İCMAL MƏQALƏ **AÇIQ GİRİŞ (OPEN ACCESS)**

İNVESTİGATİON OF *IN VITRO* ANTİOXİDANT ACTİVİTY AND *IN SILICO* BİOLOGİCAL ACTİVİTY OF FLAVONOİDS FROM *LIMONIUM CASPIUM* GROWİNG İN AZERBAIJAN

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Abstract

The antioxidant properties of the flavonoids myricetin (M1), myricitrin (M2) and myricetin-3′-O-sulfate (M3), isolated from the roots of Limonium caspium (Willd.) P.Fourn (Plumbaginaceae) native to the flora of Azerbaijan were comparatively assessed in vitro, while their biological activity assessed in silico (PASS Online, SwissADME, SwissTarget and ADMETlab 3.0). In addition, ligand–macromolecule interactions were investigated through molecular docking analysis. The antioxidant activity of the compounds was determined using the DPPH radical scavenging assay, and the IC_{50} values (μg/mL) were calculated as follows: M1 (7.81), M2 (6.34), and M3 (6.20). In silico predictions indicated that M1 exhibits pronounced antitumor activity (92.4%), whereas M2 (99.0%) and M3 (99.1%) demonstrated strong hemostatic potential.

Molecular docking studies were performed using AutoDock Vina 4.2 software. Compound M3 was selected as the ligand, and xanthine dehydrogenase was chosen as the target protein. The M3 molecule was found to interact with the active site of xanthine dehydrogenase through multiple interaction mechanisms. The overall interaction profile indicates strong and specific binding of M3 to the enzyme, suggesting its potential role as a bioactive inhibitor.

Keywords: Limonium caspium (Willd.) P. Fourn., myricetin, myricitrin,myricetin-3′-O-sulfate, antioxidant activity, in silico, molecular docking, in vitro

EXPERIMENTAL SECTION

INTRODUCTION

Plants of the genus *Limonium* are rich in biologically active phenolic compounds, and the species *Limonium caspium* (Willd.) P. Fourn. (*Plumbaginaceae*) has widely grown in the flora of Azerbaijan [1].

Antioxidants are compounds capable of neutralizing the harmful effects of free radicals and protecting cells from oxidative stress. Free radicals can damage cellular membranes, DNA, and proteins, thereby contributing to the development of cancer, cardiovascular diseases, neurodegenerative disorders, and other chronic pathologies. Phenolic compounds

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of plant origin, particularly flavonoids usually considered potent natural antioxidants. They exert their antioxidant effects both by scavenging free radicals through hydroxyl groups and by chelating metal ions. The antioxidant activity of flavonoids is commonly assessed *in vitro* using DPPH, ABTS, FRAP, and CUPRAC assays. Numerous studies have demonstrated that flavonoids isolated from *L. caspium* (Willd.) P. Fourn. exhibit pronounced antioxidant properties, highlighting their potential medical and pharmacological relevance [2].

In silico studies encompass the analysis of biological processes and molecular interactions through computer-based modeling and bioinformatics tools.

The aim of the present study is to investigate the antioxidant properties of flavonoids *in vitro*, as well as to perform a comparative *in silico* assessment of their biological activity, physicochemical properties, solubility, bioavailability, and pharmacokinetic parameters, along with molecular docking analysis of myricetin (**M1**), myricitrin (**M2**), and myricetin-3′-O-sulfate (**M3**) isolated from *L. caspium*.

MATERIALS AND METHODS

In vitro determination of antioxidant activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed according to the method described by Blois et al. and adapted for a 96-well microplate format [3]. This method assesses the effect of antioxidant-active compounds on the DPPH radical solution. The unpaired electron of DPPH accepts a hydrogen atom from the tested compound, resulting in the formation of the corresponding hydrazine. The antioxidant activity index, IC_{50}, represents the concentration of a compound required to inhibit 50% of DPPH radical absorbance at a wavelength of 517 nm; lower IC_{50} values indicate stronger antioxidant activity. Compounds **M1**, **M2**, and **M3** were initially dissolved in methanol, and serial dilutions at different concentrations (1–20 µg/mL) were prepared to determine optimized IC_{50} values.

Gallic acid (Sigma Aldrich, batch SLCN 0435) was used as a positive control, dissolved in methanol and six analytical solutions were prepared within the concentration range of 0.5–5.0 µg/mL.

A fresh DPPH solution at a concentration of 0.060 mg/mL was prepared daily by dissolving 6 mg of DPPH (Sigma Aldrich, D9132-5G, batch 0000187174) in 100 mL of methanol and allowing the solution to keep in the dark at room temperature for 1–3 hours before the analysis.

The DPPH assay was carried out in a 96-well microplate. The first row served as a blank row and contained methanol only, while the second row represented the negative control containing methanol and the DPPH reagent. In the subsequent rows, analytical solutions of each sample were added in triplicate, along with an additional row containing only the sample solution without DPPH.

