FIRST REPORT ON THE HPLC PROFILE, *In Vitro* AND IN *Silico* BIOACTIVITIES OF *Arbutus unedo* L. FRUIT ETHANOLIC EXTRACT FROM ALGERIA

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ABSTRACT

Arbutus unedo L., commonly known as the Strawberry tree, is gaining increasing interest due to its traditional, industrial, and medicinal applications. This study evaluates the *in vitro* and *in silico* biological activities of the ethanolic extract of *A. unedo* fruit, namely its antioxidant, anti-inflammatory and antibacterial properties. HPLC analysis was carried out for the determination of the main components of the extract. Antioxidant activity was assessed via DPPH radical scavenging method, ABTS, metal chelation and β-carotene/linoleic acid bleaching assays while the antiinflammatory activity was done via the inhibition of albumin denaturation method. The *in vitro* antibacterial activity was evaluated by the disk diffusion method against four ATTC strains. Molecular docking was performed using Autodock Vina PyRx docking techniques against ten bacterial protein targets. HPLC analysis identified 11 compounds whose majority components are: chlorogenic acid (22.66µg/mL) and gallic acid (15.43µg/mL). The extract exhibited strong antioxidant potential, with IC_{50} values of 0.1 ± 0.007 mg/mL for DPPH, 0.021 ± 0.02 mg/mL for ABTS, and 0.011 ± 0.006 mg/mL for iron chelation. The β-carotene/linoleic acid test showed inhibition rates ranging from 35.43% ± 0.03 to 89.42% ± 0.05. Additionally, the *in vitro* anti-inflammatory activity revealed an inhibitory effect of 92.97% compared to aspirin (97.40%) at 20 µg/mL. Naringenin, ellagic acid and chlorogenic acid are the best antibacterial candidates with binding energies of less than -8 kcal/mol and more bacterial targets bound. Ferulic acid, methyl gallate, caffeic acid, synergistic acid, and coumaric acid are the safest and pharmacokinetically favorable, while gallic acid, naringenin, and chlorogenic acid have limitations as toxicity or poor absorption. These findings support the traditional medicinal use of *A. unedo* and highlight its potential as a natural bioactive source.

Keywords: *Arbutus unedo*, HPLC analysis, antioxidant, anti-inflammatory, *in silico* antimicrobial, toxicity prediction.

INTRODUCTION

Arbutus unedo L. is an evergreen shrub with a circum-Mediterranean distribution that also reaches the Eurosiberian region in northern Iberia, Atlantic France, and a disjunct population in southern Ireland [1]. The fruits of the plant have been traditionally used as antiseptics, diuretics and laxatives in folk medicine, while the leaves have been used due to their diuretic, urinary antiseptic, antidiarrheal, astringent, depurative and antihypertensive properties [2]. Chemical investigations of leaves and fruits show the presence of essential oil, flavonoids, proanthocyanidins, iridoid glucosides, sugars, non-volatile and phenolic acids, vitamins C and E and carotenoids [3]. Antioxidants play an important role in health-promoting biochemical pathways. Phenolic compounds are popular group of secondary metabolites with wide pharmacological activities. Varied biological activities of phenolic acids were reported. Increases bile secretion, reduces blood cholesterol and lipid levels and antimicrobial activity against some bacterial strains such [4]. Nonetheless, this plant remains largely underexploited and organizations such as Food and Agriculture Organization (FAO) are currently undertaking to increase the use of this species [2]. Various inflammatory stimuli such as excessive ROS produced in the process of oxidative metabolism have been reported to initiate the inflammatory process [5]. Inflammation is an immune response that enables survival during infection in response to pathogens [6]. During inflammation, various pathogen-derived components are recognized by pathogen recognition receptors (bacteria, viruses, fungi, etc.), inducible nitric oxide synthase produces NO, excess of this NO reacts with ROS at the site of infection to form highly chemically reactive nitric oxide species [7]. The close link between oxidative stress, inflammation and infection encourages the search for resources capable of intervening to block one of these components which may exert a wide range of pharmacological activities for eventual potential health implications. This report aimed to evaluate the phytochemical, antioxidant, antibacterial activity and toxicity prediction of the main constituents of *A. unedo* fruit extract. To the best of our knowledge, this is the first report on this species from Algeria.

MATERIALS AND METHODS

Plant Material and Extract Preparation

The fruits of *A. unedo* L. were collected from Beni Aziz, Setif province (North East of Algeria) during autumn harvests. The species was identified by Dr. Nouioua Wafa from Laboratory of Phytotherapy Applied to Chronic Diseases. The ethanolic extract was prepared by macerating 76 g of the plant material in a water/ethanol mixture (20:80) at a ratio of 1:4 (powder/mixture) for three days with agitation. Then, the extract was filtered through Whatman filter paper. Then, the solvent was evaporated using a rotary evaporator under reduced pressure at 45°C. The crude extract was stored at 4°C until use [8].

HPLC Analysis

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Zorbax Eclipse plus C8 column (4.6 mm x 250 mm i.d., 5 µm) under optimized chromatographic conditions. The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–1 min (82% A); 1-11 min (75% A); 11-18 min (60% A); 18-22 min (82% A); 22-24 min (82% A). The multi-wavelength detector was monitored at 280 nm. The column was maintained at 40°C, and the injection volume was 5 µL.

Antioxidant Activity

DPPH Assay

The free radical scavenging activity was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) previously described [9]. The antiradical activity was expressed as EC_{50} (µg/mL) and calculated as follows:

$$DPPH\ scavenging\ effect\ (\%) = \frac{A0\ -\ A1}{A0} \times 100$$

Where: A_0: the absorbance of the control at 30 min, A_1: is the absorbance of the sample at 30 min.

β-Carotene Bleaching Assay

In this test, the antioxidant capacity of the extract is determined according to the method of [10]. Synthetic antioxidant BHT was used as a positive control and the absorbance of the mixture was measured at 490 nm after 0, 1, 2, 4, 6, and 24 hours. The antioxidant activity (AA) was measured in terms of successful bleaching of β-carotene by using the following equation:

$$AA\% = \frac{Abs\ sample}{Abs\ BHT} \times 100$$

Abs sample: Absorbance in the presence of the extract; Abs BHT: Absorbance in the presence of positive control BHT.

