

Toxicity, Chemical Composition, and Molecular Interactions: In-Depth Study of the Aqueous Extract of the Flesh of *Helix aspersa* Müller

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Abstract: Snails belong to the class of gastropods, and their flesh is a good source of nutrients for human consumption. In order to determine the safety of the *H. aspersa* Müller flesh aqueous extract (HAAE), a subacute toxicity study was conducted to test its effects on rats. Additionally, a chemical analysis was performed to identify its chemical composition. GC-MS was used for chemical analysis, and the subacute toxicity test was conducted, with 4 groups: a control group that received distilled water and 3 groups that received daily doses. The rats' body weight was monitored during this period, while kidney and liver weights, as well as hematological and biochemical blood parameters, were evaluated at the end of the experiment. The chemical analysis revealed that the HAAE flesh contains a variety of compounds. The toxicity study did not show any signs of subacute toxicity in rats for the doses studied. The rats' body weight and organs were not significantly modified by the administration of the HAAE. Moreover, no significant changes were observed in the biochemical and hematological parameters measured. This suggests that the HAAE does not present any danger for human consumption. In the same framework, the molecular linkage of the main volatile compounds identified on alanine aminotransferase revealed a higher binding affinity for Cholest-4-en-3-one, followed by linoleic acid. The studied molecules have therefore demonstrated promising activity with low toxicity and can be considered for further evaluation and optimization of the track.

Keywords: *H. aspersa* Müller; flesh; chemical composition; subacute toxicity; molecular docking.

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1. Introduction

Historically, natural resources were used for their therapeutic properties at the lowest cost to prevent or cure various diseases, so many people consider that natural products pose no

danger and have no lasting effects [1]. In recent years, there has been an increase in the use of natural extracts as functional or therapeutic sources for body care or disease treatment [2].

However, the increasing consumption of these extracts has raised concerns about their safety of use. It is therefore essential to evaluate their safety for optimal use. Extensive tests should be carried out to ensure their quality and effectiveness [3]. Therefore, it is common to conduct toxicity tests in order to assess the risks or side effects associated with the consumption of various natural extracts.

The information gathered from the toxicity test, which is a technique used to assess the safety of chemicals, enables the detection of hazards and the management of risks associated with their manufacture, handling, and use [4]. Ultimately, the evaluation of extract safety will enable the provision of scientific information on its various applications.

Computer-assisted drug search methods have evolved as improved technologies that can be used to search for drugs derived from biochemical compounds present in different organisms. It is crucial to use computational prediction models to guide the selection of methodologies for pharmaceutical and technological research. They have also been used to predict, *in silico*, pharmacokinetic, pharmacological, and toxicological performance [5]. The use of molecular linkage has already enabled researchers to reduce costs and the time required to study the mechanisms of activity by directing experimental studies towards the optimal active substance more quickly than conventional research methods [6]. Molecular docking, a recognition procedure between two or more molecules that involves spatial and energetic correspondence, is one of the key techniques of molecular modeling [7]. Predicting the orientation of drug candidates when linked to their target protein is enabled by the knowledge this method generates about the interactions between drugs and receptors [8]. In addition, this method simplifies systemic analysis by introducing a molecule non-covalently into the binding site of an object macromolecule, resulting in specific binding to the active sites of each ligand [9]. This technology enables the prediction of how ligands, small molecules, and peptides bind to their respective receptors, including enzymes.

Mollusks are a class of univalve organisms that constitute the second-largest phylum in the animal kingdom and include about 100,000 species [10]. *Mollusks* contain many potentially active compounds that could contribute to the production of supplements, dietary supplements, nutraceuticals, and medicines [11]. Among them are snails, which have long been important as a source of human food, thereby classifying them as foods with high nutritional value [12]. In addition, snail flesh is rich in polyunsaturated fatty acids [13] and it is rich in proteins and low in fats [14]. They are an excellent source of important biomedical products [15], and effective mechanisms for their natural immunity have been developed [16].

Various health benefits of snail consumption have been reported [17]. In addition, the bioactive compounds of *mollusks* exhibit various properties [18]. Among the most well-known land snail species is the *H. aspersa* Müller [19]. It is the species of choice for the snail breeding industry due to its ability to reproduce, fast life cycle, flavor, flexibility, and ability to survive in diverse environments and regions [20].

The study aims to evaluate the subacute toxicity of the aqueous extract of snail flesh (*H. aspersa* Müller) and to assess the putative inhibitory activity of its main compounds on the enzyme Alanine aminotransferase using molecular modeling.

2. Materials and Methods

2.2. Preparation of snail meat and extraction.

Snails of the *H. aspersa* Müller species were collected in the spring in Moulay Bousselham, Morocco. Healthy and uninfected, they were immediately transported to the laboratory. As soon as they arrived at the laboratory, the snails were placed in plastic boxes (24 cm, 32 cm, or 12 cm) and fed lettuce, carrots, and spinach. A humid environment was preserved thanks to a sponge and a wet floor. Water was also sprayed daily. The snails used in this study had a mass of 10.44 ± 3.11 g and a length of 3.35 ± 0.48 cm. The entire experiment was conducted in accordance with the instructions on veterinary care for the animal.

First of all, 150 heads are cleaned with clean water, then they are freed from their shells, their visceral masses are eliminated, their feet (with the head) are recovered, they are placed in NaCl (1.2%) to eliminate all the drool, and they are dried on absorbent paper after being cleaned with distilled water. They were then dried at 60°C for a period of 48 hours. The dry sample obtained was ground and then sieved. This results in a fine powder called snail flour [21].

The mixture of 10 g of snail flour and 100 mL of distilled water was macerated for 48 hours, then filtered twice on Whatman N°3 paper. The filtrate was evaporated to dryness, then in an oven at a temperature of 60°C. The crystals obtained were sprayed using a mortar. The fine powder collected formed the entire extract, which we stored in the refrigerator in a sterile glass jar, hermetically sealed [22].