The composition of each well was as follows:

Blank wells: 250µL methanol (MeOH)

Negative control wells: 50 µL MeOH + 200µL DPPH solution in MeOH

Sample or positive control wells: 50µL sample or positive control + 200 µL DPPH solution.

Sample blank wells: 50µL sample + 200 µL MeOH

The 96-well microplate was covered with a lid and incubated in a spectrophotometer (Agilent BioTek Epoch, USA) for 1 hour. Absorbance measurements were recorded at 517 nm. Antioxidant activity and IC_{50}

values (defined, as the concentration required inhibiting 50% of DPPH radicals) were calculated using established equations [4].

In silico assessment of biological activity.

For the comparative *in silico* assessment of the biological activity of flavonoids **M1**, **M2**, and **M3**, the PASS Online, SwissADME, SwissTargetPrediction, and ADMETlab 3.0 platforms were employed. Molecular docking simulations of ligand–macromolecule interactions were conducted using AutoDock 4.2.

Compound **M3** was selected as the ligand, while xanthine dehydrogenase was chosen as the target macromolecule. The crystal structures of the target proteins were retrieved from the Protein Data Bank (https://www.rcsb.org; PDB IDs: 1BML, 1JRO). The PDB format of the ligand (compound **M3**) was generated using MarvinSketch software (https://chemaxon.com/ marvin).

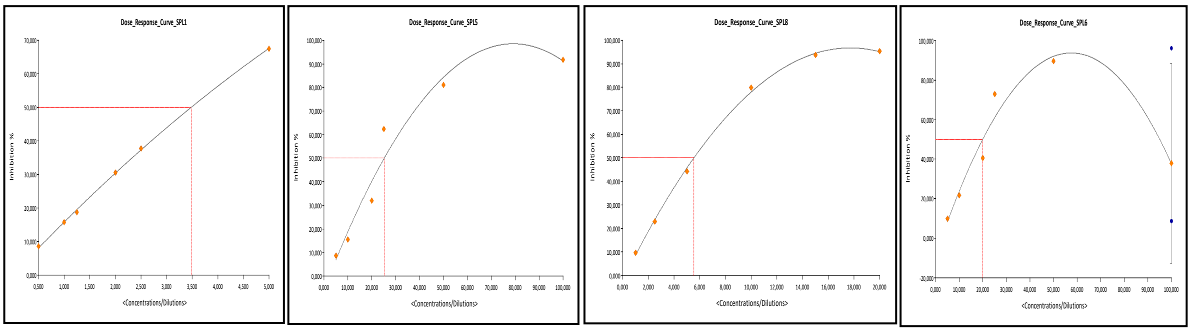
Protein preparation: the downloaded crystal structure of xanthine dehydrogenase was prepared prior to molecular docking simulations. Initially, all non-essential molecules, including co-crystallized ligands, solvent molecules, and water residues, were removed using Discovery Studio Visualizer software (https://discover.3ds.com/discovery-studio-visualizer-download). Subsequently, the cleaned protein structures were processed in AutoDock Tools version 1.4.5. During this step, polar hydrogen atoms were added to the protein structures, and Kollman partial charges were assigned to all atoms to ensure accurate representation of electrostatic interactions. The prepared protein structures were then saved in PDBQT format, which is required for molecular docking calculations, and were used as receptor models in subsequent docking studies.

Grid box parameters were set to dimensions of 80 × 80 × 80 Å. Molecular docking simulations were performed using AutoDock 4.2 via the command-line interface [5].

RESULTS AND DISCUSSION.

In vitro antioxidant activity

The antioxidant activity of methanolic solutions of **M1**, **M2**, and **M3** at various concentrations was assessed using the DPPH assay. The resulting dose–response curves are presented in Figures 1 and 2.



1 2 3 4

Fig 1. Graphical representation of the DPPH assay results (1 – gallic acid, 2 – **M1**, 3 – **M2**, 4 – **M3**).

The IC_{50} values of the DPPH assay for the flavonoids (**M1, M2,** and **M3)** isolated from methanolic extracts of *L. caspium* roots were calculated using the polynomial equation **$Y = C \cdot X^2 + B \cdot X + A$**, where **$Y = 50$** (Table 1).

TABLE 1. IC_{50} values representing the antioxidant activity of flavonoids isolated from *Limonium caspium*.

