sealed in sterile glass vials, and boiled for 30 min to eliminate any chemicals that would limit microbial growth in order to measure this activity. Then, different amounts of *H. aspersa* Müller flesh extracts were applied to each disk, and antibiotic disks were positioned on the MH medium's surface after being preinoculated by swabbing with bacterial suspensions (10^8 cfu). The infected Petri plates were then incubated at 37 °C in the dark. The inhibitory zone diameter was evaluated 24 h after incubation. For employing the latter, the bacteria have been gathered together.[46]

The results were interpreted as follows:

- Resistant: diameter ≤8 mm;
- Moderately sensitive: diameter between 9 and 14 mm;
- Sensitive: diameter between 15 and 19 mm;
- Extremely sensitive: diameter >20 mm.

4.6.4. Determination of the MIC. Using the Wiegand et al.[47] method, we calculated the lowest concentration of the snail flesh extract of *H. aspersa* Müller that inhibited the growth of the chosen bacterial strains. We added 20 mL of fresh bacterial culture (1×10^4 cfu) to different concentrations of the snail flesh extract (10, 20, 40, 60, 80, and 100 mg mL^{-1}). MIC was defined as the lowest concentration of the *H. aspersa* Müller flesh extract that prevented the formation of turbidity. Dimethyl sulfoxide was used as a negative control.

4.7. Statistical Analysis. The data was subjected to a one-way ANOVA followed by Duncan's test ($\alpha = 5$) for multiple comparisons and determination of significance levels using statistical software (SPSS, ver. 20). The average value of three repetitions is presented. Values of $p < 0.05$ were considered statistically significant.

AUTHOR INFORMATION

Corresponding Authors

Marouane Aouji − *Laboratory of Natural Resources and Sustainable Development, Department of Biology, Faculty of Sciences, Ibn Tofail University, Kenitra 14000, Morocco;* orcid.org/0000-0002-5884-0016; Email: aouji.marouane@uit.ac.ma

Hamada Imtara − *Faculty of Medicine, Arab American University Palestine, Jenin 44862, Palestine;* orcid.org/0000-0002-8410-3212; Email: hamada.imtara@aaup.edu

Authors

Malak Zirari − *Laboratory of Organic Chemistry Catalysis and Environment, Department of Chemistry, Faculty of Sciences, Ibn Tofail University, Kénitra 14000, Morocco*

Amine Rkhaila − *Laboratory of Plant, Animal and Agro-Industry Productions, Department of Biology, Faculty of Sciences, Ibn Tofail University, Kenitra 14000, Morocco*

Bouchra Bouhaddioui − *Laboratory of Natural Resources and Sustainable Development, Department of Biology, Faculty of Sciences, Ibn Tofail University, Kenitra 14000, Morocco*

Ramzi A. Mothana − *Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia*

Omar M. Noman − *Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia*

Mahmoud Tarayrah − *National Center for Research in Human Genomics, Evry Courcouronnes 91000, France*

Rachid Bengueddour − *Laboratory of Natural Resources and Sustainable Development, Department of Biology, Faculty of Sciences, Ibn Tofail University, Kenitra 14000, Morocco*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c04042

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

https://doi.org/10.1021/acsomega.4c04042
ACS Omega 2024, 9, 34754−34764

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors extend their appreciation to the Researchers Supporting Project (RSP2024R119), King Saud University, Riyadh, Saudi Arabia, for funding this work.

■ ABBREVIATIONS

H. aspersa	*Helix aspersa*
GC−MS	gas chromatography−mass spectrometry
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	ferric reducing antioxidant power
TAC	total antioxidant capacity
cfu	colony forming unit
ANOVA	analysis of variance
Ex Chl	chloroform extract
Ex AcEt	ethyl acetate extract
Ex EtOH	ethanol extract

■ REFERENCES

(1) Luo, F. L.; Xing, R.; Wang, X. Q.; Peng, Q.; Li, P. Proximate composition, amino acid and fatty acid profiles of marine snail Rapana venosa meat, visceral mass and operculum. *J. Sci. Food Agric.* **2017**, *97*, 5361−5368.

(2) Shanmugam, M.; Mody, K. H. Heparinoid-active sulphated polysaccharides from marine algae as potential blood anticoagulant agents. *Curr. Sci.* **2000**, *79*, 1672−1683.