2.2. GC-MS analysis.

H. aspersa Müller flesh extract was analyzed using gas chromatography-mass spectrometry (GC-MS) in accordance with the method outlined by Aouji et al. [23], under the following conditions: Injector port temperature is 250°C. The oven temperature starts at 40°C and is increased at 8°C/min for 18 minutes, until it reaches 260°C. The BR-5ns FS capillary column (30m x 0.25mm ID x 0.25m) was utilized. In undivided mode, the injection volume of helium is 1.0 mL/min. The whole analysis took 100 minutes. 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 then it quadrupled to 150°C. With a 3-minute solvent delay, the electron multiplier voltage (EM voltage) was held at 1100 V above the self-regulatory limit.

2.3. Study animals.

The Wistar albino rats used in this investigation were weighed, grouped, and housed in cages at the Faculty of Sciences of Ibn Tofail University, under a constant temperature of 25°C and a 12-hour light/dark cycle. Throughout the experiment, the rats had unrestricted access to food and water. The National Academy of Sciences' eighth edition of "Guide for the care and use of laboratory animals" served as the basis for the experiment's execution. Every attempt has been made to reduce the number of animals utilized in the study as well as their suffering.

2.4. Evaluation of subacute toxicity.

The subacute toxicity study was conducted according to OECD Guideline 407 [24]. 20 albino Wistar rats were randomly divided into four groups of five females as follows: the

control group received distilled water at a rate of 1 mL/100 g by weight; the other groups received an HAAE solution at rates of 30, 75, and 150 mg/kg by weight.

The treatment lasted 28 days; the rats were fed and hydrated at will, then weighed every 7 days before gavage. At the end of the experiment, the rats were kept on an empty stomach all night, then a blood sample from the abdominal aorta was taken for the determination of hematological and biochemical parameters, followed by dissection under anesthesia with chloral hydrate (50 mg/kg). The organs removed were the liver and the kidneys, which were rinsed with a 0.9% salt solution, then weighed.

The protocol was approved by the ethics committee of the Faculty of Sciences, Ibn Tofail University- Kenitra, under decision number 2023/07, on 25.07.2023. In accordance with institutional procedures.

2.4.1. Blood and serum.

One milliliter of rat venous blood was separated from the rest of the blood for hematological examination. To extract the serum for biochemical examination, the remaining portion was centrifuged for 10 minutes at 3000 rpm.

2.4.2. Organ coefficient (relative weight).

The formula for calculating the relative weight of each organ was used after the organs were thoroughly weighed and dissected:

$$\text{Organ coefficient (g/100g)} = \frac{\text{Organ weight}}{\text{Rat body weight}} \times 100 \quad (1)$$

2.4.3. Biochemical analysis of serum.

Several biochemical parameters were assayed. For the transaminase enzymatic activities, ALT and AST were assayed using the optimized UV-IFCC kinetic method with the ALT-GPT and AST-GOT kits, respectively. The first absorbance of the samples was measured after 1 minute against the white at 340 nm, and new readings were taken every minute for 3 minutes, as well as the levels of blood glucose and cholesterol by the glucose oxidase-peroxidase and cholesterol oxidase-peroxidase methods, respectively.

The enzymatic activity was obtained from the following formula:

$$\text{Activity (U/L)} = \frac{\Delta A}{mn} \times 1746 \quad (2)$$

Where ΔA = Variation of absorbance between 2-time intervals, $\Delta A/mn$ = Change in sample absorbance per minute, and 1746 = multiplication factor.

The cholesterol and glucose concentration of the sample is calculated according to the following formula:

$$C_{ech} = \frac{A_{ech}}{A_{standard}} \times C_{standard} \quad (3)$$

2.4.4. Hematological analysis.

The following hematological constants are evaluated by an automatic hematology counter (Neckman Coulter Dxh 800), the Leukocytes (WBC), the Lymphocytes (LYMPH), the <https://nanobioletters.com/>

Monocytes (MONO), the red blood cells (RBC), the hemoglobin level (HGB), the Average Globular Volume (AGV), the hematocrit (HCT), the Average Corpuscular Hemoglobin Content (ACHC), the white blood cell count (WBCC), platelets (PLT), Medium Platelet Volume (MPV), basophilic polynuclear (BASO), neutrophilic polynuclear (NEUT) and eosinophilic polynuclear (EOS).

2.5. Molecular docking.

2.5.1. Toxicity analysis.

The toxicity analysis was predicted using the web program ProTox-III [25]. A crucial step in the creation of new drugs is the prediction of small-molecule toxicity by ProTox-III. Numerous criteria, including LD₅₀, projected toxicity class, immunotoxicity, neurotoxicity, hepatotoxicity, mutagenicity, carcinogenicity, and cytotoxicity, were considered for the toxicity analysis in the current study.

2.5.2. ADMET studies.

To ascertain the molecule's pharmacodynamic characteristics, the ADMET analysis is crucial. Using a web-based server called SWISSADME, the therapeutic qualities of the most likely natural substances and medications were ascertained [26]. Ligand smiles have been acquired from PubChem and entered into SWISSADME.

2.5.3. Preparation of proteins.

The Protein Data Bank (PDB) provided the crystalline structure of alanine aminotransferase (ALT) (Figure 1). It was then loaded into AutoDock, a molecular docking program [9]. Using the Biovia Discovery Studio 2024 software (Accelrys, San Diego, CA, USA), the ligand molecule was first removed from the active site to further prepare the protein. Next, as suggested in the literature [27], the water molecules were removed. After that, hydrogen atoms were added to the enzyme structures using AutoDockTools 4.2.6 version 1.5.7. The macromolecule was then assigned partial atomic charges (Kollman charges) and converted to a pdbqt file [28].