Curve name	A	B	C	R^2	X
Gallic acid	0.103	16.3	-0.553	0.999	3.48
M1	-1.16	7.76	-0.155	0.998	7.81
M2	-2.35	11.2	-0.316	0.999	6.34
M3	-0.103	9.55	-0.237	0.999	6.2

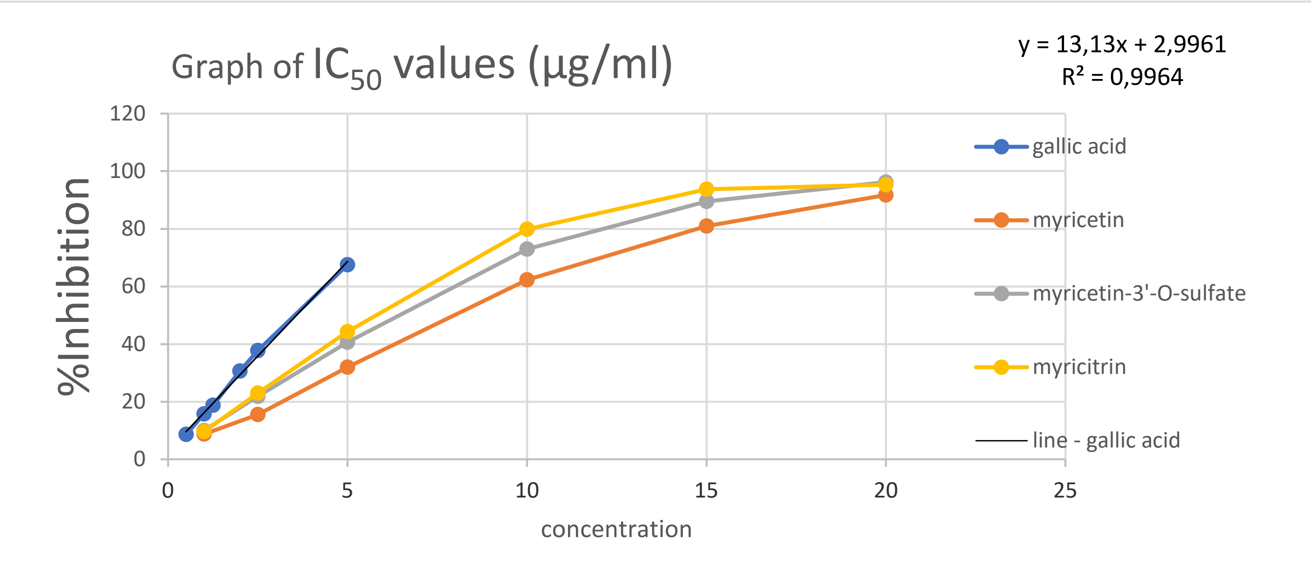


Fig 2. Dependence of the percentage of inhibition on the concentration of the tested compounds.

***In silico* assesment of the biological activity of the compounds using computational tools.**

The biological activity of compounds M1, M2, and M3 was investigated in silico using the PASS Online software (Tables 2–4).

TABLE 2**. Predicted biological activity of compound M1**.**M1 (canonical structure):**
: OC1=CC(O)=C2C(OC(=C(O)C2=O)C2=CC(O)=C(O)C(O)=C2)=C1)

Pa	Pi	Biological activity	Pa	Pi	Biological activity
0,924	0,005	Antineoplastic	0,792	0,011	Membrane permeability inhibitor
0,913	0,005	HIF1A expression inhibitor	0,783	0,004	Antimutagenic

0,889	0,006	Chlordecone reductase inhibitor	0,769	0,006	Peroxidase inhibitor
0,894	0,013	Membrane integrity agonist	0,795	0,034	Ubiquinol-cytochrome-c reductase inhibitor
0,868	0,003	Prostate cancer treatment	0,763	0,004	HMOX1 expression enhancer
0,847	0,004	Kinase inhibitor	0,750	0,005	UGT1A9 substrate
0,794	0,008	2-Dehydropantoate-2-reductase inhibitor	0,759	0,016	TP53 expression enhancer

According to the program predictions, compound **M1** exhibits pronounced antitumor activity (92.4%), anticancer activity against prostate cancer (86.8%), antimutagenic activity (78.3%), as well as other biological activities.

TABLE 3. **Predicted biological activity of compound M2.M2** (**canonical structure**): C[C@@H]1O[C@@H](OC2=C(OC3=CC(O)=CC(O)=C3C2=O)C2=CC(O)=C(O)C(O)=C2)[C@H](O)[C@H](O)[C@H]1O)

Pa	Pi	Biological activity	Pa	Pi	Biological activity
0,990	0,001	Hemostatic	0,972	0,000	Morphine 6-dehydrogenase inhibitor
0,985	0,001	Membrane permeability inhibitor	0,972	0,002	Lipid peroxidase inhibitor
0,985	0,001	Membrane integrity agonist	0,967	0,000	Iodide peroxidase inhibitor
0,983	0,001	Cardioprotectant	0,964	0,002	UDP-glucuronosyltransferase substrate
0,979	0,001	Monophenol monooxygenase inhibitor	0,962	0,001	Vasoprotector
0,978	0,001	Free radical scavenger	0,955	0,001	Anticarcinogenic
0,977	0,001	CYP1A inducer	0,954	0,002	Anaphylatoxin receptor antagonist

According to the prediction results, compound **M2** demonstrates strong hemostatic (99.0%), cardioprotective (98.3%), vasoprotective (96.2%), and other biological properties.