(3) Cheung, R. C.; Ng, T. B.; Wong, J. H. Marine peptides: Bioactivities and applications. *Mar. Drugs* **2015**, *13*, 4006−4043.

(4) Zhukova, N. V. Lipids and fatty acids of nudibranch mollusks: potential sources of bioactive compounds. *Mar. Drugs* **2014**, *12* (8), 4578−4592.

(5) Benkendorff, K. Molluscan biological and chemical diversity: Secondary metabolites and medicinal resources produced by marine molluscs. *Biol. Rev.* **2010**, *85* (4), 757−775.

(6) Wendel, M.; Heller, A. R. Anticancer actions of omega-3 fatty acids-current state and future perspectives. *Anti-Cancer Agents Med. Chem.* **2009**, *9* (4), 457−470.

(7) Blunt, J. W.; Copp, B. R.; Hu, W. P.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. Marine natural products. *Nat. Prod. Rep.* **2007**, *24*, 31.

(8) Tincu, J. A.; Taylor, S. W. Antimicrobial peptides from marine invertebrates. *Antimicrob. Agents Chemother.* **2004**, *48*, 3645−3654.

(9) Nagash, Y. S.; Nazeer, R. A.; Kumar, N. S. In vitro antioxidant activity of solvent extracts of molluscs (Loligo duvauceli and Donax strateus) from India. *World J. Fish Mar. Sci.* **2010**, *2*, 240−245.

(10) Olivera, B. M. Conus peptides: biodiversity-based discovery and exogenomics. *J. Biol. Chem.* **2006**, *281* (42), 31173−31177.

(11) Matusiewicz, M.; Kosieradzka, I.; Niemiec, T.; Grodzik, M.; Antushevich, H.; Strojny, B.; Gołębiewska, M. In Vitro Influence of Extracts from Snail Helix aspersa Muller on the Colon Cancer Cell Line Caco-2. *Int. J. Mol. Sci.* **2018**, *19*, 1064.

(12) Grilla, A.; LaJeunesse, C.; McMaster, D.; Morgan, D. *Feasibility of Snail Farming as a Model for Small Urban Farms to Expand into Niche Markets for Increased Profitability*; Worcester Polytechnic Institute: Worcester, MA, USA, 2016.

(13) Kulawik, P.; Özogul, F.; Glew, R.; Özogul, Y. Significance of antioxidants for seafood safety and human health. *J. Agric. Food Chem.* **2013**, *61* (3), 475−491.

(14) Arias, C. A.; Murray, B. E. Antibiotic-Resistant Bugs in the 21st Century-A Clinical Super-Challenge. *N. Engl. J. Med.* **2009**, *360*, 439−443.

(15) Anand, T. P.; Edward, J. K. P. Antimicrobial activity in the tissue extracts of five species of cowries cypraea spp. (Mollusca: Gastropoda) and an ascidian didemnum psammathodes (Tunicata: Didemnidae). *Ind. J. Mar. Sci.* **2002**, *31*, 239−242.

(16) Szkucik, K.; Ziomek, M.; Paszkiewicz, W.; Drozd, Ł.; Gondek, M.; Knysz, P. Fatty acid profile in fat obtained from edible part of land snails harvested in Poland. *J. Vet. Res.* **2018**, *62*, 519−526.

(17) Meyer-Rochow, V. B. Therapeutic arthropods and other, largely terrestrial, folk-medicinally important invertebrates: a comparative survey and review. *J. Ethnobiol. Ethnomed.* **2017**, *13* (1), 9−31.

(18) Zhang, H.; Birch, J.; Pei, J.; Mohamed Ahmed, I. A.; Yang, H.; Dias, G.; Abd El-Aty, A. M.; Bekhit, A. E. D. Identification of six phytochemical compounds from Asparagus officinalis L. root cultivars from New Zealand and China using UAE-SPE-UPLC-MS/MS: effects of extracts on H_2O_2-induced oxidative stress. *Nutrients* **2019**, *11*, 107−117.

(19) Kang, D. G.; Lee, H. S. An improved method in screening of superoxide and hydroxyl radical scavenging activities of plant medicinal extracts. *Korean J. Pharmacog.* **2001**, *32*, 253−256.

(20) Rafińska, K.; Pomastowski, P.; Rudnicka, J.; Krakowska, A.; Maruśka, A.; Narkute, M.; Buszewski, B. Effect of solvent and extraction technique on composition and biological activity of Lepidium sativum extracts. *Food Chem.* **2019**, *289*, 16−25.