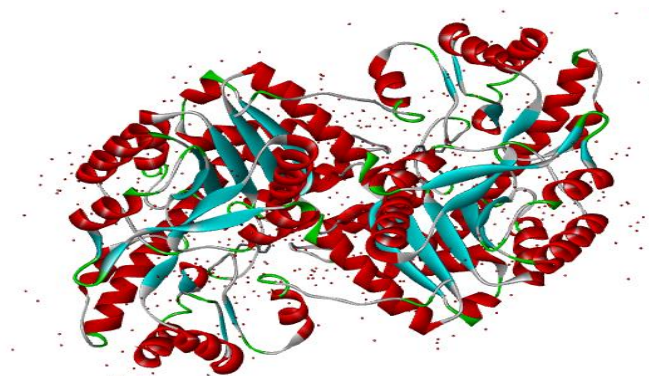


Figure 1. 3D structure of the protein.

2.5.4. Preparation of ligands.

The discovered ligand biomolecules were retrieved from the PubChem-NCBI directory in 3D Standard Data Format (SDF). The ligands of 3D SDF files have been converted into

Protein Data Bank (PDB) format using PyMOL. These ligand molecules were saved in pdbqt format after being downloaded separately using AutoDock Tools 4.2.6 version 1.5.7.

2.5.5. Molecular docking analysis

The molecular docking analysis of all selected bioactive compounds was performed using AutoDock Vina [9] with the standard scripting method; the grid measurements were stored in a config format.txt file. The docking calculations were carried out with the genetic algorithm and the local search parameters (LGA). A grid centered on the co-crystallized ligands for the protein was generated using the following grid settings: resolution of 60 Å × 60 Å × 60 Å, with a grid spacing of 0.500 and coordinates of 0.023, 41.812, and 24.297 for the x, y, and z axes, respectively. For the dynamic simulation, every binding affinity was evaluated and considered. The various postures of complex structures and the interactions between amino acids were studied using Biovia Discovery Studio (Ver. 2024).

2.6. Statistical analysis.

Results are expressed as an average ± standard deviation ($n=5$). Using statistical software (SPSS, ver. 27), the data were analyzed using a one-way ANOVA, followed by Duncan's test ($\alpha=5$) for multiple comparisons and significance testing. $p<0.05$ values were regarded as statistically significant.

3. Results and Discussion

3.1. Chemical analysis.

A total of 49 bioactive compounds were identified from GC-MS analysis of HAAE by comparing their mass spectral fragmentation patterns and retention times (RTs) with those of known compounds in the NIST II library (Table 1 and Figure 2).

Table 1. GC-MS analysis of aqueous extract of *H. aspersa* Müller flesh

RT	Compounds name	Area %
34.113	9-Fluoroundecane	00.129
42.098	(Z)-2-nonadecene	00.111
42.947	2,4-Di-tert-butylphenol	00.332
43.826	Methyl octadec-6,9-dien-12	00.119
47.752	cyclo(leucyl-prolyl) cyclo(prolyl-valyl)	00.434
52.090	Cyclotetradecane	01.614
53.474	,tau,-Muurolol	00.745
54.895	n-Hexadecanoic acid	05.160
58.414	Heptadecanoic acid	00.618
59.841	9,12-Octadecadienoic acid	04.241
60.092	Linoleic acid	11.919
60.213	Oxirane, [(dodecyloxy)methyl]	03.983
60.463	9-Octadecenoic acid (Z)	04.072
60.781	5,8,11,14-Eicosatetraenoic acid	06.027
62.855	Octadecanoic acid	00.281
63.739	Octanoic acid	00.305
63.970	11,14-Eicosadienoic acid	00.341
64.182	Z, Z, Z-8,9-Epoxyeicosa-5,11,14-trienoic acid	00.854
65.641	1,2-O-[2'-Hydroxyoctadecyl]g	01.436
66.050	Octadecane	00.622
66.335	Palmitamide (Hexadecanamide)	00.123
66.612	Ethyl 3-hydroxyoctadecanoate	01.205

RT	Compounds name	Area %
67.915	Methyl 7,11,14-eicosatrienoacid	00.676
68.070	Methyl dehydroabietate	00.559
68.766	Fumaric acid	00.508
69.817	Docosanoic acid	00.401
70.632	2-Isopropyl-4-methyl-1,3-dioxolane	00.160
71.415	1,1-Dimethoxydecane	00.411
72.630	Cholesta-5,7-dien-3-ol, dimethyl	00.617
74.311	Benzenepropanoic acid, 3,5-b	00.341
74.559	Tricosanoic acid	01.536
76.486	Cholesta-3,5-diene	01.630
76.724	Cholesta-4,6-dien-3-ol, (3 β)	00.327
77.180	Cholest-5-en-3-ol (3 β)	06.852
77.602	Cholesta-3,5-dien-7-one	00.236
77.989	Ergosta-5,22-dien-3-ol	00.159
78.309	Cholesta-5,7-dien-3-ol	00.184
79.238	Dihydrobrassicasterol	00.233
79.773	Stigmastan-6,22-dien, 3,5-dedihydro-	00.052
80.914	Clionasterol	00.394
82.234	Desmosterol	00.286
83.419	Ethyl iso-allocholate	00.135
83.697	Cholest-4-en-3-one	05.971
84.203	Cholesta-4,6-dien-3-one	00.264
84.938	β -Sitosterol	00.109
85.556	4-Campestene-3-one	00.240
87.044	γ -Sitostenone	00.205
89.262	Tris(2,4-di-tert-butylphenyl)	00.457
103.451	Phenol, 2,4-bis(1,1-dimethylethyl)	09.332
	Not identified	23.054

Phenol, 2,4-bis(1,1-dimethyl), linoleic acid, Oxirane, [(dodecyloxy)methyl], 9,12-Octadecadienoic acid, Cholest-4-en-3-one and n-Hexadecanoic acid were considered the most abundant.