TABLE 4. **Predicted biological activity of compound M3. M3** (**canonical structure**): OC1=CC(O)=C2C(=O)C(OS(O)(=O)=O)=C(OC2=C1)C1=CC(O)=C(O)C(O)=C1)

Pa	Pi	Biological activity	Pa	Pi	Biological activity
0,991	0,001	Hemostatic	0,929	0,001	SULT1A3 substrate
0,958	0,001	Beta-carotene 15,15'-monooxygenase inhibitor	0,928	0,004	HIF1A expression inhibitor
0,959	0,003	CYP2C12 substrate	0,919	0,002	Kinase inhibitor

0,955	0,002	Benzoate-CoA ligase inhibitor	0,916	0,003	Membrane permeability inhibitor
0,944	0,003	Chlordecone reductase inhibitor	0,913	0,003	UDP-glucuronosyltransferase substrate
0,943	0,002	Sulfotransferase substrate	0,878	0,001	Coenzyme-B sulfoethylthiotransferase inhibitor
0,931	0,002	Peroxidase inhibitor	0,876	0,004	2-Dehydropantoate 2-reductase inhibitor

According to the program predictions, compound **M3** exhibits pronounced hemostatic activity (99.1%) along with other biological activities [5].

Investigation of M1, M2, and M3 using the SwissADME program

Figure 3 presents the bioavailability radar diagrams for compounds **M1, M2,** and **M3**. The radar plots illustrate the main physicochemical parameters affecting oral bioavailability, including LIPO (lipophilicity), SIZE (molecular size), POLAR (polarity), INSOLU (solubility), INSATU (degree of saturation), and FLEX (molecular flexibility). Localization of the red polygon within the pink optimal area indicates a higher likelihood of drug-like properties. The distribution of these parameters differs among **M1, M2,** and **M3**, suggesting variations in their bioavailability potential. Overall, the radar diagrams provided by the SwissADME program allow a comparative assesment of the compounds from an ADME perspective.

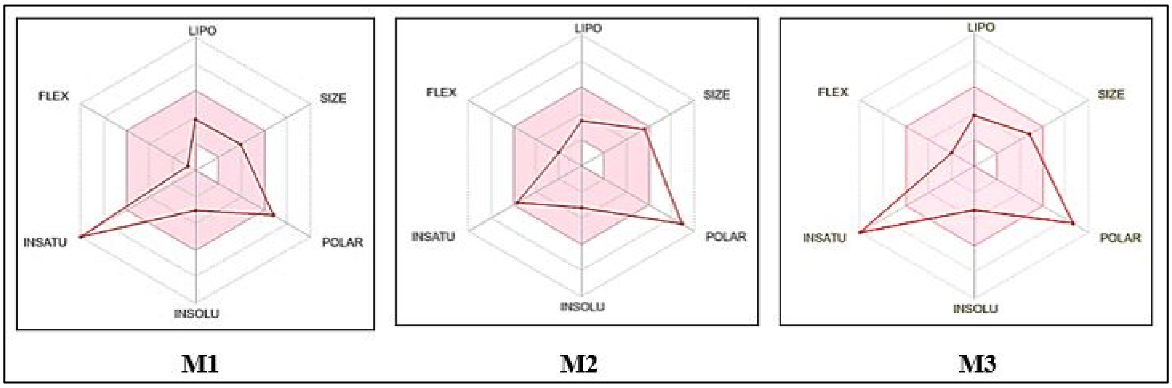


Fig 3. Investigation of compounds **M1, M2,** and **M3** using the SwissADME program.

Physicochemical properties, water solubility, pharmacokinetics, lipophilicity, drug-likeness, and medicinal chemistry parameters were predicted using the SwissADME software (Tables 5–9).

TABLE 5. **Physicochemical properties M1**, **M2** and **M3**

Physicochemical Properties	Substances		
	M1	M2	M3
Molecular weight	318.24 g/mol	464. 38 g/mol	398. 30 g/mol
Num. heavy atoms	23	33	27

Num. arom. heavy atoms	16	16	16
Fraction Csp3	0.00	0.29	0.00
Num. rotatable bonds	1	3	3
Num. H-bond acceptors	8	12	11
Num. H-bond donors	6	8	6
Molar Refractivity	80.06	111.02	90.26
TPSA	151. 50 Å²	210. 51 Å²	203.34 Å²

TABLE 6. **Solubility M1**, **M2 and M3 in water**

	Substances		
	M1	**M2**	**M3**
Log S(ESOL)	-3.01	-3.20	-3.32
Solubility	3.14e-01 mg/ml; 9.88e-04 mol/l	2.92e-01 mg/ml; 6.29e-04 mol/l	1.91e-01 mg/ml; 4.80e-04 mol/l
Class	Soluble	Soluble	Soluble
Log S (Ali)	-3.96	-4.50	-5.09
Solubility	3.50e-02 mg/ml; 1.10e-04 mol/l	1.46e-02 mg/ml; 3.15e-05 mol/l	3.26e-03 mg/ml; 8.18e-06 mol/l
Class	Soluble	Moderately soluble	Moderately soluble
Log S(SILICOS-IT)	-2.66	-1.49	-2.27
Solubility	6.98e-01 mg/ml; 2.19e-03 mol/l	1.49e+01 mg/ml; 3.21e-02 mol/l	2.14e+00 mg/ml; 5.38e-03 mol/l
Class	Soluble	Soluble	Soluble

