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Mechanism Prediction of Gastro-protective Effects of the *Biebersteinia multifida* against Ethanol-Induced Gastric Ulcers; an Insilco study

Running title: Insilco study of Gastro-protective Effects of the *Biebersteinia multifida*

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ABSTRACT

Introduction: Peptic ulcer disease (PUD) is one of the most common diseases of the gastrointestinal tract. Herbal remedies are alternative therapies that have different gastro protective mechanisms, the gastro protective effect of *Biebersteinia multifida* (BM) was evaluated previously.

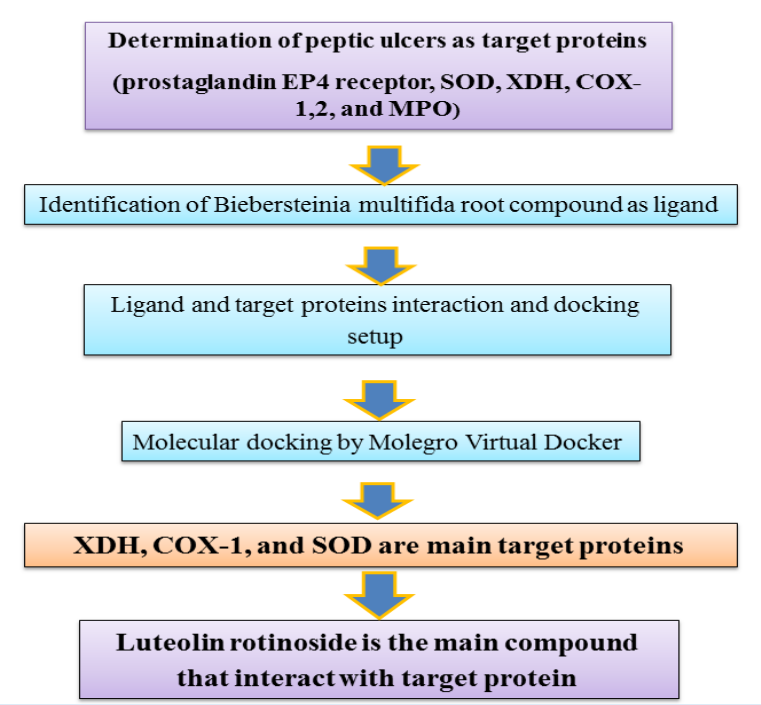
Objective: This study was performed to predict the possible mechanism of action of BM bioactive compounds on ameliorating ethanolic PUD in an Insilico study by molecular docking.

Methods: Bioactive compounds in BM were screened by Molegro Virtual Docker 6.0 (MVD) for their effects on factors affecting the healing and progression of ethanolic PUD.

Results: The principal bioactive compound in BM root that has an ameliorating effect in PUD is the flavonoid luteolin rutinoside. This compound establishes the lowest binding energy with the enzymes xanthine oxidase, cyclooxygenase-2, and superoxide dismutase, respectively.

Conclusion: *Biebersteinia multifida* probably affects the factors involved in ethanolic PUD due to the presence of flavonoids luteolin rutinoside and thus plays a role in the healing of PUD.

Keywords: Molecular Docking, *Biebersteinia multifida*, Peptic ulcer, Molegro Virtual Docker



INTRODUCTION: Peptic ulcer disease (PUD) is one of the most common diseases of the gastrointestinal tract. The pathophysiology of this disease is caused by an imbalance of protective and destructive factors in the gastric mucosa. Also, factors such as infection, smoking, stress, long-term use of non-steroidal anti-inflammatory drugs (NSAIDs), and excessive alcohol consumption play a role in causing PUD [1, 2]. One of the animal models for gastric ulcers is the use of ethanol [3], which causes disorders of the gastric mucosa and bleeding. Ethanol-induced ulcers invoke inflammatory agents (including neutrophils) in the site of injury, leading to the overproduction of reactive oxygen species (ROS) and other inflammatory mediators, resulting in oxidative damage [4]. The resulting oxidative stress stimulates lipid peroxidation and increases the level of malondialdehyde (MDA) in gastric tissue [5]. In addition, activation of nuclear factor kappa B (NF-κB) occurs during gastric mucositis. The production of proinflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and induced nitric oxide synthase (iNOS) is controlled by NF-κB [6, 7].

A bunch of previous studies suggested that functional food products (FFPs) and functional foods (FFs) are the potential to prevent disease progression, optimize health, and sometimes have therapeutic aspects. The status of the FFC's definition is not currently recognized by the Food and Drug Administration (FDA). To uniform the development of functional food products (FFPs) that could improve the

health of people struggling with chronic diseases, academic research institutes like the Functional Food Institute/Functional Food Center (FFC), government agencies, and many Functional Food Scientists are engaged in organized research in this field [8]. Antacids, antibiotics, H2 receptor antagonists, and proton-pump inhibitors (PPIs), are widely used in gastritis [9]. Long-term treatment with these drugs has some adverse effects such as acute interstitial nephritis, chronic kidney disease, collagenous colitis, gastric carcinoid tumor, gastric fundic mucosal hypertrophy, changes in the gut microbiome, small intestinal bacterial overgrowth, gastric fundic gland polyps, and gastric cancer [10]. Due to these adverse effects, alternative treatments are needed. Herbal remedies such as chili peppers, berries, grapes, dill, celery, spinach, and Shirazi thyme [11] are alternative therapies that have different gastroprotective mechanisms, including stimulation of mucosal proliferation, inhibition of acid production, and antioxidant properties [12]. According to previous research, this category of plants, due to their unique compounds (such as capsaicin, catechin, hesperetin, gallic acid, quercetin, kaempferol, and luteolin), are effective in improving digestive functions and diseases [13].