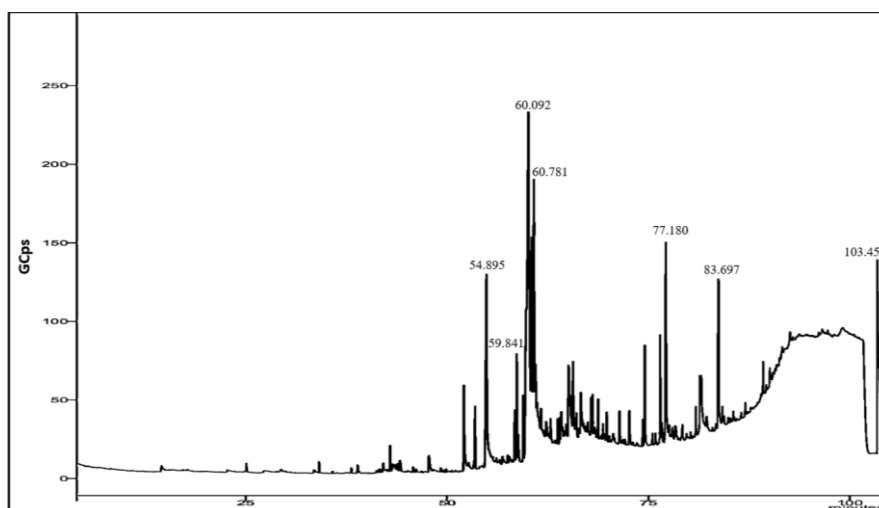


Figure 2. GC-MS chromatogram for the aqueous extract of the flesh of *H. aspersa* Müller.

To conclude, the aqueous extract of *H. aspersa* Müller flesh has a rich chemical composition, and the identification of these elements provides a robust scientific basis for understanding its biological characteristics. The nutritional value and the potentially therapeutic effects of this product, as an anti-inflammatory, antioxidant, and possibly hepatoprotective agent, are supported by its content of essential fatty acids, sterols (including phytosterols and cholesterol derivatives), and antioxidant compounds.

3.2. Subacute toxicity.

The study of subacute toxicity is extremely useful for assessing the safety of test substances and their long-term risks [29]. Given that acute toxicology testing revealed no harmful effects [21], another investigation was conducted to assess the subacute toxicity of HAAE. Based on this, three different doses, including 30, 75, and 150 mg/kg bw, were used to evaluate the health risk over 28 days of HAAE.

Following assessment of behavioural and weight-growth parameters, organ weight, and biochemical and haematological data, the effects of daily oral administration of HAAE at repeated doses were assessed.

3.2.1. Effects of HAAE on behavioral parameters.

No behavioral changes were observed in the rats during the 28 days of the study, regardless of the dose administered. In addition, the weight gain of the treated rats was not significantly different from that of the control group. The body weight of all groups increased gradually over time, indicating that the administration of HAAE had no impact on the normal growth of the animals (Figure 3).

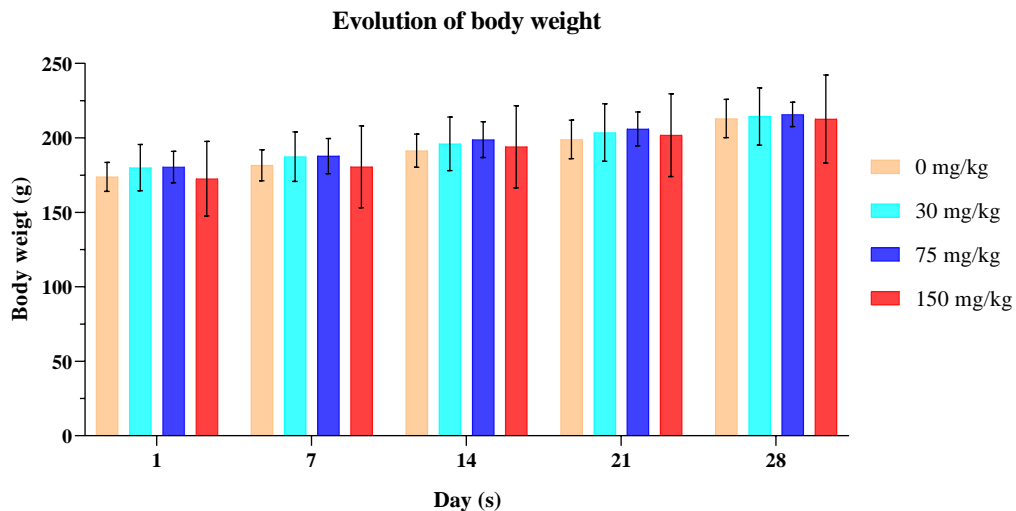


Figure 3. Effect of HAAE on rat body weight.

Due to disruptions in the metabolism of proteins, lipids, or carbohydrates, a decrease in appetite frequently results in weight loss [30].

3.2.2. Effects of HAAE on relative organ weights.

The relative weight of the organs is a sensitive indicator of toxicity. Analysis of the main organs, such as the liver and kidneys, revealed no alterations in weight, color, or texture compared to the control group (Table 2).

Table 2. Aqueous extract effects on relative organ weights in rats.

	weight of rats	Kidneys		Liver	
		Weights (g)	Live weight (%)	Weights (g)	Live weight (%)
Control	213.0 ± 12.83	1.75 ± 0.25	0.87 ± 0.04 ^a	8.68 ± 0.76	4.09 ± 0.33 ^a
30 mg/Kg	214.4 ± 19.42	1.86 ± 0.17	0.87 ± 0.05 ^a	9.07 ± 0.80	4.25 ± 0.32 ^a
50 mg/Kg	215.8 ± 08.14	1.98 ± 0.26	0.92 ± 0.10 ^a	9.13 ± 0.61	4.23 ± 0.23 ^a
150 mg/Kg	212.6 ± 29.57	2.28 ± 0.40	1.02 ± 0.15 ^a	9.31 ± 0.72	4.43 ± 0.47 ^a

Means of the same column that share a letter do not show a significant difference at the 5% risk threshold.