TABLE 7. **Pharmacokinetics M1**, **M2** and **M3**

Pharmacokinetics	**Substances**		
	M1	**M2**	**M3**
GI absorption	Low	Low	Low
BBB permeant	No	No	No
P-gp substrate	No	No	Yes
CYP1A2 inhibitor	Yes	No	Yes
CYP2C19 inhibitor	No	No	No
CYP2C9 inhibitor	No	No	No
CYP2D6 inhibitor	No	No	No
CYP3A4 inhibitor	Yes	No	No
Log K_p (skin permeation)	-7.40 cm/s	-8.77 cm/s	-7.86 cm/s

TABLE 8. **Lipophilicity M1**, **M2** and **M3**

Substances	**Lipophilicity**					
	Log $P_{o\backslash w}$ (LOGP)	Log $P_{o\backslash w}$ (XLOGP3)	Log $P_{o\backslash w}$ (WLOGP)	Log $P_{o\backslash w}$ (MLOGP)	Log $P_{o\backslash w}$ (SILICOS-IT)	Consensus Log $P_{o\backslash w}$
M1	1.08	1.18	1.69	-1.08	1.06	0.79
M2	1.71	0.51	0.19	-2.32	-0.46	-0.07
M3	-0.43	1.22	2.25	-1.50	-0.52	0.20

TABLE 9. **Medicinal chemistry prediction results M1, M2, and M3**

Substances	**Medicinal Chemistry**			
	PAINS	Brenk	Leadlikeness	Synthetic accessibility
M1	1 alert: catechol_A	1 alert: catechol	Yes	3.27
M2	1 alert: catechol_A	1 alert: catechol	No; 1 violation: MW>350	5.32
M3	1 alert: catechol_A	2 alerts: catechol, sulfonic_acid_2	No; 1 violation: MW>350	3.58

Research of M1, M2 and M3 using the SwissTarget program

The SwissTarget program allows predicting macromolecular targets of a substance (Fig. 4).

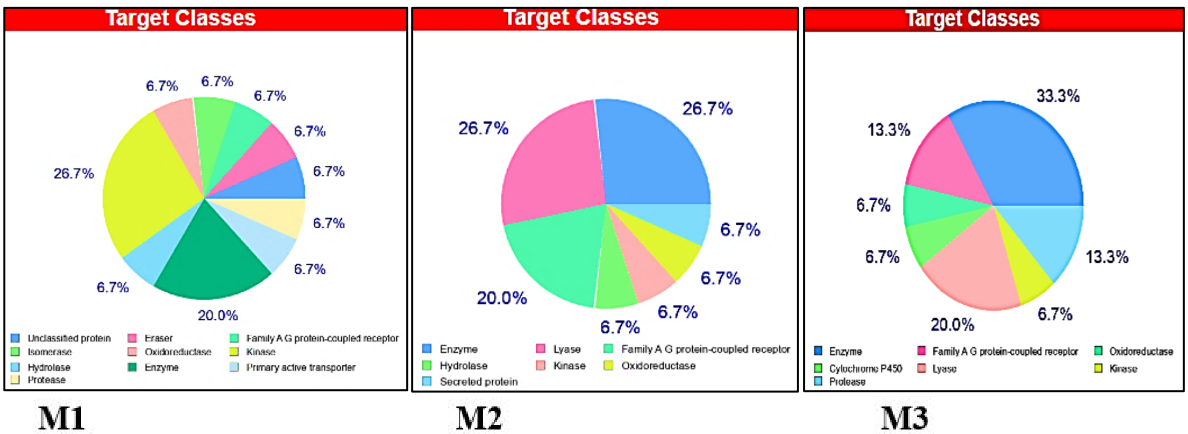


Fig.4. Macromolecular enzyme targets

Thus, it was determined that the macromolecules with which **M1** is most likely to interact are kinase (26.7%) and enzyme (20%). The macromolecules most likely to bind with **M2** are lyase (26.7%), enzyme (26.7%), and G protein-coupled receptor family A (20%).

Considering the high antioxidant activity and the rare occurrence of compound **M3** compared to **M1** and **M2** (Table 1), special attention was given to this compound. The macromolecule with which **M3** is most likely to bind and exhibit antioxidant properties was identified as xanthine dehydrogenase, belonging to the oxidoreductase class. Under pathological conditions such as ischemia, xanthine dehydrogenase converts into xanthine oxidase, which actively produces free radicals. It is hypothesized that **M3**, by binding to xanthine dehydrogenase in the body, prevents the conversion of the enzyme into xanthine oxidase.