(21) USDA Agricultural Research Service. *1992−2016 Dr. Duke's Phytochemical and Ethnobotanical Databases*, 2016.

(22) Gomot, A. Biochemical composition of Helix snails: influence of genetic and physiological factors. *J. Molluscan Stud.* **1998**, *64* (2), 173−181.

(23) Özogul, Y.; Özogul, F.; Olgunoglu, A. I. Fatty acid profile and mineral content of the wild snail (Helix pomatia) from the region of the south of the Turkey. *Eur. Food Res. Technol.* **2005**, *221* (3−4), 547−549.

(24) Ghedadba, N.; Bousselsela, H.; Hambaba, L.; Benbia, S.; Mouloud, Y. Evaluation of the antioxidant and antimicrobial activities of the leaves and flowered tops of Marrubium vulgare L. *Phytothérapie* **2014**, *12* (1), 15−24.

(25) Kouachi, M.; Naimi, D.; Djadouri, D.; Bendahra, I. Preventive effect of Helix aspersa crude extract against chemo-induced colonic damages in rats. *Int. J. Pharma Sci. Res.* **2017**, *8* (6), 2458−2468.

(26) Gogas, A.; Laliotis, G. P.; Ladoukakis, E.; Trachana, V. Chemical composition and antioxidant profile of snails (Cornu aspersum aspersum) fed diets with different protein sources under intensive rearing conditions. *J. Anim. Feed Sci.* **2021**, *30* (4), 391−398.

(27) Chekroun-Bechlaghem, N.; Belyagoubi-Benhammou, N.; Belyagoubi, L.; Gismondi, A.; Nanni, V.; Di Marco, G.; Canuti, L.; Canini, A.; El Haci, I. A.; Atik Bekkara, F. Phytochemical analysis and antioxidant activity of Tamarix africana, Arthrocnemum macrostachyum and Suaeda fruticosa, three halophyte species from Algeria. *Plant Biosyst.* **2019**, *153* (6), 843−852.

(28) Borquaye, L. S.; Darko, G.; Oklu, N.; Anson-Yevu, C.; Ababio, A. Antimicrobial and antioxidant activities of ethyl acetate and methanol extracts of Littorina littorea and Galatea paradoxa. *Cogent Chem.* **2016**, *2* (1), 1161865.

(29) Subhapradha, N.; Ramasamy, P.; Sudharsan, S.; Seedevi, P.; Moovendhan, M.; Srinivasan, A.; Shanmugam, V.; Shanmugam, A. Preparation of phosphorylated chitosan from gladius of the squid Sepioteuthis lessoniana (Lesson, 1830) and it's in vitro antioxidant activity. *Bioact. Carbohydr. Diet. Fibre* **2013**, *1*, 148−155.

(30) Nazeer, R. A.; Naqash, S. Y. In vitro antioxidant activity of two molluscs, Loligo duvauceli Orbigny and Donax cuneatus Linnaeus, by solvent extraction methods. *Mediterr. J. Nutr. Metabol.* **2013**, *6*, 17−21.

(31) Subavathy, P.; Janet, S. M. B. Molecular characterization of protein and antioxidant capacity of Turbo brunneus R. Cypraea annulus L. and Babylonia spirata L. *Int. J. Pharm. Sci. Res.* **2020**, *11*, 3285−3293.

(32) Aouji, M.; Rkhaila, A.; Bouhaddioui, B.; Zirari, M.; Harifi, H.; Taboz, Y.; Lrhorfi, L. A.; Bengueddour, R. Chemical composition, mineral profile, anti-bacterial, and wound healing properties of snail slime of Helix aspersa Müller. *Biomed* **2023**, *13* (4), 10−19.