These results suggest that the administration of HAAE did not cause any damage or organ dysfunction. In toxicity investigations, the relative weight of organs is considered a reasonably sensitive indicator [31].

3.2.3. Effects of HAAE on hematological parameters.

The hematopoietic system is very sensitive to toxic substances; its evaluation is crucial for the safety of a product [32]. The hematological analyses showed no significant differences ($p>0.05$) in blood parameters between the treated rats and the control group (Table 3). This indicates that HAAE does not have a toxic effect on the blood composition.

Table 3. Effects of HAAE on hematological and biochemical constants.

Parameters	0 mg/Kg	30 mg/Kg	75 mg/Kg	150 mg/Kg
WBC ($10^3/ml$)	01.06 ± 0.12 ^a	01.09 ± 0.11 ^a	00.10 ± 0.10 ^a	00.98 ± 0.14 ^a
WBCC ($10^3/ml$)	03.32 ± 0.06 ^a	03.60 ± 0.07 ^a	03.66 ± 0.24 ^a	03.60 ± 0.30 ^a
RBC ($10^6/ml$)	07.59 ± 0.00 ^a	07.44 ± 0.38 ^a	07.26 ± 0.23 ^a	07.39 ± 0.40 ^a
HGB (g/dl)	13.13 ± 0.22 ^a	13.40 ± 0.35 ^a	13.17 ± 0.46 ^a	13.10 ± 0.11 ^a
HCT (%)	38.63 ± 0.47 ^a	38.50 ± 0.46 ^a	38.32 ± 0.26 ^a	38.23 ± 0.09 ^a
AGV (fL)	50.50 ± 0.34 ^a	50.70 ± 0.91 ^a	50.80 ± 0.20 ^a	50.23 ± 0.63 ^a
ACHC (pg)	17.35 ± 0.53 ^a	17.32 ± 0.06 ^a	17.33 ± 0.42 ^a	17.10 ± 0.35 ^a
ACHC (g/dl)	34.04 ± 0.59 ^a	33.82 ± 0.17 ^a	33.10 ± 0.52 ^a	34.40 ± 0.26 ^a
MPV (fL)	06.50 ± 2.23 ^a	06.69 ± 0.19 ^a	06.45 ± 0.27 ^a	06.56 ± 0.44 ^a
NEUT (%)	12.45 ± 0.20 ^a	12.43 ± 0.22 ^a	12.54 ± 0.15 ^a	12.63 ± 0.29 ^a
LYMPH (%)	83.77 ± 0.25 ^a	83.63 ± 0.31 ^a	84.08 ± 0.19 ^a	83.06 ± 1.01 ^a
MONO (%)	00.34 ± 0.17 ^a	00.28 ± 0.03 ^a	00.31 ± 0.10 ^a	00.36 ± 0.06 ^a
EOS (%)	02.06 ± 0.01 ^a	01.93 ± 0.12 ^a	01.84 ± 0.09 ^a	02.03 ± 0.20 ^a
BASO (%)	01.10 ± 0.10 ^a	01.24 ± 0.20 ^a	01.12 ± 0.07 ^a	01.20 ± 0.11 ^a
PLT ($10^3/ml$)	877.33 ± 5.38 ^a	876.27 ± 04.02 ^a	876.93 ± 8.38 ^a	877.59 ± 10.37 ^a
ALT (UI/l)	56.16 ± 3.53 ^a	56.75 ± 4.54 ^a	56.75 ± 6.11 ^a	57.04 ± 6.19 ^a
AST (UI/l)	133.28 ± 2.20 ^a	134.15 ± 3.94 ^a	133.86 ± 2.02 ^a	133.57 ± 3.15 ^a
Blood Glucose	1.25 ± 0.20 ^a	1.23 ± 0.25 ^a	1.24 ± 0.13 ^a	1.25 ± 0.21 ^a
Cholesterol	0.94 ± 0.03 ^a	0.96 ± 0.05 ^a	0.97 ± 0.05 ^a	0.96 ± 0.02 ^a

WBC: Leukocytes; LYMPH: Lymphocytes; MONO: Monocytes; RBC: Hematies; HGB: Hemoglobin; AGV: Average Globular Volume; HCT: Hematocrit; ACHC: Average Corpuscular Hemoglobin Content; WBCC: White Blood Cell Count, ACHC: Average Corpuscular Hemoglobin Concentration; PLT: Platelets; MPV: Medium Platelet Volume; NEUT: Neutrophil Polynuclear; BASO: Basophilic Polynuclear; and EOS: Eosinophilic Polynuclear; At the 5% level, the means of the same line with the same letter do not differ significantly.

Mukinda and Syce [33] state that blood parameter analysis is important because it provides information on intravascular effects such as haemolysis, haematopoietic function (assessment of myeloid line cells), and the occurrence of allergies (white blood cell studies).

3.2.4. Effects of HAAE on biochemical parameters.

Biochemical analyses were performed to evaluate liver and kidney functions. The ALT and AST enzymes, which are indicators of liver damage, did not show a significant change in the blood of the treated rats ($p>0.05$). Similarly, glucose and total cholesterol levels remained stable in all groups (Table 3). These results confirm that HAAE had no significant effect on liver and kidney metabolic function or on glucose metabolism.

According to Ozer et al. [34], the liver, in particular, has sensitive indicators of potential tissue damage, including AST and ALT levels. Whereas AST is extensively distributed

throughout the liver and heart, ALT is found in the cytoplasm of hepatocytes. Thus, liver damage may cause the bloodstream's AST and ALT activity to increase.