Comparative study of M1, M2, and M3 using ADMETlab 3.0.

This program predicted several parameters that differ from those determined by SwissADME regarding physicochemical properties (Table 10).

TABLE 10. **Comparative physicochemical properties of M1, M2, and M3**

Physicochemical properties				
Property	**Value M1**	**Value M2**	**Value M3**	**Comment**
nRing	4.0	4.0	3.0	Number of rings. Optimal:0~6
MaxRing	10.0	10.0	10.0	Number of atoms in the biggest ring. Optimal:0~18
nHet	12.0	12.0	12.0	Number of heteroatoms. Optimal:1~15
fChar	0.0	0.0	0.0	Formal charge. Optimal:-4 ~4
nRig	24.0	24.0	20.0	Number of rigid bonds. Optimal:0~30
Flexibility	0.125	0.125	0.15	Flexibility = nRot /nRig
Stereo Centers	5.0	5.0	0.0	Stereo Centers. Optimal: ≤ 2
Melting point	230.593	230.593	299.085	Melting points below 25°C are classified as liquids, while melting points above 25°C are classified as solids.
Boiling point	319.71	319.71	386.217	A normal boiling point below 25°C is categorized as a gas.

Molecular docking of compound M3

The compound **M3** was used as the ligand, while xanthine dehydrogenase was selected as the macromolecular target. The 2D interaction structure is presented in Figure 5, and the 3D structures are shown in Figures 6 and 7. The binding energy of compound **M3** upon interaction with the xanthine dehydrogenase enzyme is provided in Table 11.

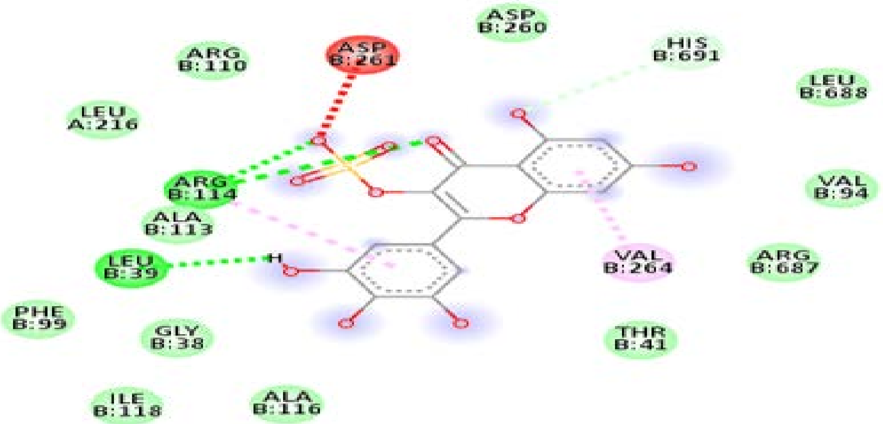


Fig. 5. 2D Structure of the docking result of compound **M3** with the xanthine dehydrogenase enzyme

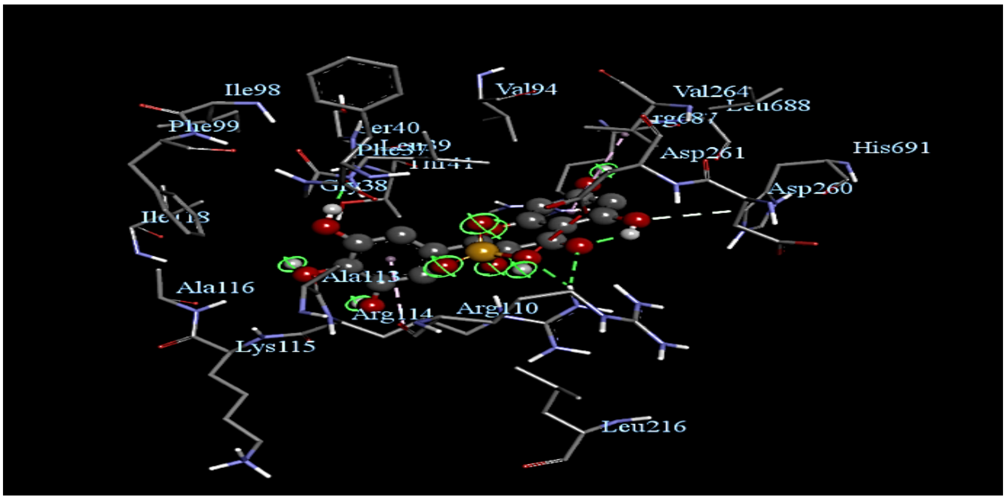


Fig. 6. 3D Structure of the interaction between compound **M3** and the xanthine dehydrogenase enzyme (protein–ligand binding)