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ABTS radical cation decolorization assay

The radical scavenging assay against ABTS was measured according to [11]. The extent of decolorization as percentage inhibition of the ABTS radical cation is determined as a function of concentration and time and calculated relative to the reactivity of

Trolox as the standard. To determine the IC_{50} values, a dose response curve was plotted. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity.

Ferrous ion chelating activity

The Ferrous ion chelating effect of the extracts was determined according to the detailed method of [12]. In this method, the inhibition of the formation of the Fe^{2+}-ferrozine complex is utilized after treating samples with Fe^{2+} ions. Subsequently, the absorbance of the Fe^{2+} ferrozine complex was measured at 562 nm to assess the extent of inhibition in the formation of this complex. The chelating activity was expressed as a percentage using the following equation:

$$CA\ (\%) = \frac{AC - ATS}{AC} \times 100$$

CA : Chelating activity, AC : Abs of control, ATS : Abs of test sample. To determine the IC_{50} values, a dose response curve was plotted. IC $_{50}$ is defined as the effective concentration of the test material that is required to chelate 50% of iron ions.

***In vitro* anti-inflammatory activity**

The anti-inflammatory activity was studied using the albumin denaturation technique according to [13] with slight adjustments, the experimental procedure involves separating the egg white, and adjusting its volume with Tris-HCl buffer to create a 1:100 dilution. After gentle stirring and filtration, equal volumes of the egg white solution are distributed into tubes. Subsequently, the extract and aspirin are added to each tube, followed by incubation at 74°C for 15 minutes. Then, the samples are cooled, and their absorbance at 650 nm is measured. The percentage inhibition of protein denaturation was calculated as follows:

$$I\% = \frac{Abs\ Control\ - Abs\ sample}{Abs\ Control} \times 100$$

Antibacterial activity

The antimicrobial activity was assessed against four bacterial strains (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 2035, *Bacillus subtilis* ATCC6633 *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922) in addition to a yeast strain (*Candida albicans* ATCC 1024) by the disk diffusion method as reported by the EUCAST [14]. Gentamicin (10 µg) was used as positive control for bacteria. The aromatogram is performed by substituting the antibiotic with paper discs impregnated with the extract at the content of. 20 µg (10 µg /ml). Then inhibition zones are measured in mm.

***In silico* antibacterial activity**

Target choice

The target proteins (Table 1) were selected due to their direct involvement in key biological functions such as adhesion, virulence, quorum sensing regulation, membrane permeability, and antibiotic resistance. Their functional diversity allows for a multi-target approach to maximize the chances of effectively interfering with the bacterial infection process while limiting the emergence of resistance.

Table 1: Microbial proteins used as targets in molecular docking

Protein	Role	Oeganisme	PDB code
Bacterial Dynamin-Like Protein Lipid Tube Bound	Mediates membrane fission, fusion, and restructuring events	*E. coli*	2w6d
Adhesin protein	Adhesion properties of pathogens to their target receptors	*E. coli*	6GTW
LasR	Quorum sensing	*P. aeruginosa*	6MVM
NorA	Efflux pump	*S aureus*	9B3L
OmpC	Porins involved in a wide range of virulence	*E.coli*	2J1N
OmpF	Porins involved in a wide range of virulence	*E.coli*	2ZFG
Acr	Blocks the activity of Cas endonucleases, thereby preventing recognition and cutting of target DNA	*E.coli*	4DX5
ABC ATP-Binding Cassette **Sav1866**	Multidrug ABC transporter which facilitates the export of diverse cytotoxic drugs across cell membranes	*S aureus*	2HYD
Histidine Kinase WALK	Participates in the regulation of efflux pumps and modulates biofilm formation, making bacteria more resistant to treatment.	*B.subtilis*	3SL2
Lipoteichoicacidsynthase	Lipoteichoic acid biosynthesis	*S aureus*	2W5Q

Phytocompounds

The compounds revealed by the HPLC analysis were the subject of the *in silico* antibacterial study to determine their binding affinities. The 3D structures of the phytocompounds with their compound identifiers CID were obtained in SDF format files from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and Molview (https://molview.org/?cid=5478883) then converted to PDB format by Open Babel tools version 2.4.1.

The 3D structures of the target proteins were obtained in PDB format from the Protein Data Bank (https://www.rcsb.org/). Autodock Vina in PyRx was used to generate the best predicted binding modes and corresponding binding energies of the phytoconstituents. The interactions were visualized in 3d and 2d forms using BIOVIA Discovery Studio visualizer 2024 v.24.1.0.23298 software. Bonds between the ligands and interacting residues are depicted with a distance range of Å.

Pharmacokinetics and toxicity prediction

Lipinski's method was employed to assess the drug-like properties of phytocompounds, which sets limits on four specific physicochemical parameters [15]. These are the characteristics of an orally active drug: the octanol-water partition coefficient (milogP) and the number of hydrogen bond donors (n-OH and n-NH) should not exceed 5, and the number of hydrogen bond acceptors (n-ONs) should be less than 10. The molecular weight (MW) should be below 500 D, and no more than one violation should occur [16]. Molinspiration Cheminformatics free web services(https://www.molinspiration.com , Slovensky Grob, Slovakia) and SwissADME free online tools (http://www.swissadme.ch/) were used to predict physicochemical and pharmacokinetic parameters, while OSIRIS Property Explorer online tools (https://www.organic-chemistry.org/prog/peo/) were used to predict toxicity risks.

RESULTS

Identification and quantification of phenolic compounds

The ethanolic maceration was chosen because it uses sustainable raw materials or environmentally friendly solvents (i.e., ethanol and water) and provides an extract rich in bioactive compounds [17]. The extraction of *A. unedo* fruits resulted in a crude extract with a yield of 38.1% of the dry weight. The HPLC chromatogram identifying phenolic compounds is shown in figure 1, the chromatogram determined nine representative peaks.