Biebersteinia is a genus of the family Geraniaceae, including an herbaceous species called *Biebersteinia multifida* (BM) that grows in Iran and is known as the Adamak. This species is also found in Syria and Central Asia. Morphologically, this plant has a stem 20 to 70 cm long, lacquered leaves, flowers formed in loose clusters, flower-strengthened calyx, and yellowish petals slightly shorter than the sepals [14]. In the previous studies, beneficial effects of BM that can be effective in wound healing have been mentioned, such as anti-inflammatory and pain-relieving, antibacterial, anti-hemolytic, and antioxidant effects. Raeesi et al., considering such a history of the plant, investigated it in the healing of ethanol peptic ulcers [15-17]. Pharmacodynamics active ingredients in Biebersteinia species and natural products isolated from it have been studied and include flavonoids (luteoline glucoside and rutinoside), guanidine (galegine), an alkaloid (vasicinone), phenylpropanoid (umbelliferone, scopoletin, ferulic acid), terpenoid (geniposide), polysaccharides (glucan-A, glucan-B, and glucan-C), fatty acids (myristic, palmitic, stearic, and arachidic acids), as well as various essential oil compounds [14].

Molecular docking studies determine the interaction between two molecules to find the best orientation of a ligand in a complex with minimal energy. A statistical scoring function is applied to analyze the results. This statistical scoring function converts the interaction energy into numerical values called docking scores for calculation. The docking results include 3D shapes of the ligand attached to the macromolecule that assay with tools such as Chimera, Pymol, and Rasmol [18].

In a previous study, the gastro protective effect of BM was evaluated. Researchers attributed this effect to its antioxidant activity and acceleration of nitric oxide (NO) production in the body. However, the exact mechanism is not well defined [19]. In this insilico study, we investigate the effect of various components of BM (as a ligand) on factors involved in gastric protection and gastric degradation (as protein targets) by molecular docking. This method predicts the effective composition of BM according to its specific protein target. This Insilco study aimed to investigate the possible mechanisms of the gastro protective effect of hydro alcoholic extract *Biebersteinia multifida*.

METHODS:

(i) Suitable structure of the target protein and Ligand selection: A list of factors involved in the ethanol-induced gastric ulcer was prepared as target proteins. Their crystal structures were retrieved from the RCSB PDB database at (https: //www.rcsb .org). Structures that were designed by the x-ray method and their resolution was less than 2.5 angstroms were preferred. The chemical formula of the desired ligands, including effective bioactive compounds in the extract and

essential oil of BM root [20, 21], was drawn using Chem Draw (Figure 1) and imported to Molegro Virtual Docker 6.0 (MVD) workspace in 'sdf' format and measured their interactions by MVD software. The MVD docking scoring function is based on PLP, in which the direction of hydrogen bonding is considered. In addition, a re-ranking procedure is applied to the highest-ranked poses to further increase docking accuracy [22]. During this study, 10 solutions were obtained from 10 independent dockings and then re-ranked.

(ii) Ligand and target protein interaction and docking setup: To prepare the structure of proteins and ligands for the docking process, they should be modified. MVD has tools that detect bands, orientations, hydrogen bonds, charges, and flexible bonds in the ligand. On the flip side, since the structure of PDB can contain wrongs in protein residues, they can identify, mature, optimize, and repair by tools and directions given in MVD. The cavities must be identified to determine which part of the target protein is most likely to interact with the protein and ligand. With the help of MVD, potential binding sites (cavities) can automatically detect by the cavity detection algorithm. In the docking algorithm was a maximum iteration of 1500 and a simplex evolution size of 50.

The binding cavities of crystal structure of COX-1 (PDB ID: 6Y3C), COX-2 (PDB ID: 5F1A), E2 prostaglandin receptor (EP4) (PDB ID: 5WYW), Xanthine oxidase (XDH) (PDB ID: 2E1Q), Myeloperoxidase (MPO) (PDB ID: 6BMT), and SOD (PDB ID: 5K02) were predicted by MVD. The binding site was set inside a restricted sphere of X: -17.76, Y:-45.92, Z:-2.33 for COX-1; X: 53.71, Y:22.44, Z:-203.6 for COX-2; X:-36.37, Y:-88.49, Z:4.05 for EP4; X:28.36 , Y:20.90, Z:184.87 for XDH; X:9.20 , Y:30.09, Z:141.35 for MPO; X:-8.03 , Y:36.89, Z:57.49 for SOD. These binding sites had a radius of 23 Å for COX2, XDH, and MPO; 15 Å for COX-1 and EP4; 13 Å for SOD. A grid resolution for all was 0.30 Å and the algorithm used was MolDock simplex Evolution (MolDock SE). For each docking, the best orientation for the ligand-protein complex was analyzed and hydrogen bonds were identified and labeled. Ligand energy using MolDock Score (GRID), a linear combination of E-inter (steric, Vander Waals, hydrogen bonding, and electrostatic interactions) and E-intra (torsion, sp2-sp2, hydrogen bonding, Vander Waals, and electrostatic interaction) inspected and analyzed. The docking simulation was run at least 100 times for 10 poses and the best poses were chosen based on the Rerank score, MolDock score, and interaction energy [23].