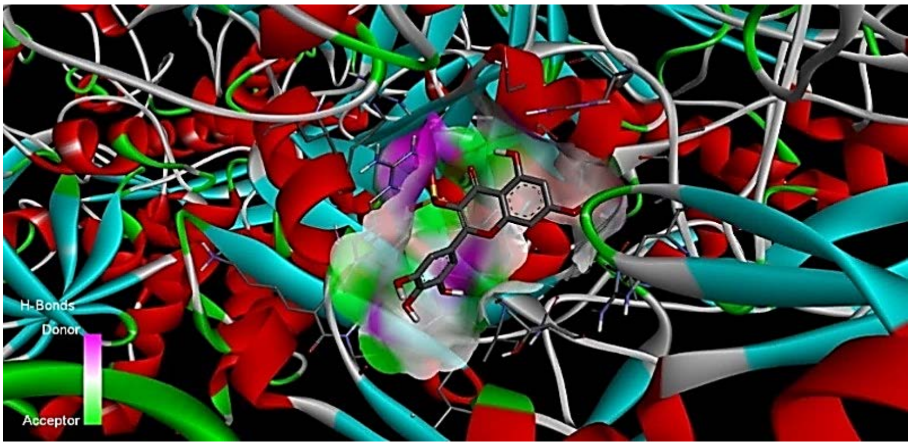


Fig. 7. 3D Structure of the docking result of compound **M3** with the xanthine dehydrogenase enzyme

TABLE 11. **Binding energy of the interaction between compound M3 and the xanthine dehydrogenase enzyme (Protein–ligand complex)**

Mode	Affinity, kcal/mol	Dist from best mode	
		RMSD, Å rmsd l.b.	RMSD, Å rmsd u.b.

1	-7.7	0.000	0.000
2	-7.4	2.344	3.817
3	-7.3	2.487	7.184
4	-7.2	2.440	4.079
5	-7.2	2.024	6.603
6	-7.1	34.574	35.835
7	-6.7	23.384	25.440
8	-6.7	3.128	5.012
9	-6.7	17.218	21.753

Interaction of molecule **M3** (ligand) with xanthine dehydrogenase enzyme in 2D format. The analysis revealed the following key interactions:

1. **Van der Waals interactions:** Molecule **M3** forms weak Van der Waals contacts with several amino acid residues, primarily with Leu (A:216, B:688), Val (B:94, B:264), Ile (B:118), Ala (B:116), Gly (B:38), and Phe (B:99). These interactions contribute to the stable binding of the ligand within the enzyme's active site.
2. **Hydrogen bonds:**
   Hydrogen bonds are formed between molecule **M3** and the amino acid residues Arg (B:110, B:114). These bonds provide high selectivity for ligand binding at the active site and help stabilize the complex.
3. **Carbonhydrogen bonds:**
   The figure illustrates carbon-hydrogen bonds formed between the aromatic and hydroxyl groups of the ligand and the residues Val (B:264) and Thr (B:41).
4. **π-alkyl interactions:**
   π-Alkyl interactions occur between the aromatic π-systems and alkyl groups with residues Ala (B:113) and Arg (B:114). These hydrophobic interactions enhance the ligand's firm positioning in the active center.
5. **Negative (unfavorable) interaction:**
   It is important to note that a negative interaction is observed with residue Asp (B: 261), which may cause electrostatic repulsion of a part of the ligand molecule and potentially weaken the binding strength.

Overall, molecule **M3** binds to the active site of xanthine dehydrogenase through several different interaction mechanisms. Hydrogen bonds and π-alkyl interactions play a particularly significant role in complex stabilization. Van der Waals and carbon-hydrogen contacts provide additional stabilizing effects. Although the negative interaction may partially affect the binding energy, the overall interaction profile indicates strong and specific binding of **M3** to the enzyme.

CONCLUSION

In this study has been carried out the comparative *in vitro* investigation of the antioxidant properties, as well as *in silico* assessment of the biological activity, physicochemical properties, solubility, bioavailability, pharmacokinetic parameters, and drug-likeness of flavonoids **M1, M2,** and **M3** isolated from *Limonium caspium* species growing along the Caspian Sea coast in Azerbaijan.

In vitro antioxidant activity testing.

The antioxidant activities of flavonoids **M1**, **M2**, and **M3** were examined using the DPPH assay. As a result of the analysis, the

IC_{50} antioxidant activity values were calculated, and the following results were obtained: **M1** (7.81 μg/ml), **M2** (6.34 μg/ml), and **M3** (6.2 μg/ml), indicating that the substances possess a strong antioxidant effect. Gallic acid was used as a positive control with an IC_{50} value of 3.48 μg/mL.

In silico biological activity study.