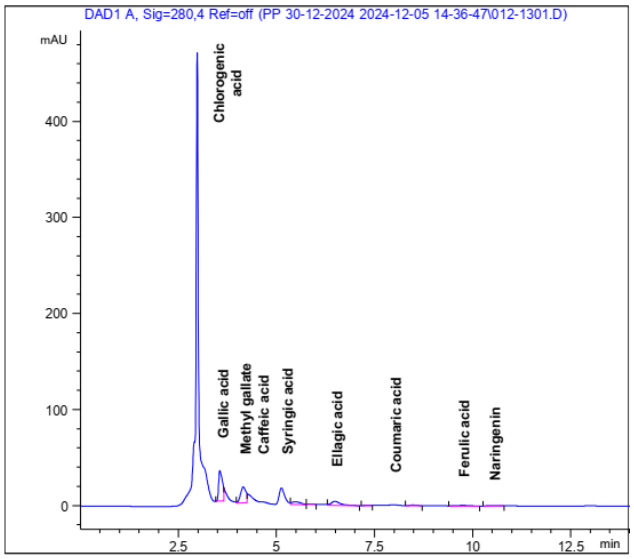


Figure 1: HPLC chromatogram of *A. unedo* fruits ethanolic extract

The HPLC analysis revealed a diverse range of phenolic acids (table 2), with chlorogenic acid (22.66 µg/g) and gallic acid (15.43 µg/g) being the predominant compounds. These results highlight the significant presence of phenolic acids, which likely contribute to the extract's antioxidant, antimicrobial, and anti-inflammatory activities observed in biological assays.

Table 2: Phenolic composition of *A. unedo fruits* ethanolic extract

Compound	Concentration (µg/mL)	CID
Chlorogenic acid	22.66	1794427
Gallic acid	15.43	370
Syringic acid	3.84	10742
Methyl gallate	2.72	7428
Ferulic acid	0.58	445858
Ellagic acid	0.47	5281855
Caffeic acid	0.39	689043
Naringenin	0.43	439246
Coumaric acid	0.34	637542

Antioxidant tests

The antioxidant potential of *A. unedo* fruit extract was evaluated using various biochemical assays, including DPPH free radical scavenging, metal chelation, total antioxidant capacity, and lipid peroxidation inhibition, which are all recognized for their effectiveness in the evaluation of antioxidant activity.

DPPH radical scavenging activity

A. unedo fruit extract displayed an antioxidant activity in a dose-dependent manner. The IC_{50} value obtained for the ethanolic extract was 0.1 ± 0.007 mg/mL, indicating a lower antioxidant activity compared to BHT (IC_{50}= 0.011 ± 0.001 mg/mL) and quercetin (IC_{50}= 0.0023 ± 0.00 mg/mL), which is supported by the percentages of the antioxidant relative reduction (ARR).

Table 3: DPPH scavenging activity of *Arbutus unedo* extract and standards (Each value represents the mean of n = 3 ± SD)

	IC_{50} (µg/mL)	% of ARR
BHT	0.011±0.001	95.27 ±0.005
Quercetin	0.023±0.000	85.94 ±0.004
A. arbutus	0.10±0.007	71.77 ±0.002

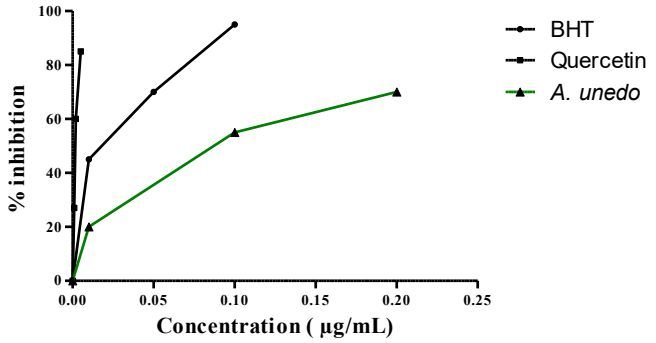


Figure 2: DPPH scavenging activity

β-carotene/linoleic acid bleaching assay

The polarity-dependent β-carotene bleaching test is useful for evaluating the antioxidant power of natural extracts [18]. As shown in figure 3, the extract showed potent activity. The inhibitory activity of *A. unedo* extract ranges from 35.43% to 89.42%, which is lower than that of the BHT. However, at higher concentrations, the extract shows a remarkable inhibitory activity of about 97.95%, suggesting a strong antioxidant potential.

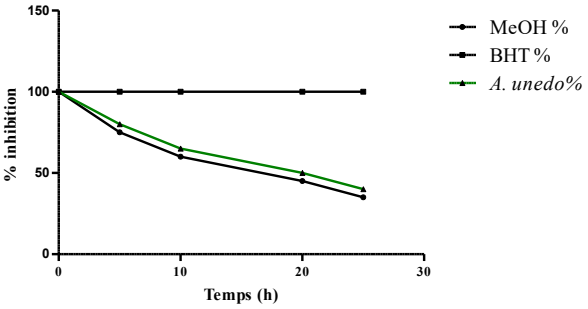


Figure 3: Antioxidant activity of *A. unedo* extract compared to the BHT

ABTS scavenging capacity

The results presented in figure 4 show that the IC_{50} value of the ethanolic extract of *A. unedo* is 0.021 ± 0.02 mg/mL, which is higher than that of the standard antioxidant BHT (IC_{50} = 0.014 ± 0.00 mg/mL), indicating slightly lower antioxidant efficiency compared to the synthetic reference compound.

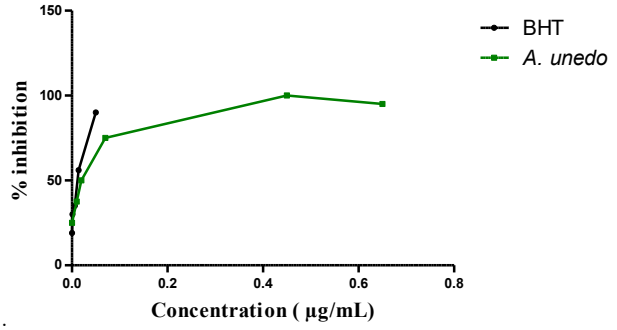


Figure 5: Antioxidant activity of *A. unedo* extract by the ABTS test

Ferric ion reducing power

The reducing power of the extracts may serve as a significant indicator of its potential antioxidant activity. For the ethanolic extract of *A. unedo*, the EC_{50} value was 0.011 ± 0.006 mg/mL demonstrating a strong reducing power but it remains lower than that of the well-known metal chelator EDTA (EC_{50} = 0.002 ± 0.00 mg/mL).