(33) More, K.; Tayade, S.; Gawande, P.; Manik, S.; Shelke, D. Antioxidant and antimicrobial potential of Canavalia gladiata (Jacq.) DC. leaves and seeds: GC-MS based metabolic profiling. *Ind. J. Nat. Prod. Res.* **2022**, *13* (2), 163−169.

https://doi.org/10.1021/acsomega.4c04042
ACS Omega 2024, 9, 34754−34764

(34) Rota, M. C.; Herrera, A.; Martínez, R. M.; Sotomayor, J. A.; Jordán, M. J. Antimicrobial activity and chemical composition of Thymus vulgaris, Thymus zygis and Thymus hyemalis essential oils. *Food Control* **2008**, *19* (7), 681−687.
(35) Helal, G. A.; Sarhan, M. M.; Abu Shahla, A. N. K.; Abou El-Khair, E. K. Effects of Cymbopogon Citratus L. Essential Oil on the Growth, Lipid Content and Morphogenesis of Aspergillus Niger ML_2-Strain. *J. Basic Microbiol.* **2006**, *46*, 456−469.
(36) Prashar, A.; Hili, P.; Veness, R. G.; Evans, C. S. Antimicrobial Action of Palmarosa Oil (Cymbopogon Martinii) on Saccharomyces Cerevisiae. *Phytochemistry* **2003**, *63*, 569−575.
(37) Ulagesan, S.; Kim, H. J. Antibacterial and antifungal activities of proteins extracted from seven different snails. *Appl. Sci.* **2018**, *8*, 1362.
(38) Aouji, M.; Imtara, H.; Rkhaila, A.; Bouhaddioui, B.; Alahdab, A.; Parvez, M. K.; Saleh Alzahrani, M.; Aicha Lrhorfi, L.; Bengueddour, R. Nutritional Composition, Fatty Acids Profile, Mineral Content, Antioxidant Activity and Acute Toxicity of the Flesh of Helix aspesra Müller. *Molecules* **2023**, *28*, 6323.
(39) Deepachandi, B.; Weerasinghe, S.; Andrahennadi, T. P.; Karunaweera, N. D.; Wickramarachchi, N.; Soysa, P.; Siriwardana, Y. Quantification of Soluble or Insoluble Fractions of Leishmania Parasite Proteins in Microvolume Applications: A Simplification to Standard Lowry Assay. *Int. J. Anal. Chem.* **2020**, *2020*, 1−8.
(40) Nielsen, S. S. Phenol-Sulfuric Acid Method for Total Carbohydrates. In *Food Analysis Laboratory Manual*; Nielsen, S. S., Ed.; Springer: Boston, 2010; pp 47−53.
(41) Negrulescu, A.; Patrulea, V.; Mincea, M. M.; Ionascu, C.; Vlad-Oros, B. A.; Ostafe, V. Adapting the reducing sugars method with dinitrosalicylic acid to microtiter plates and microwave heating. *J. Braz. Chem. Soc.* **2012**, *23*, 2176−2182.
(42) Zargoosh, Z.; Ghavam, M.; Bacchetta, G.; Tavili, A. Effects of ecological factors on the antioxidant potential and total phenol content of Scrophularia striata Boiss. *Sci. Rep.* **2019**, *9*, 16021.
(43) Zirari, M.; Aouji, M.; Zouarhi, M.; Dermaj, A.; Erramli, H.; Hmouni, D.; El Mejdoub, N. Antioxidant activity, phytochemical screening and GC-MS profile of Abies marocana Trab. *Int. J. Sec. Metab.* **2024**, *11* (1), 121−133.
(44) Aouji, M.; Rkhaila, A.; Bouhaddioui, B.; Khalid, G.; Lrhorfi, L. A.; Bengueddour, R. Antioxidant activity, biochemical composition and physicochemical properties of Helix aspersa Müller snail slime. *Int. J. Chem. Bioch. Sci.* **2023**, *23* (3), 53−62.
(45) Hudzicki, J. Kirby-Bauer disk diffusion susceptibility test protocol. *Am. Soc. Microbiol.* **2009**, *15*, 1−23.
(46) EL Moussaoui, A.; Bourhia, M.; Jawhari, F. Z.; Salamatullah, A. M.; Ullah, R.; Bari, A.; Majid Mahmood, H.; Sohaib, M.; Serhii, B.; Rozhenko, A.; Aboul-Soud, M. A. M.; Ezzeldin, E.; Mostafa, G. A. E.; Bousta, D.; Bari, A. Chemical Profiling, Antioxidant, and Antimicrobial Activity against Drug-Resistant Microbes of Essential Oil from Withania frutescens L. *Appl. Sci.* **2021**, *11*, 5168.
(47) Wiegand, I.; Hilpert, K.; Hancock, R. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat. Protoc.* **2008**, *3*, 163−175.

https://doi.org/10.1021/acsomega.4c04042
ACS Omega 2024, 9, 34754−34764