Computational tools including PASS Online, SwissADME, SwissTarget, and ADMETlab 3.0 were applied to predict the biological activities of flavonoids **M1, M2,** and **M3**. According to PASS Online predictions, **M1** exhibits significant antitumor activity (92.4%), anticancer activity against prostate cancer (86.8%), antimutagenic activity (78.3%), among other effects. **M2** demonstrated high hemostatic (99.0%), cardioprotective (98.3%), vasoprotective (96.2%), and additional activities. **M3** showed pronounced hemostatic activity (99.1%) along with other notable biological properties. Molecular docking was performed using AutoDock Vina 4.2, where compound **M3** was used as the ligand and xanthine dehydrogenase was selected as the protein target. It is suggested that **M3** may bind to xanthine dehydrogenase in the body, potentially preventing its conversion into xanthine oxidase.

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AZƏRBAYCANDA BİTƏN *LIMONIUM CASPIUM* BİTKİSİNDƏN ALINMIŞ FLAVONOİDLƏRİN *IN VITRO* ANTİOKSİDANT AKTİVLİYİNİN VƏ *IN SILICO* BİOLOJİ AKTİVLİYİNİN TƏDQİQİ

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Xülasə

Azərbaycan florasından *Limonium caspium* (Willd.) P.Fourn (*Plumbaginaceae*) köklərindən alınmış mirisetin (**M1**), mirisitrin (**M2**) və mirisetin-3′-O-sulfat (**M3**) flavonoidlərinin antioksidant xassələri *in vitro* şəraitdə müqayisəli şəkildə öyrənilmiş, bioloji aktivlikləri isə *in silico* üsullarla (PASS Online, ProTox 3.0, SwissADME, SwissTarget və ADMETlab 3.0) proqnozlaşdırılmışdır. Bundan əlavə, molekulyar dokinq proqramı vasitəsilə ligand–makromolekul qarşılıqlı təsirləri araşdırılmışdır. DPPH testi ilə maddələrin IC_{50} antioksidant fəallıqları (mkq/ml) hesablanmışdır: **M1** – 7,81; **M2** – 6,34; **M3** – 6,2. Proqnozlaşdırılmışdır ki, **M1** yüksək şiş əleyhinə aktivliyə (%92,4), **M2** (%99,0) və **M3** (%99,1) isə yüksək hemostatik (qankəsici) xüsusiyyətlərə malikdir.

Molekulyar dokinq AutoDock Vina 4.2 proqramından istifadə edilməklə həyata keçirilmişdir. Tədqiq olunan ligand kimi **M3** birləşməsi, hədəf zülal kimi isə ksantindehidrogenaza seçilmişdir. **M3** molekulu ksantindehidrogenaza fermentinin aktiv mərkəzi ilə müxtəlif qarşılıqlı təsir mexanizmləri vasitəsilə bağlanmışdır və qarşılıqlı təsirlərin ümumi profili **M3**-ün fermentlə güclü və spesifik bağlanmasını göstərir.

Açar sözlər: *Limonium caspium (Willd.) P.Fourn.,* mirisetin, mirisitrin, mirisetin 3′-O-sulfat, antioksidantlıq, *in silico,* molekulyar dokinq, *in vitro*

ИССЛЕДОВАНИЕ *IN VITRO* АНТИОКСИДАНТНОЙ АКТИВНОСТИ И *IN SILICO* БИОЛОГИЧЕСКОЙ АКТИВНОСТИ ФЛАВОНОИДОВ *LIMONIUM CASPIUM*, ПРОИЗРАСТАЮЩЕГО В АЗЕРБАЙДЖАНЕ

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Резюме

Антиоксидантные свойства флавоноидов мирицетина (**M1**), мирицитрина (**M2**) и мирицетин-3'-*O*-сульфата (**M3**), выделенных из корней *Limonium caspium* (Willd.) P.Fourn (*Plumbaginaceae*) из флоры Азербайджана были сравнительно изучены *in vitro*,

а биологическая активность *in silico* (PASS Online, ProTox 3.0, SwissADME, SwissTarget и ADMETlab 3.0). Также изучены взаимоотношении лиганд – макромолекула с помощью программы молекулярного докинга. Методом DPPH рассчитана антиоксидантная активность IC50 веществ (мкг/мл): **М1**(7.81), **М2**(6.34) и **М3**(6.2). Предсказано, что **М1** обладает (%) выраженными противоопухолевыми (92.4), а **М2** (99.0) и **М3** (99.1) кровоостанавливающими свойствами.

Молекулярный докинг был выполнен с использованием программы AutoDock Vina 4.2. В качестве исследуемого лиганда использовалось соединение **М3**, а в качестве белка-мишени была выбрана ксантиндегидрогеназа. Молекула **М3** связывается с активным сайтом фермента ксантиндегидрогеназы посредством нескольких различных механизмов взаимодействия и общий профиль взаимодействий свидетельствует о сильном и специфическом связывании **М3** с ферментом.

Ключевые слова: *Limonium caspium (Willd.) P. Fourn.*, мирицетин, мирицитрин, мирицетин-3′-О-сульфат, антиоксидантность, *in silico*, молекулярный докинг, *in vitro.*