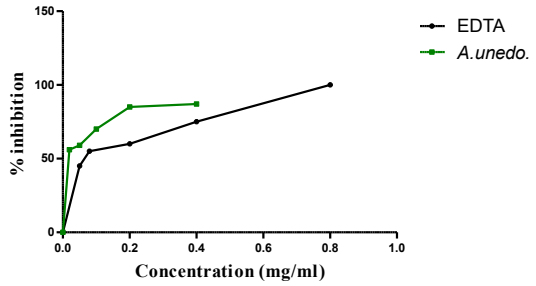


Figure 4: Antioxidant activity of *A. unedo* extract by the reducing power test

Antiinflammatory activity

Protein denaturation is a well-established contributor to inflammation, and the ability of plant extracts to inhibit this process is considered a key indicator of their anti-inflammatory potential (Chandra et al., 2012). In this study, the ethanolic extract of *A. unedo* was evaluated for its capacity to inhibit heat-induced albumin denaturation. The results showed that the inhibition percentage ranged from 45.18% ± 1.19 to 92.97% ± 4.31 across concentrations of 2.5 to 20 µg/mL. In comparison, aspirin, a well-known nonsteroidal anti-inflammatory drug (NSAID), exhibited a concentration-dependent inhibition of protein denaturation, reaching a significantly higher inhibition rate of 97.74% at 20 µg/mL (figure 6).

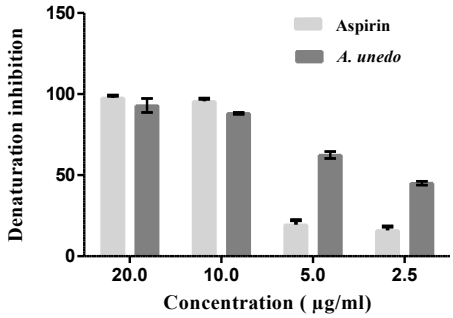


Figure 6: Inhibition of albumin denaturation

Antimicrobial Activity

The evaluation of the antimicrobial activity of the plant extract was carried out by the agar diffusion method, comparing the diameters of the inhibition zones with those induced by gentamicin (table 4). The results show that the extract exhibits variable antimicrobial activity depending on the strains tested. Against Gram-positive bacteria, the extract showed moderate activity against *S. aureus* (15.2 ± 0.5 mm and 12.12 ± 0.23 mm) and *E. faecalis* (13.8 ± 0.3 mm), with an efficacy, however, lower than that of gentamicin (28 to 32 mm). On the other hand, against *B. subtilis*, an inhibition zone of 16.5 ± 0.4 mm was observed, reflecting a more marked activity; although still lower than that of the antibiotic (38 mm). For Gram-negative bacteria, the extract demonstrated significant activity against *K. pneumoniae* (11.3 ± 0.6 mm), but less effective than gentamicin (36 mm). However, higher activity than gentamicin was observed against *P. aeruginosa* (17.46 ± 0.12 mm vs. 8.5 ± 1.23 mm) and *E. coli* (17.86 ± 0.22 mm vs. 8.23 ± 0.35 mm).

Table 4: Diameters of the inhibition zones induced by the extract in mm

Microorganism	Extract	Gentamicin
S. aureus	15.2 ± 0.5	32
E. faecalis	13.8 ± 0.3	28
B. subtilis	16.5 ± 0.4	38
K. pneumoniae	11.3 ± 0.6	36
P. aeruginosa	17.46± 0.12	8.5
S. aureus	12.12± 0.23	12.12
E.coli	17.86± 0.22	8.23

***In silico* antimicrobial study**

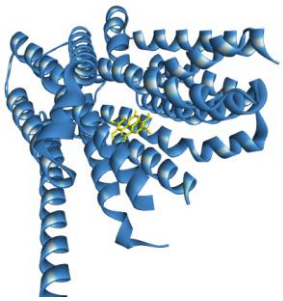
Molecular docking analysis of binding energies identified several phytocompounds with high affinity for target bacterial proteins (Table 5). Among the ligands tested, ellagic acid showed exceptional affinity toward LasR (-10.8 kcal/mol) and Walk (-10.4 kcal/mol), two proteins involved in quorum sensing regulation and membrane signaling, respectively, suggesting a potential antivirulence effect. Similarly, chlorogenic acid showed strong interaction with FimH (-9.4 kcal/mol), a bacterial adhesion protein, and Walk (-9.7 kcal/mol), highlighting its interest in inhibiting adhesion and signaling. Naringenin also showed good affinity with several targets, including NorA (-8.8 kcal/mol), an efflux pump associated with antibiotic resistance, suggesting a potential resistance-modulating effect. Additionally, methyl gallate showed a significant interaction with LasR (-10.3 kcal/mol), comparable to that of ellagic acid. In contrast, other compounds such as gallic acid, ferulic acid, and syringic acid showed lower binding energies (generally greater than –7 kcal/mol), reflecting lower affinity toward the targeted proteins.