RESULTS

(i) Ligand selection: The effective bioactive compounds in the extract and essential oil of BM root (14 compounds) were obtained from previous research [20, 21] (figure 1).

(ii) Docking results: The results showed that the target proteins had the highest interaction with luteolin rutinoside because they had the lowest binding energy with this bioactive compound. Therefore, the main bioactive compound of BM root that has an ethanolic healing effect in gastric ulcers is likely luteolin rutinoside. Also, among the target proteins, there was the lowest binding energy between luteolin rutinoside and XDH (-171 KJ / mol). The Thr 354 with a binding energy of -16.6 kJ/mol has the highest contribution to creating hydrogen bonding, and Glu 263 with a binding energy of -23.2 kJ/mol has the highest contribution to creating an ester bond between luteolin rotinoside and XDH (Table 1). Following the XDH, Cox-2 (-159.7 Kj /mol) and SOD (-154.26 Kj/mol) had the lowest binding energy to luteolin rutinoside (Tables 2 and 3). The result of interactions between target proteins and luteolin rutinoside are summarized in table 4 also shown in Figure 2.

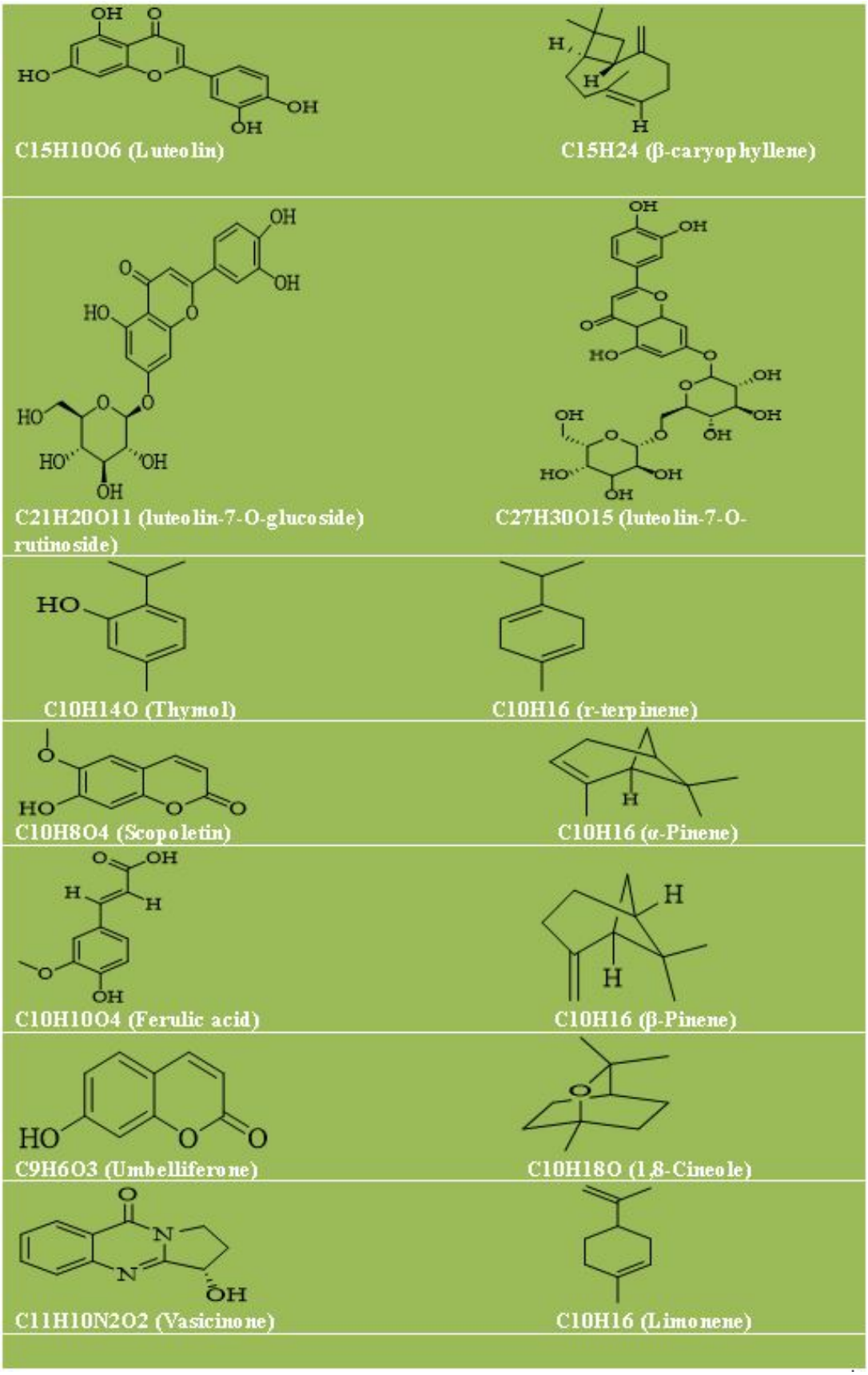
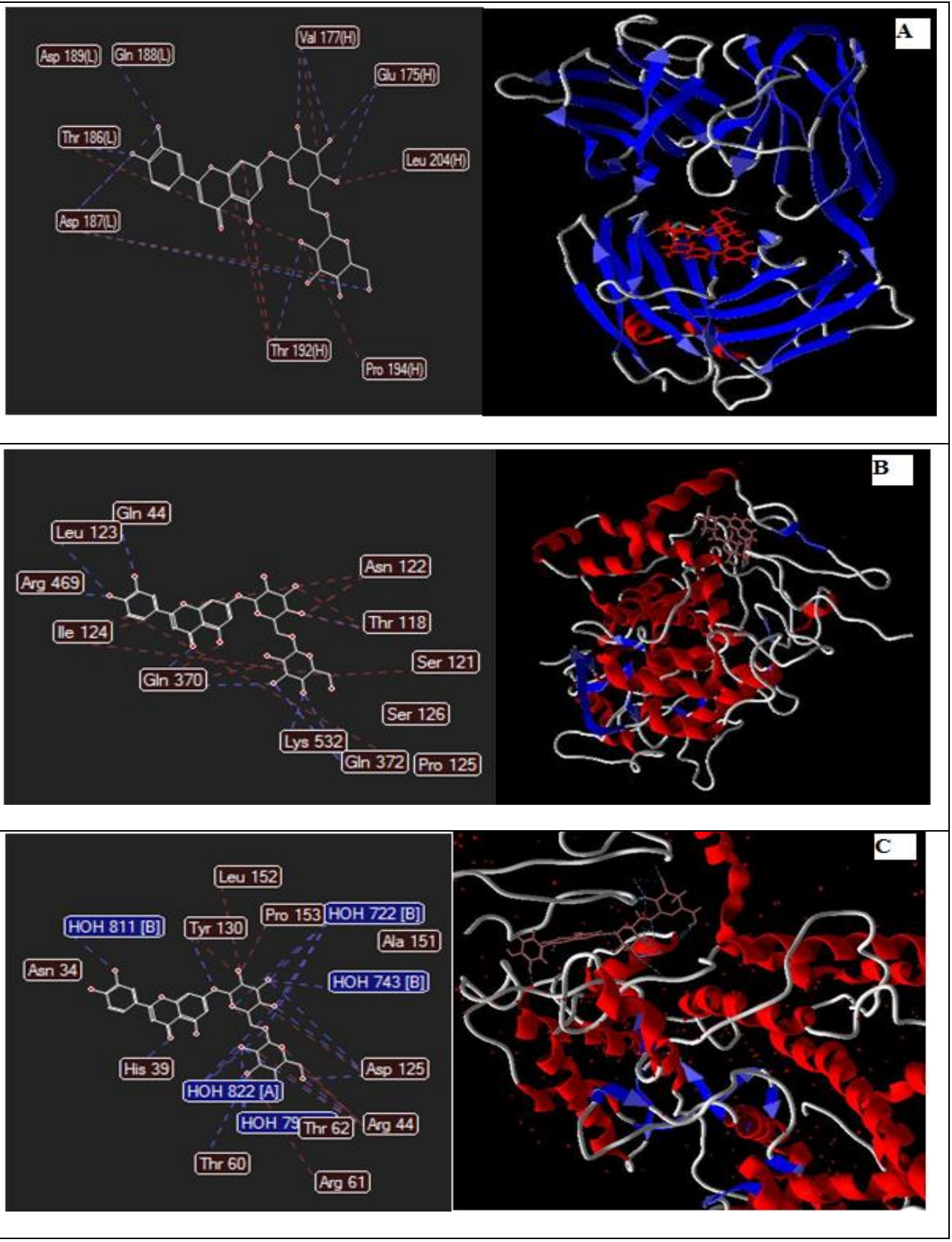