The kidney's function is to purify the blood and remove waste, while the hepatocytes' function is to neutralise toxins, whether they originate within or outside the body (detoxification). Because the liver and kidneys are essential to an organism's survival, their function must be considered when assessing the toxicity of medications and extracts [35].

They are produced in the cytoplasm of these organs' cells and released into the bloodstream when those cells sustain damage. These enzymes rise in myopathy, myocardial infarction, rhabdomyolysis, and AST, particularly when haemolysis is present. ALT is more specific for liver disease, although AST is a little more sensitive. The increase in AST and ALT levels in the blood is due to their release following damage to liver cells [35].

3.3. ADMET analyze.

In the present study, the pharmacokinetic and physicochemical properties of the natural compounds of *H. aspersa* Müller ((1) 5,8,11,14-Eicosatetraenoic acid, (2) 9,12-Octadecadienoic acid, (3) 9-Octadecenoic acid (Z), (4) Linoleic acid, (5) Cholest-4-en-3-one, (6) Cholest-5-en-3-ol, (7) n-Hexadecanoic acid, (8) Oxirane, [(dodecyloxy)methyl], (9) Phenol, 2,4-bis(1,1-dimethylethyl)) were carried out. All the molecules analyzed followed Lipinski's rule of five. Intestinal absorption is the essential prerequisite for evaluating the apparent effectiveness of an oral medication. In the present study, 5,8,11,14-Eicosatetraenoic acid, 9,12-Octadecadienoic acid, 9-Octadecenoic acid (Z), Linoleic acid, n-Hexadecanoic acid, Oxirane, [(dodecyloxy)methyl], Phenol, 2,4-bis(1,1-dimethylethyl), showed high gastrointestinal absorption, while Cholest-4-in-3-one, (6)Cholest-5-in-3-ol had low gastrointestinal absorption. The 57 isozymes of the human cytochrome P450 family (phase I enzymes) metabolise around two-thirds of all medications; isozymes 1A2, 3A4, 2C9, 2C19, and 2D6 account for 80% of this process, according to research on HAAE's subacute toxicity. The majority of these CYPs are located in the liver and control Phase I responses.

In this study, the probability of being a substrate or an inhibitor is defined by the output value, which varies from 0 (No) to 1 (Yes). The accessible synthesis assessment evaluates the ability to synthesize a large number of chemical compounds that should advance drug discovery. The scores of this method vary from 1 (easy to achieve) to 10 (difficult to achieve). In this research, the synthetic accessibility levels of Cholest-4-en-3-one and Cholest-5-en-3-ol were 5.79 and 5.98. Table 4 shows the results of the ADMET analysis.

Table 4. ADMET results and toxicity analysis of biocompounds.

Sub. N°	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Mw (g/mol)	304.47	280.45	282.46	280.45	384.64	386.65	256.42	242.40	242.40
TPSA (Å ²)	37.30	37.30	37.30	37.30	17.07	20.23	37.30	21.76	21.76
H-bond acceptor	2	2	2	2	1	1	2	2	2
H-bond donors	1	1	1	1	0	1	1	0	0
Molar refr	98.13	89.46	89.94	89.46	122.65	123.61	80.80	74.27	74.27
XLOGP	6.98	6.98	7.64	7.64	8.37	8.72	7.17	4.96	4.96
iLOGP	4.64	4.14	4.01	4.01	4.76	4.89	3.85	4.13	4.13
MLOGP	4.75	4.47	4.57	4.57	6.23	6.34	4.19	2.88	2.88
WLOGP	6.22	5.88	6.11	5.88	7.60	7.39	5.55	4.32	4.32
Lipinski	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ghose	No	No	No	No	No	No	Yes	Yes	Yes
Veber	No	No	No	No	Yes	Yes	No	No	No
Egan	No	No	No	No	No	No	Yes	Yes	Yes
Muegge	No	No	No	No	No	No	No	Yes	Yes
Bioavailability sc	0.85	0.85	0.85	0.85	0.55	0.55	0.85	0.55	0.55

Sub. N°	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
GI absorption	High	High	High	High	Low	Low	High	High	High
BBB per	No	Yes	No	No	No	No	Yes	Yes	Yes
P-gp substrate	No	No	No	No	No	No	No	No	No
CYP1A2 inh	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes
CYP2C19 inh	No	No	No	No	No	No	No	No	No
CYP2C9 inh	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
CYP2D6 inh	No	No	No	No	No	No	No	Yes	Yes
CYP3A4 inh	No	No	No	No	No	No	No	No	No
Log Kp (cm/s)	-3.20	-3.05	-2.60	-2.60	-2.70	-2.47	-2.77	-4.26	-4.26
PAINS	0	0	0	0	0	0	0	0	0
Brenk	1	1	1	1	0	1	0	1	1
Leadlikeness	No	No	No	No	No	No	No	No	No
Syn accessibility	3.26	3.10	3.07	3.07	5.79	5.98	2.31	3.68	3.68
LD ₅₀ (mg/kg)	10000	10000	48	10000	1190	890	900	7800	800
Toxicity class	6	6	2	6	4	4	4	6	4
Nephr	0.55	0.55	0.55	0.55	0.90	0.92	0.53	0.69	0.83
Hepat	0.55	0.55	0.55	0.55	0.69	0.85	0.52	0.89	0.82
Carcin	0.64	0.64	0.64	0.64	0.62	0.59	0.63	0.78	0.53
Muta	1.0	1.0	1.0	1.0	0.97	0.96	1.0	0.54	0.86
Immun	0.98	0.96	0.99	0.96	0.96	0.99	0.99	0.56	0.84
Cyto	0.71	0.71	0.71	0.71	0.93	0.95	0.74	0.83	0.80

Hepat: Hepatototoxicity; *Carcin:* Carcinogenicity; *Muta:* Mutagenicity; *Immun:* Immunotoxicity; *Cytot:* Cytotoxicity; *Nephr:* Nephrotoxicity.