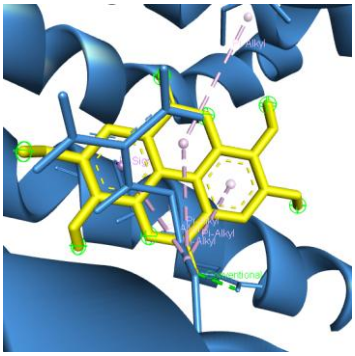
Table 5: Phytocompounds scoring results

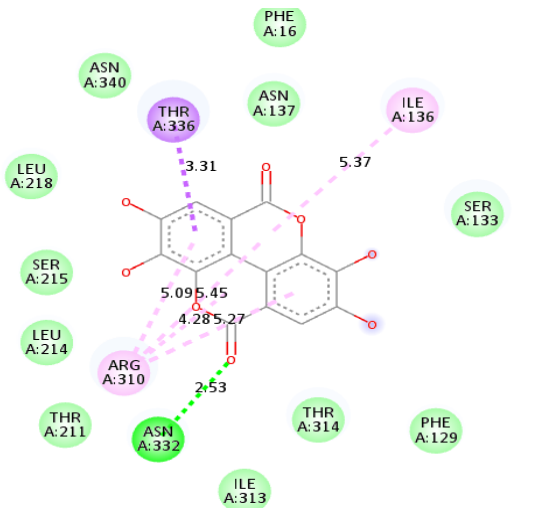
Ligand / Protein	Naringenin	Ferulic acid	Coumaric acid	Ellagic acid	Syringic acid	Caffeic acid	Methyl gallate	Chlorogenic acid	Gallic acid
Binding energies (kcal/mol)									
2w6d	-7.9	-6.4	-7.3	-8.7	-6.2	-7.6	-7.0	-7.2	-6.0
ABC	-7.6	-5.9	-5.5	-8.6	-5.2	-5.8	-5.4	-6.5	-5.4
AcrB	-7.9	-6.0	-5.6	-7.6	5.7	-6.1	-5.9	-7.3	-5.9
FimH	-7.1	-6.7	-6.2	-7.3	-6.2	-6.7	-6.3	-9.4	-6.8
LasR	-10.2	-7.3	-7.5	-10.8	-6.6	-7.6	-5.6	-10.2	-7.2
Lipo	-8.2	-6.9	-6.6	-9.4	-5.9	-7.0	-7.1	-7.2	-7.1
NorA	-8.0	-6.2	-6.0	-8.2	-5.8	-6.4	-5.8	-7.4	-5.6
OmpC	-7.6	-5.8	-5.9	-7.9	-5.5	-6.1	-5.4	-7.7	-5.4
OmpF	-7.5	-5.8	-5.7	-8.5	-5.4	-5.9	-5.3	-7.5	-5.6
Walk	-9.2	-7.3	-7.0	-10.4	-6.6	-7.1	-6.6	-9.7	-6.3

Ellagic acid exhibits strong interactions with LasR, OmpF, NorA, Walk, ABC, lipoteichoicacidsynthase, and Bacterial Dynamin-Like Protein Lipid Tube Bound (Figure 7) suggesting a potential role in inhibiting membrane transport and the qurum-sensing mechanism. Notably, Ellagic acid formed hydrogen bonds with polar amino acids (Glu 273, Gln 334, Arg 333, Arg 414, Asn 794, Arg 61, Asp 65, Thr 75, Thr 501, His293, Thr 295, Asn 332, Asp 121, Tyr 124, Thr 241,Tyr and Lys 243) demonstrating the most favorable binding interactions. Although less potent than ellagic acid, Naringin is involved in the active sites of LasR, Lipo, NorA, and Walk proteins, with hydrogen bonds involving purely polar amino acids (Thr 75, Arg 61, Asn 564, His 293, Thr 295, and Asp 533). Similarly, Chlorogenic acid docked with FimH, LasR, and Walk reveals the involvement of, primarily, polar amino acids (Gln 133, Asp 54, Asp 47, Tyr 48, Tyr 137, Tyr 64, Asp 533, Tyr 507, Lys 506, and Asn 503).