Figure 1. The effective bioactive compounds in the extract and essential oil of *Biebersteinia multifida root*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Gln 188(L)
Val 177(H)
Glu 175(H)
Thr 186(L)
Leu 204(H)
Asp 187(L)
Thr 192(H)
Pro 194(H)
A
Gln 44
Leu 123
Asn 122
Arg 469
Thr 118
Ile 124
Ser 121
Gln 370
Ser 126
Lys 532
Gln 372
Pro 125
B
Leu 152
Pro 153
HOH 722 [B]
HOH 811 [B]
Tyr 130
Ala 151
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Arg 44
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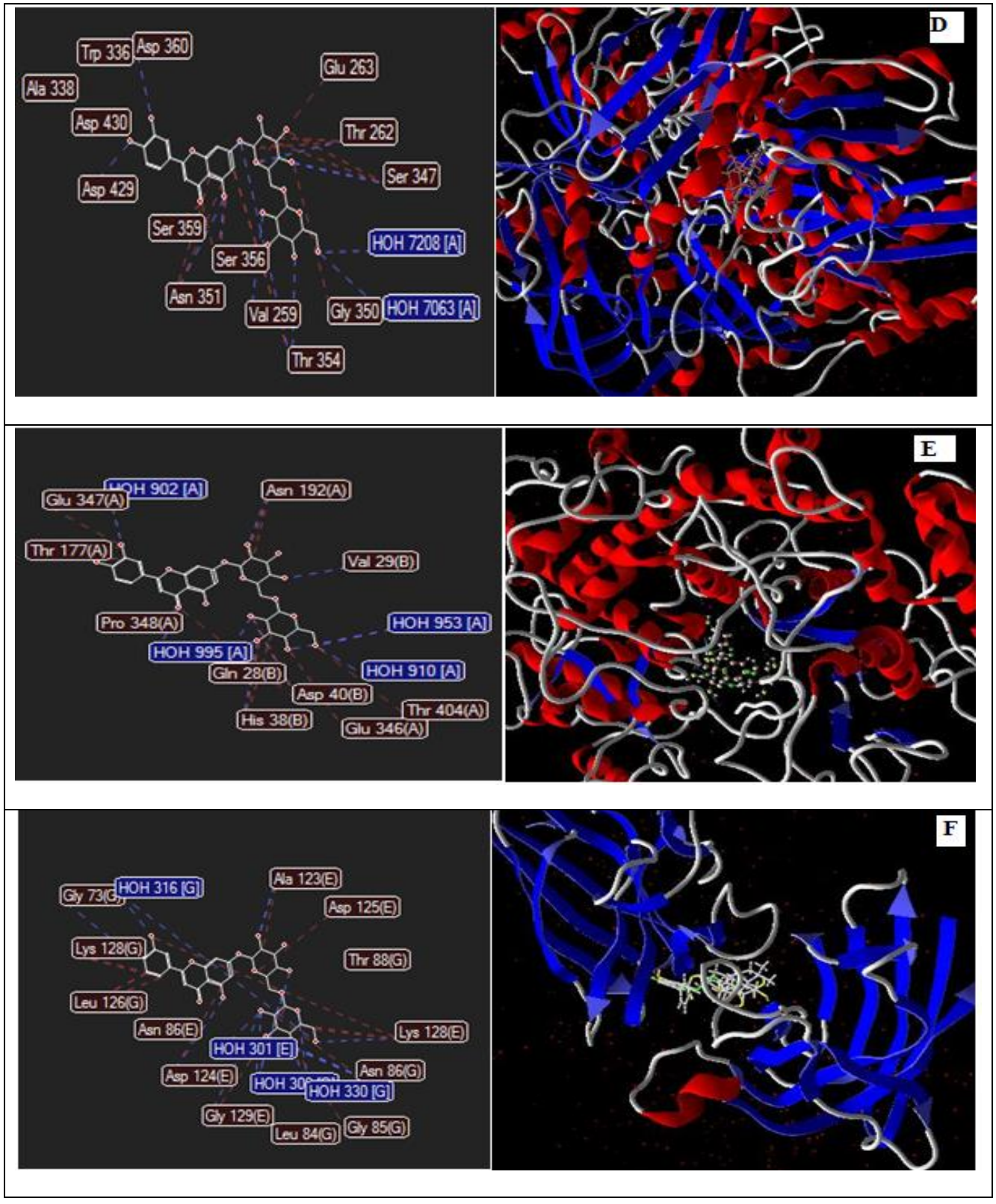

Figure 2. Interaction of amino acid residues of ethanolic gastric ulcer target proteins with flavonoid compound luteolin rutinoside via hydrogen and esteric bonds, A) interaction with prostaglandin receptor E2 (EP4), B) Cox-1, C) Cox-2, D) XDH, E) MPO, F) SOD. Target proteins depicted in the secondary structure view and ligand in the stick view; COX-1: cyclooxygenase-1, COX-2: and cyclooxygenase-2, XDH: xanthine oxidase, MPO: myeloperoxidase, SOD: superoxide dismutase.