3.4. Toxicity analysis.

Certain toxicity characteristics have been evaluated with ProTox-III. The predictive models are developed from *in vitro* experiments (Tox21 tests, Ames bacterial mutation tests, HepG2 cytotoxicity tests, and immunotoxicity tests) and *in vivo* experiments (carcinogenicity and hepatotoxicity).

The bioactive compounds chosen for this study were inactive in terms of carcinogenicity, mutagenicity, cytotoxicity, and nephrotoxicity, but it was expected that they would exhibit hepatotoxicity and immunotoxicity. In the same way, Cholest-4-en-3-one showed activity with regard to hepatotoxicity and immunotoxicity. In addition, an active activity of Cholest-5-en-3-ol has been observed in terms of immunotoxicity. Table 4 shows the predicted probabilities of the toxicity analysis.

3.5. Evaluation of molecular docking.

Now, molecular docking is used in many applications, such as predicting biological activity and analyzing interactions between peptides and small-molecule proteins [36]. The main objective of the docking analysis is to evaluate the interaction between the protein molecule and the ligand. The interaction between the protein macromolecule and the ligand is described by a binding site, and the bioactive compounds with the lowest binding energy are those that have the greatest importance.

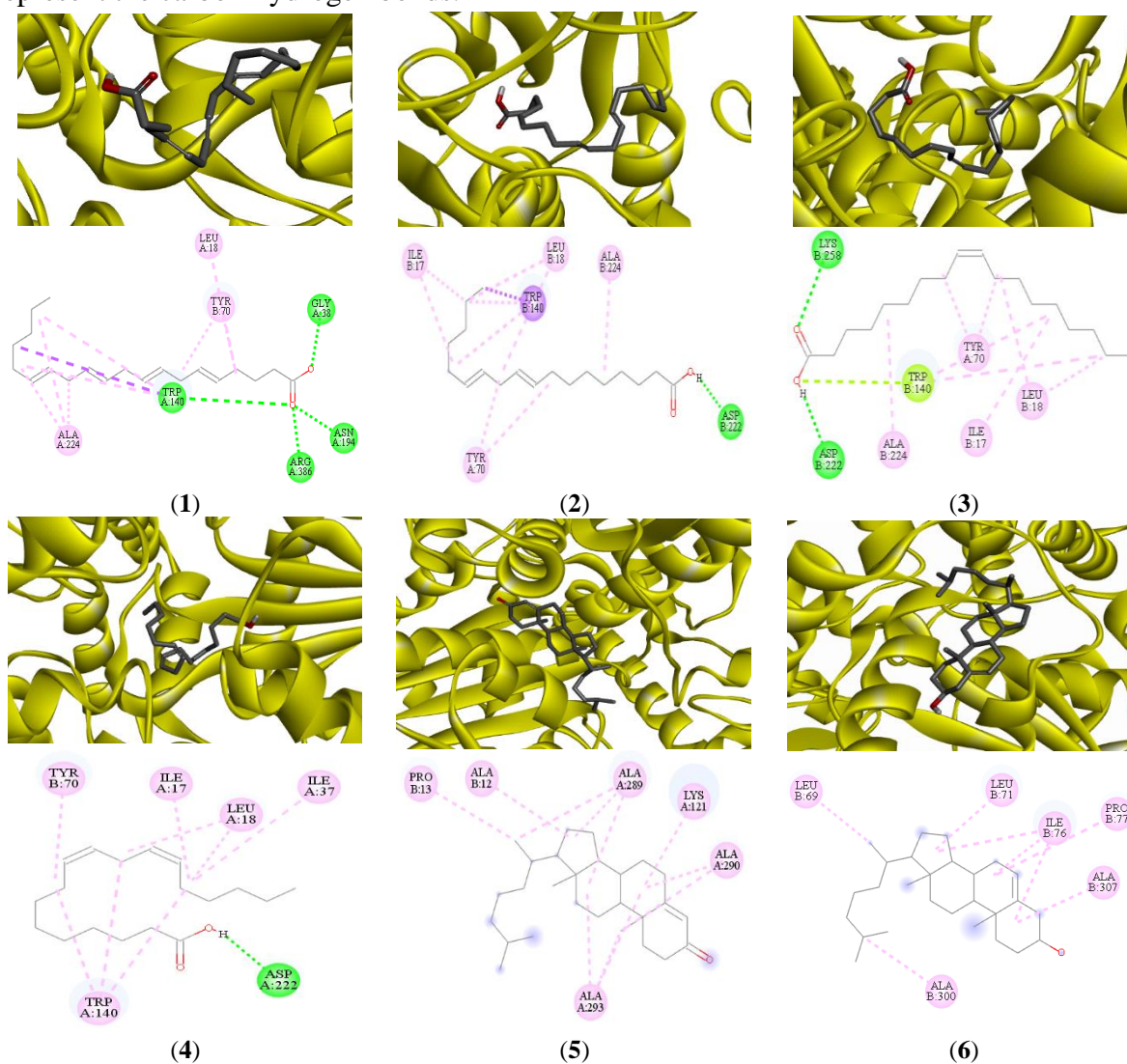
Table 5. Docking scores of the compounds studied in the active sites of ALT.