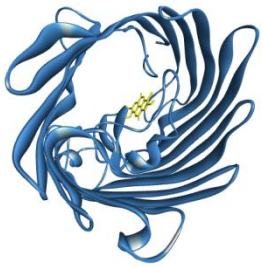
Ellagic acid/NorA

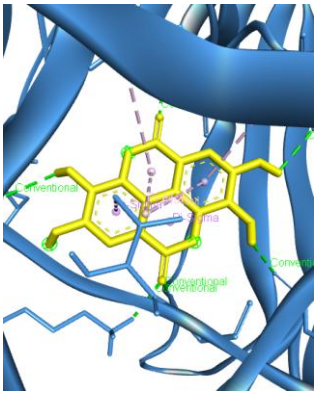


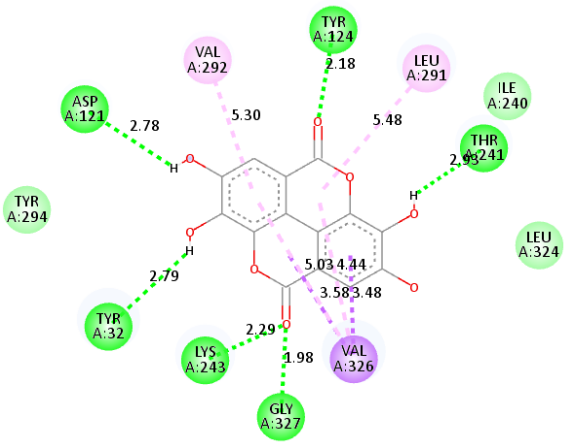




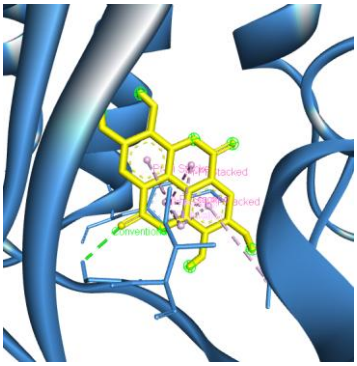
Ellagic acid/OmpF

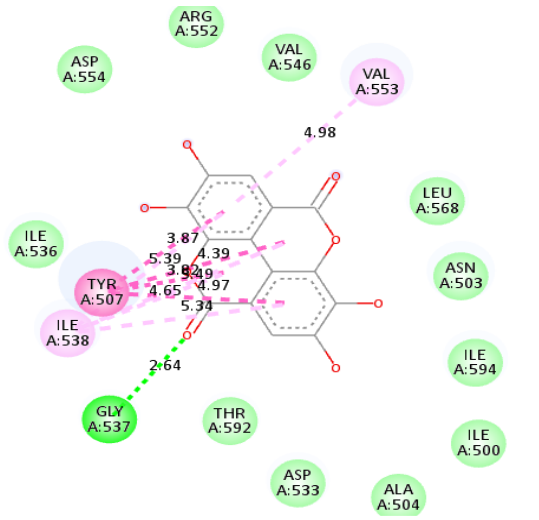




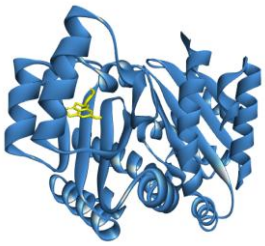


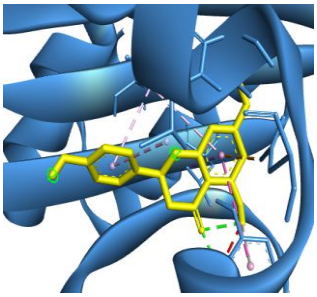
Ellagic acid/Walk

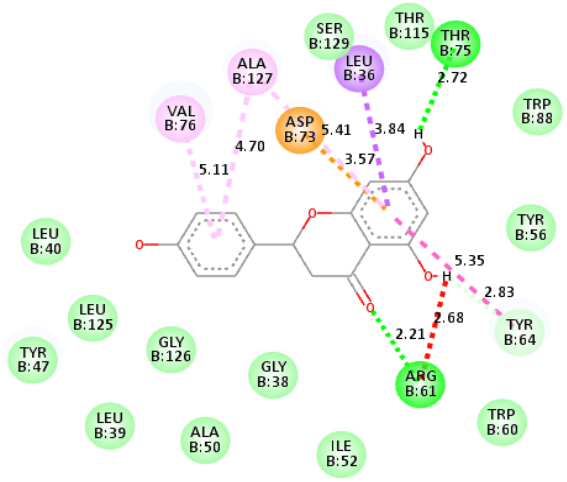




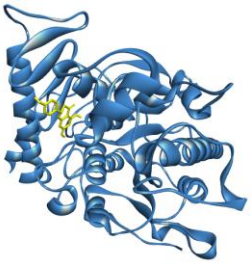
Naringin/LasR

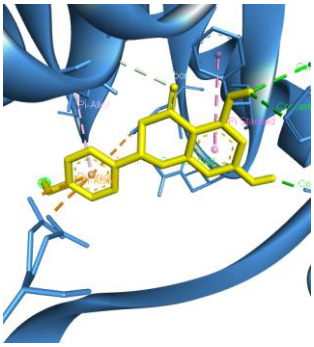


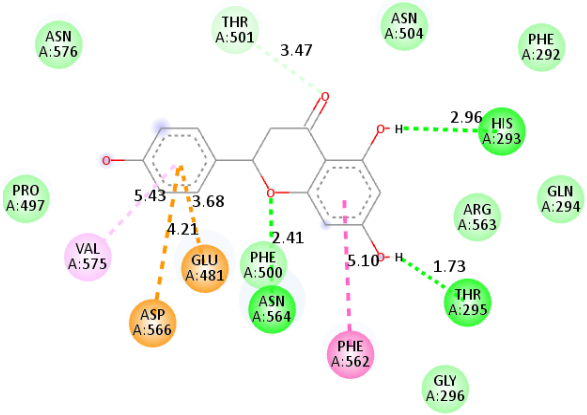




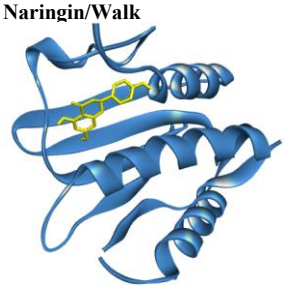
Naringin/Lipo

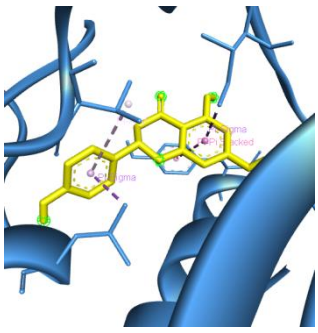


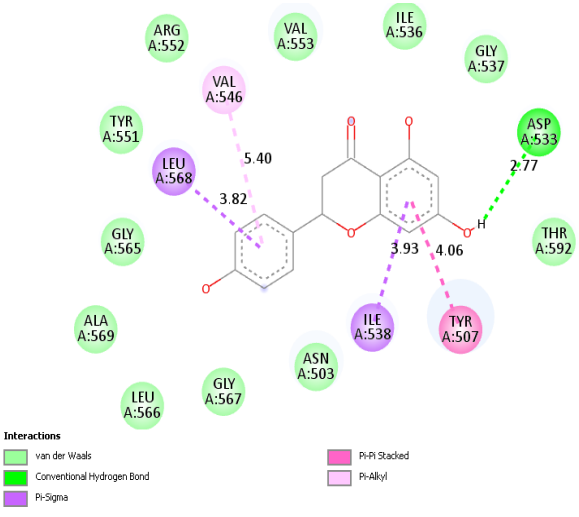




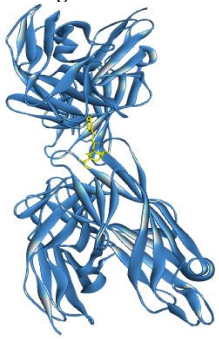
Naringin/Walk

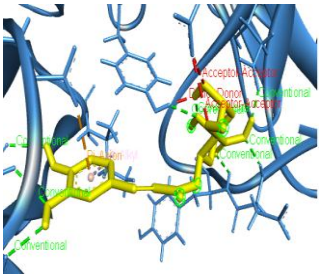


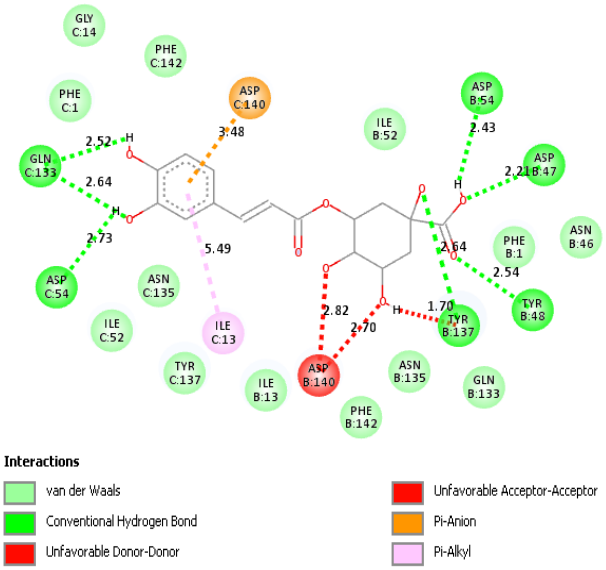




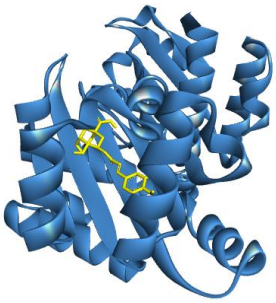
Chlorogenic acid/ FimH

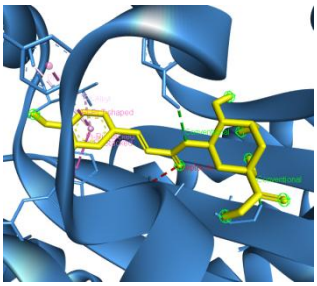


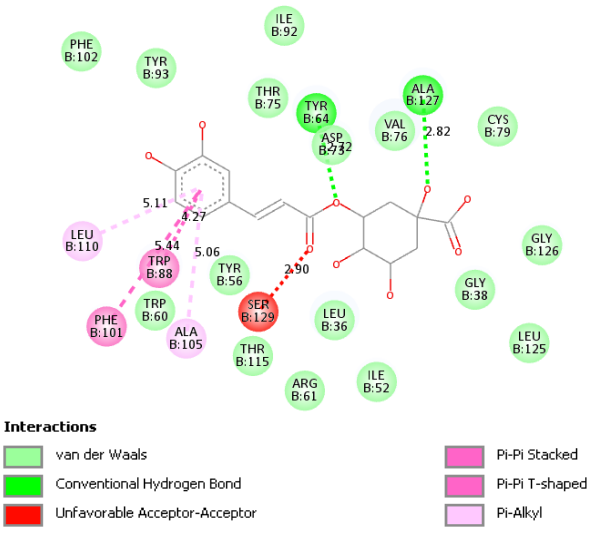




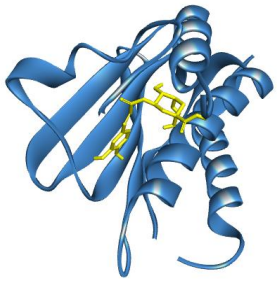
Chlorogenic acid/ LasR

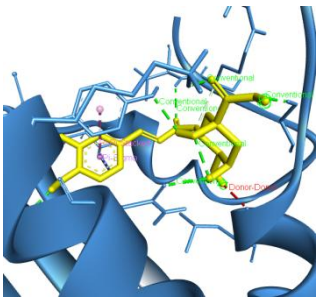






Chlorogenic acid/ Walk





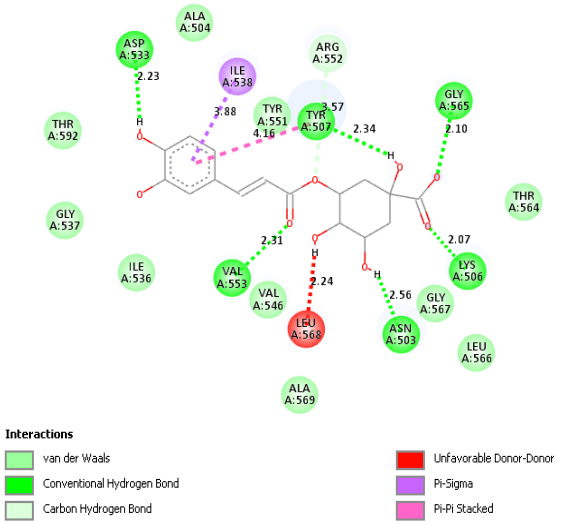


Figure 7: Interaction and bond distances of ligands inside the active site pocket as shown by molecular surface maps

Toxicity prediction

All the compounds comply with Lipinski's rule of five except Chlorogenic acid. The intestinal absorption predictions reveal that most compounds demonstrate high absorption potential, while chlorogenic acid shows low absorption. Log Kp values less than -6 indicate very low transdermal permeability which is presented by all predicted compounds (Kp values -6.17 to -8.76 cm/s), which limits their direct topical application. The CYP450 enzyme interaction profile shows that naringenin, gallic and elagic acids may undergo metabolism by CYP3A4 and CYP1A2, which could lead to low clearance *in vivo*. The other compounds, with no significant effect on CYP 450, potentially offer more stable pharmacokinetic profiles. All predicted compounds have good water solubility, which favors oral or parenteral formulation. This finding is particularly relevant when combined with the low molecular weights and the promising