Table 1 Molecular interactions of *Biebersteinia multifida* and xanthine oxidase

Ligand name	Residual interaction with hydrogen bond	Hydrogen bond energy interaction (Kj/mol)	Residual interaction with Esteric bond	Esteric bond energy interaction (Kj/mol)	Moldock score (Kj/mol)	Rerank score	Hydrogen bonds (Kj/mol)
1,8-Cineole	Asn 261 Thr 262	-12.07 -10.4	Ser 347 Gly 260 Asn 261 Thr 262 Gly 350 Ile 264 Val 258 Val 259	-6.2 -8.8 -12.07 -10.4 -7.4 -2.3 -4.5 -10	-64.8	-51.97	-2.31
α-Pinene	None	0	Val 258 Asn 261 Glu 263	-5.2 -7.1 -9.3	-64.52	-56.5	0
Thymol	Ile 264 Glu 263	-6.4 -9.8	Leu 257 Gly 350 Thr 262 Ala 346 Asn 261 Ile 264 Glu 263	-2 -6..2 -5.5 -2.6 -7.3 -6.4 -9.8	-77.2	-64.32	-4.16
r-terpinene	None	0	Gly 260	-5.5	-83.2	-71.12	0
β-Pinene	None	0	Asn 261 Glu 263	-8.4 -9.1	-65.65	-58.17	0
Limonen	None	0	None	0	-83.2	-71.00	0
Vasicinone	Gly 260 Ser 347	-9.7 -14.3	Val 259 Ser 347 Gly 260 Glu 263	-7.3 -14.4 -9.8 -10	-103.4	-80.72	-5.29
Ferulic acid	Glu 263 Gly 260 Val 259 Asn 261	-19 -7.5 -7.5 -9.2	Glu 263 Gly 260 Val 259 Asn 261	-19 -7.5 -7.5 -9.2	-101.8	-85.73	-7.944
Luteolin	Asn 351 Thr 354 Ser 356 Ser 347 Val 259 Gly 260	-17.4 -13.4 -7.7 -11.7 -9.3 -9.4	Ser 356 Thr 354 Ser 359 Gly 350 Ser 347 Asn 261 Thr 262 Glu 263 Val 259 Gly 260	-7.7 -13.4 -3.5 -11.9 -11.7 -6.5 -14.5 -18.7 -9.3 -9.4	-123.9	-95.56	-15.10
Scopoletin	Gly 260 Ser 347 Asn 261 Val 259 Thr 262	-9.4 -13.4 -8.6 -10.3 -15.8	Gly 260 Ser 347 Asn 261 Val 259 Thr 262 Glu 263	-9.4 -13.4 -8.6 -10.3 -15.8 -6.2	-100.26	-56.039	-12.193
luteolin-7-O-glucoside	Val 259 Gly 260 Asn 261 Ile 264 Glu 263 Glu 402 Ser 399 Ala 255 Leu 404 Lys 249 Asp 254	-10.8 -3.7 -7.6 -11 -7.3 -7.3 -5.15 -5.3 -10.23 -9.3 -4.5	Val 259 Gly 260 Asn 261 Ile 264 Glu 263 Glu 402 Ser 399 Ala 255 Leu 404 Lys 249 Asp 254 Leu 257 Leu 398	-10.8 -3.7 -7.6 -11 -7.3 -7.3 -5.15 -5.3 -10.23 -9.3 -4.5 -23.17 -4.5	-152.5	-137.53	-20.20

Umbelliferone	Gly 260 Ser 347 Asn 261 Val 259	-8.3 -10.5 -7.3 -9.6	Gly 260 Ser 347 Asn 261 Val 259 Ala 346 Glu 263	-8.3 -10.5 -7.3 -9.6 -2.7 -7.1	-85.92	-63.49	-7.57
β-aryophyllene	None	0	Asn -1.2 Leu 257 Thr 354	-1.2 -11.4 -5.4	-96.55	-78.11	0
luteolin-7-O-rutinoside	Asp 360 Asp 429 Ser 356 Asn 351 Val 259 Thr 354 Ser 347 Thr 262	-7.3 -6.3 -7.2 -27 -9.4 -16.6 -9.9 -11.12	Asp 360 Asp 429 Ser 356 Asn 351 Val 259 Thr 354 Ser 347 Thr 262 Trp 336 Asp 430 Ala 338 Ser 359 Gly 350 Glu 263	-7.3 -6.3 -7.2 -27 -9.4 -16.6 -9.9 -11.12 -1.7 -6 -7 -1.7 -17.9 -23.2	-171.0	-143.2	-17.22

The total energy (the sum of internal ligand energies, protein interaction energies, and soft penalties), H-Bond is the hydrogen bonding energy between protein and ligand. Esteric is the esteric interaction energy between protein and ligand. Electro is the sum of short-range (r < 4.5 °A) and long-range (r > 4.5 °A) electrostatic protein-ligand interaction energy.

Table 2 Molecular Interactions of *Biebersteinia multifida* and cyclooxygenase-2

Ligand name	Residual interaction with hydrogen bond	Hydrogen bond energy interaction (Kj/mol)	Residual interaction with Esteric bond	Esteric bond energy interaction (Kj/mol)	Moldock score (Kj/mol)	Rerank score	Hydrogen bonds (Kj/mol)
1,8-Cineole	His 39 Gln 461	-20.5 -7.6	His 39 Gln 461	-20.5 -7.6	-55.94	-51.98	-3.22
α-Pinene	None	0	None	0	-52.23	-48.48	0
Thymol	Ser 530	-5.8	Ser 530 Leu 384	-5.8 +2.2	-71.48	-47.53	-2.5
r-terpinene	None	0	Leu 384	-1.08	-69.66	-60.76	0
β-Pinene	None	0	Gly 526 Leu 352 Ala 527	-8.4 -5.44 -6.7	-53.50	-48.95	0
Limonen	None	0	None	0	-68.55	-60.53	0
Vasicinone	Ser 530	-6.03	Leu 352 Val 349 Ser 530 Leu 384	-14.7 -6.9 -6.03 +0.96	-88.44	-68.45	-2.5
Ferulic acid	Leu 352 Ser 530	-17.83 -6.08	Leu 352 Ser 530 Val 349 Tyr 385	-17.83 -6.08 -2.7 -4	-91.13	-77.59	-4.36
Luteolin	Gln 192 Ile 517 Phe 518 Ser 353 His 90 Tyr 355 Tyr 385 Ser 530	-6.1 -5.6 -15.4 -17.2 -5.2 -9.08 -5.8 -6.2	Gln 192 Ile 517 Phe 518 Ser 353 His 90 Tyr 355 Tyr 385 Ser 530 Ala 516 Val 523	-6.1 -5.6 -15.4 -17.2 -5.2 -9.08 -5.8 -6.2 -1.3 -16	-125.03	-105.5	-10.82