Compounds	Score d'amarrage	Ki
5,8,11,14-Eicosatetraenoic acid	-3.9 kcal/mol	17.67 mM
9,12-Octadecadienoic acid	-5.4 kcal/mol	593.45 μM
9-Octadecenoic acid (Z)	-4.6 kcal/mol	3.32 mM
Linoleic acide	-7.2 kcal/mol	402.79 μM
Cholest-4-en-3-one	-7.4 kcal/mol	15.97 μM
Cholest-5-en-3-ol	-6.9 kcal/mol	13.17 μM
n-Hexadecanoic acid	-5.08 kcal/mol	189.02 μM
Oxirane, [(dodecyloxy)methyl]	-4.3 kcal/mol	505.10 μM
Phenol, 2,4-bis(1,1-dimethylethyl)	-6.72 kcal/mol	11.83 μM

In this research, the biomolecules of the aqueous extract of the snail *H. aspersa* Müller's flesh were targeted for analysis against alanine aminotransferase. Table 5 presents the results obtained for the binding affinities and the inhibition constant (K_i).

In the present study, Cholest-4-en-3-one showed the highest binding affinity score of -7.4 kcal/mol, followed by linoleic acid (-7.2 kcal/mol). Similarly, 5,8,11,14-Eicosatetraenoic acid produced the lowest binding energy of -3.9 kcal/mol. In summary, a docking score of -7.4 kcal/mol is a very promising value, placing the Cholest-4-in-3-one molecule as a potential candidate for significant biological activity, similar to certain drugs or ligands known for their strong binding affinities.

In addition to the study of binding energies, protein-ligand interactions were also studied. In Figure 4, the green balls and rods represent the hydrogen bonds, the purple balls and rods represent the hydrophobic bonds (Pi-Pi/Pi-sigma/amide-Pi interactions), the pink balls and rods represent the hydrophobic interactions (stack of Pi-alkyl/alkyl interactions), the golden balls and rods represent the hydrophobic bonds (Pi-sulfur), and the white balls and rods represent the carbon-hydrogen bonds.



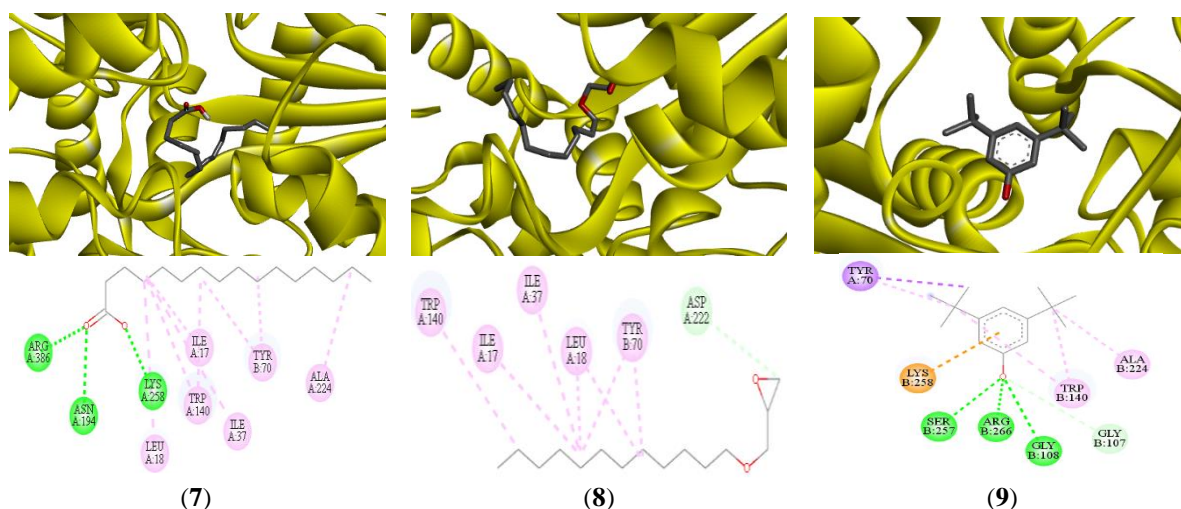


Figure 4. 3D and 2D molecular interactions and the major components.

Figure 4 shows the interactions of the major components with the ALT receptor. The higher binding affinity of Cholest-4-en-3-one is attributed to alkyl interactions with Ala A:293, Ala A:290, Ala B:12, Pro B:13, Ala A:289, and Lys A:290. Similar alkyl interactions with Tyr B:70, Ile A:17, Ile A:37, Leu A:18, and Trp A:140 are shown for linoleic acid, but the latter has a unique conventional hydrogen bond with Asp A:222.

4. Conclusions

This study provided a complete evaluation of the safety profile and the bioactive potential of the aqueous extract of the snail flesh *H. aspersa* Müller. The results of the subacute toxicity study clearly show that there is no toxicity associated with oral administration of the extract at doses of 30, 75, and 150 mg/kg in rats. The absence of significant changes in behavioral parameters, body weight, organ weight, and biochemical and hematological parameters significantly strengthens its safety profile. At the same time, chemical analyses revealed the richness of the extract in bioactive compounds. Molecular modeling has highlighted the inhibitory potential of some of these compounds, in particular Cholest-4-en-3-one and linoleic acid, against the enzyme alanine aminotransferase, suggesting therapeutic potential for the management of liver disorders. As a result, HAAE can be considered a safe substance for consumption, suggesting its possible use as a food supplement or as a source of bioactive molecules. Nevertheless, to fully confirm its safety and explore its clinical applications, it is imperative to conduct additional studies, in particular chronic toxicity, reprotoxicity, and genotoxicity, as well as to validate the inhibitory properties on ALT by in vitro tests. This future research will enable better understanding of the extract's mechanisms of action and optimize its use for human health.

Author Contributions

Conceptualization, M.A.; methodology, M.A. and M.Z.; software, M.A., M.Z., G.K., and S.R.; validation, M.A. and M.Z.; formal analysis, M.A.; investigation, M.A.; resources, H.H.; data curation, M.A. and A.R.; writing—original draft preparation, M.A.; writing—review and editing, M.A., M.Z., and A.R.; visualization, A.R. and L.A.L.; supervision, R.B. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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