antibacterial potential. Chlorogenic, methyl gallate, and ellagic acids do not present any predictive mutagenic, tumorigenic, irritant, or reprotoxic risks. This reinforces their pharmacological potential as safe candidates. However, potential mutagenic (gallic, caffeic, syringic and ferulic acids) tumorigenic (caffeic acid, ferulic acid) and reproductive (Gallic, caffeic, coumaric and ferulic acids) concerns warrant further investigation.

Table 6. Calculated physicochemical and pharmacokinetic parameters of the docked phytocompounds

Compound		**Gallic acid**	**Chlorogenic acid**	**Methyl gallate**	**Caffeic acid**	**Syringic acid**	**Ellagic acid**	**Coumaric acid**	**Ferulic acid**	**Naringenin**
Physicochemical and pharmacokinetic parameters (Molinspiration Cheminformatics)										
miLogP < 5		0.59	-0.45	0.85	0.94	1.20	0.94	1.43	1.25	2.12
TPSA (oA) < 500		97.98	164.74	86.99	77.75	76.00	141.33	57.53	66.76	86.99
MW < 500 (g/mol)		170.12	354.31	184.15	180.16	198.17	302.19	164.16	194.19	272.26
V		135.10	296.27	152.63	154.50	170.15	221.78	146.48	172.03	230.26
nON < 10		5	9	5	4	5	8	3	4	5
nOHNH < 5		4	6	3	3	2	4	2	2	3
Lipinski's violation		0	1	0	0	0	0	0	0	0
Solubility and pharmacokinetics properties (SwissADME)										
Water solubility		S to VS	S to VS	S to VS	S to VS	S to VS	S	S	S	S
BBB permeant		No	No	No	No	No	No	Yes	Yes	No
Gastrointestinal absorption		High	Low	High	High	High	High	High	High	No
Log K_p: Skin permeation: cm/s		-6.84	-8.76	-6.8	-6.58	-6.77	-7.36	-6.26	-6.41	-6.17
Cytochromes inhibitors	CYP1A2	No	No	No	No	No	Yes	No	No	Yes
	CYP2C19	No	No	No	No	No	No	No	No	No
	CYP2C9	No	No	No	No	No	No	No	No	No
	CYP2D6	No	No	No	No	No	No	No	No	No
	CYP3A4	Yes	No	No	No	No	No	No	No	Yes
Toxicity risks (OSIRIS Property Explorer)										
Mutagenic		Yes	N	N	Yes	Yes	N	N	Yes	N
Tumorigenic		N	N	N	Yes	MR	N	N	Yes	N
Irritant		N	N	N	N	N	N	N	N	N
Reproductive effective		Yes	N	N	Yes	N	N	Yes	Yes	N

miLogP: Logarithm of partition coefficient between n-octanol and water. TPSA: Topological polar surface area. MW: Molecular weight. MV: Molecular volume. nON: Number of hydrogen bond acceptors. nOHNH: Number of hydrogen bond donors. No: No effect, N: no indication found. S: Soluble. VS: very soluble. MR medium risk.