Scopoletin	Leu 352	-18.6	Leu 352 Phe 518 Leu 384 Ser 353	-18.6 -8 +2.11 -6.07	-84.62	-70.61	-0.68
luteolin-7-O-glucoside	His 39 Arg 44 Pro 154 Asn 34 Cys 36	-14.5 -14.7 -12 -5.9 -12.8	His 39 Arg 44 Pro 154 Asn 34 Cys 36 Arg 469 Gly 45 Gly 135	-14.5 -14.7 -12 -5.9 -12.8 -2.3 -6.4 -9.6	-141.77	-134.04	-14.39
Umbelliferone	Cys 41 Gln 461 His 39	-7.8 -8.4 -28.4	Cys 41 Gln 461 His 39	-7.8 -8.4 -28.4	-77.43	-67.83	-5.70
β-aryophyllene	None	0	His 39 Cys 47	-11.8 -15	-92.76	-74.68	0
luteolin-7-O-rutinoside	Asn 34 His 39 Thr 60 Arg 44 Asp 125 Ala 151 Tyr 130	-6 -9.8 -4.6 -27.3 -17 -5.3 -19.7	Asn 34 His 39 Thr 60 Arg 44 Asp 125 Ala 151 Tyr 130 Arg 61 Pro 153 Leu 152 Thr 62	-6 -9.8 -4.6 -27.3 -17 -5.3 -19.7 -3.4 -10.3 -4.6 -5.1	-159.7	-146.7	-15.37

The total energy (the sum of internal ligand energies, protein interaction energies, and soft penalties), H-Bond is the hydrogen bonding energy between protein and ligand. Esteric is the esteric interaction energy between protein and ligand. Electro is the sum of short-range (r < 4.5 °A) and long-range (r > 4.5 °A) electrostatic protein-ligand interaction energy.

Table 3 Molecular Interactions of *Biebersteinia multifida* and superoxide dismutase

Ligand name	Residual interaction with hydrogen bond	Hydrogen bond energy interaction (Kj/mol)	Residual interaction with Esteric bond	Esteric bond energy interaction (Kj/mol)	Moldock score (Kj/mol)	Rerank score	Hydrogen bonds (Kj/mol)
1,8-Cineole	Lys 128	-16.24	Lys 128 Asp 125	-16.24 -4.03	-31.53	-31.81	-0.79
α-Pinene	None	0	Ala 123	-5.14	-35.54	-34.97	0
Thymol	Gly 129 Asn 86	-7.68 -12.69	Gly 129 Asn 86	-7.68 -12.69	-51.51	-48.46	-2.5
r-terpinene	None	0	Ala 123	-3.98	-47.69	-43.47	0
β-Pinene	None	0	Asn 139 Ala 123	-12.93 -5.53	-35.35	-34.67	0
Limonen	None	0	Ala 123	-2.55	-47.38	-43.51	0
Vasicinone	Asn 86	-12.12	Asn 86 Gly 129	-12.12 -7.64	-69.45	-66.37	-2.93
Ferulic acid	Ala 123 Gly 129	-9.89 -7	Gly 129 Asn 131 Asp 125 Ala 123 Asn 139	-7 -3.58 -10.75 -9.89 -17.16	-78.20	-69.74	-5.96
Luteolin	Asn 86 Lys 122 Aasn 139 Lys 128	-10.2 -8.6 -17.6 -8.76	Asn 86 Leu 84 Gly 129 Lys 128 Ala 123 Lys 122	-10.2 -2.4 -13.75 -8.76 -2.69 -8.6	-96.70	-89.90	-11.83

			Asn 139	-17.59			
Scopoletin	None	0	Asn 131	-4.22	-64.99	-61.83	-0.77
luteolin-7-O-glucoside	Lys 122	-10.91	Gly 129	-7.27	-112.23	-94.98	-11.37
	Asn 139	-25.83	Asn 86	-9.02			
	Thr 88	-8.90	Leu 84	-4.07			
	Ala 123	-8.67	Ala 123	-8.67			
	Leu 84	-4.07	Thr 88	-8.90			
			Lys 122	-10.91			
			Asn 139	-25.83			
Umbelliferone	Gly 129	-6.96	Gly 129	-6.96	-56.00	-51.22	-3.01
			Asn 131	-3.52			
			Ala 123	-4.26			
β-aryophyllene	None	0	Asn 139	-19.28	-69.69	-57.27	0
			Ala 123	-2.66			
luteolin-7-O-rutinoside	Ala 123	-13.7	Lys 128 (E)	-13.51	-154.26	-80.54	-17.74
	Gly 73	-5.8	Gly 73	-5.8			
	Asp 124	-10.05	Lys 128 (G)	-13.44			
	Gly 129	-12.61	Leu 126	-6.95			
	Asn 86	-2.02	Asp 124	-10.05			
	Lys 128	-13.44	Asn 86 (E)	-16.72			
			Leu 84	-2.73			
			Asn 86 (G)	-16.72			
			Gly 85	-1.16			
			Gly 129	-12.61			
			Thr 88	-3			
			Asp 125	-29.28			
			Ala 123	-13.7			