DISCUSSION

A. unedo is of increasing interest because of its common traditional, industrial, chemical and pharmaceutical uses [19]. It possesses pharmacologically active compounds that have been traditionally used for medicinal purposes due to the high content of its fruit phytochemicals, mainly polyphenolic compounds [20]. The phenolic profiles of the different species of *A.unedo* extracts reported in the literature do not differ much, the polyphenols detected in our extracts exist in almost all extracts studied outside Algeria [21–25]. These polyphenols exhibit diverse structures that contribute probably to their antioxidant and anti-inflammatory properties [22]. In plant extracts, polyphenols performe the antioxidant activity [26] which originate from their properties of proton loss, chelate formation, and dismutation of radicals [27]. The phenolic hydroxyl group structure reacts easily with free radicals and can form hydrogen radicals with an antioxidant effect to eliminate the activity of hydroxyl radicals and superoxide anions [28]. In the DPPH test, the IC_{50} of our extract was 0.10±0.007 µg/mL lower compared to BHT and quercétine but better than [29] (0.83 mg/mL), [30] (0.25 mg/mL) and [26] (7.956 mg/mL) for the leaves aqueous extract. The extract showed a remarkable inhibition of lipid peroxidation of about 97.95%, suggesting a strong antioxidant potential which is in agreement with previous studies [25,31]. Polyphenols act as free radical scavengers in a lipid system, thus preventing oxidative damage in biological membranes. The best antioxidant activity was revealed by the ABTS test (0.021mg/mL), which was higher than the BHT. This can be attributed to the fact that polyphenols act synergistically, multiplying the sites of interaction with oxidative species unlike BHT, which has a specific action. Anyway, it is recommended that a combination of assays for scavenging electron or radical, such as DPPH, ABTS, or FRAP, and for the assays associated with lipid peroxidation be used [32], although the different antioxidant levels may reflect a relative difference in the ability of antioxidant compounds in extracts to reduce the free radical DPPH, ABTS, and oxidative bleaching [25]. The proteins denaturation is the cause of inflammation and rheumatoid arthritis [26]. Therefore, combating protein denaturation can decrease inflammation. *A. unedo* fruit extract showed an inhibition close to that of Aspirin especially at 20 µg/mL (92.97% and 97.74% respectively) better than values found by [33] (58.73 %) at 500 µg/m from the fruit methanolic extract [26] (74.28%) at 500 µg/mL in the leaves water extract. It seems that the main compounds in the extract are responsible for this effect; according to [34], the chlorogenic acid increased the denaturation temperature of BSA which may indicate a stabilization of this protein against heat denaturation due to a stronger binding interactions. On the other hand, binding of gallic acid with protein increased protein intramolecular packing and induced higher thermal stability [35]. *S. aureus*, *B. subtilis P. aeruginosa* and *E.coli* were strains susceptible to our extract. These results corroborate those of [36,37] on *E. coli*, *S. aureus* and *B. cereus*. However, [36] results reveal the resistance of *P. aeruginosa* (0mm) which suggests that our extract could act via alternative mechanisms (membrane disruption, metal chelation, enzyme inhibition), making these strains sensitive despite their resistant profile. Moreover, [37] found an activity on *E. faecalis* and *K. pneumoniae*, but no effect on the Gram negative strains which were susceptible in our case. The recent advances in computational approaches have significantly developed the rationale for identifying and designing pharmacologically active natural molecules that can target proteins of interest [38]. These molecular targets are associated with a wide spectrum of infectious mechanisms. In this study, ten key proteins involved in the bacterial virulence process were the subject of molecular docking of all components of the extract. The high binding affinities observed for Naringenin, ellagic and chlorogenic acids, suggest strong interaction with essential target proteins, such as LasR and Walk. These proteins often play a role in metabolic regulation or antibiotic resistance, suggesting that these compounds could disrupt pathways critical for bacterial survival. The multi-targeting observed for some compounds such is particularly promising in a context where resistance to conventional antibiotics poses a major challenge. By acting on several proteins simultaneously, these molecules could reduce the risk of resistance development, a strategy similar to that of antibiotic combinations used clinically. These compounds were reported t obe antibacterial [39–42]. Furthermore, chlorogenic acid could disorder many physiological pathways, mainly including the ones of antagonizing biofilms [43], downregulating ribosomal subunits, affecting lipid metabolism [44]. Chlorogenic acid increased intracellular membrane permeability and resulted in the leakage of intracellular materials which resulted in the decrease of LPS contents of *P. aeruginosa* and supported the detachment of outer membrane [45]. As for its safety, [46], claims that the *A. unedo* extract can replace synthetic antioxidants and colorants and might lead to the design of healthy food products. The combination of binding affinities with these pharmacokinetic/toxicity properties suggest that compound like methyl gallate may represent the most balanced candidates, showing favorable binding affinities, good absorption profiles, minimal CYP interactions and no toxicity.

CONCLUSION

The study revealed that the ethanolic extract of *A. unedo* is rich in phenolic compounds, as confirmed by HPLC analysis. It exhibited strong antioxidant and anti-inflammatory activities, with high reducing power and lipid peroxidation inhibition. The extract also inhibited protein denaturation, suggesting promising anti-inflammatory potential. Thie *in silico* study identifies ellagic acid, chlorogenic acid, and naringenin as promising candidates for antibacterial drug development. Their strong and broad-spectrum binding affinities warrant further experimental validation to elucidate their mechanisms of action and therapeutic potential. These findings highlight its pharmacological interest for natural therapeutic applications but future work should focus on isolation, *in vitro* testing, and synergy studies to optimize their efficacy.

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