The total energy (the sum of internal ligand energies, protein interaction energies, and soft penalties), H-Bond is the hydrogen bonding energy between protein and ligand. Esteric is the esteric interaction energy between protein and ligand. Electro is the sum of short-range (r < 4.5 °A) and long-range (r > 4.5 °A) electrostatic protein-ligand interaction energy.

Table 4. Molecular interactions of luteolin rutinoside (as main ligand) and target proteins (Cox-1, Cox-2, EP4, MPO, SOD, and XDH)

Target protein	Residual interaction with hydrogen bond	Hydrogen bond energy interaction (Kj/mol)	Residual interaction with Esteric bond	Esteric bond energy interaction (Kj/mol)	Moldock score (Kj/mol)	Rerank score	Hydrogen bonds (Kj/mol)
EP4	Asp 187	-21	Gln 188	-4.4	-128.9	-111.38	-13.44
	Thr 192	-31.5	Asp 189	-10.12			
	Val 177	-9.5	Thr 192	-31.5			
	Glu 175	-7.3	Glu 175	-7.3			
			Val 177	-9.5			
			Leu 204	-1.8			
			Asp 187	-21			
			Thr 186	-15.3			
			Pro 194	-13.3			
COX-1	Gln 44	-4.6	Gln 44	-4.6	-122.03	-73.55	-20.8
	Leu 123	-8	Leu 123	-8			
	Arg 469	-3.2	Arg 469	-3.2			
	Gln 370	-22.3	Gln 370	-22.3			
	Thr 118	-10.5	Thr 118	-10.5			
	Gln 372	-13.7	Gln 372	-13.7			
	Lys 532	-5.5	Lys 532	-5.5			
			Ile 124	-1.6			
			Asn 122	-28			

			Ser 121 Ser 126 Pro 125	-4.5 -16.33 -9.45			
COX-2	Asn 34 His 39 Thr 60 Arg 44 Asp 125 Ala 151 Tyr 130	-6 -9.8 -4.6 -27.3 -17 -5.3 -19.7	Asn 34 His 39 Thr 60 Arg 44 Asp 125 Ala 151 Tyr 130 Arg 61 Pro 153 Leu 152 Thr 62	-6 -9.8 -4.6 -27.3 -17 -5.3 -19.7 -3.4 -10.3 -4.6 -5.1	-159.7	-146.7	-15.37
XDH	Asp 360 Asp 429 Ser 356 Asn 351 Val 259 Thr 354 Ser 347 Thr 262	-7.3 -6.3 -7.2 -27 -9.4 -16.6 -9.9 -11.12	Asp 360 Asp 429 Ser 356 Asn 351 Val 259 Thr 354 Ser 347 Thr 262 Trp 336 Asp 430 Ala 338 Ser 359 Gly 350 Glu 263	-7.3 -6.3 -7.2 -27 -9.4 -16.6 -9.9 -11.12 -1.7 -6 -7 -1.7 -17.9 -23.2	-171.0	-143.2	-17.22
MPO	Asn 192 Val 29 Gln 28 Asp 40 His 38	-10.55 -9.2 -18.27 -8.1 -8.6	Asn 192 Val 29 Gln 28 Asp 40 His 38 Glu 347 Thr 177 Pro 348 Thr 404 Glu346	-10.55 -9.2 -18.27 -8.1 -8.6 -6.3 -5.6 -6.4 -3.06 -40.7	-124.4	-51.96	-8.935
SOD	Ala 123 Gly 73 Asp 124 Gly 129 Asn 86 Lys 128		Lys 128 (E) Gly 73 Lys 128 (G) Leu 126 Asp 124 Asn 86 (E) Leu 84 Asn 86 (G) Gly 85 Gly 129 Thr 88 Asp 125 Ala 123	-13.51 -5.8 -13.44 -6.95 -10.05 -16.72 -2.73 -16.72 -1.16 -12.61 -3 -29.28 -13.7	-154.26	-80.54	-17.74

DISCUSSION In this study, to predict the ameliorating mechanism of the gastric protection of BM root, the molecular docking method was applied by using the MVD software. Protein-ligand binding simulation is a computational method that seeks to locate a ligand at the protein target binding site [18]. The ligand library in this study includes natural compounds that had been isolated from the extract and essential oil of BM. On the other hand, target proteins are various enzymes and factors involved in the ethanolic gastric ulcer process due to their destructive or healing effects. After identifying the target proteins' active sites, they were all docked with the bioactive compounds in the ligand library. The docking results are interpreted on the assumption that the more negative the binding energy values between the

selected composition of each ligand and the target protein, the more thermodynamically binding energy is desirable [24]. The estimation of the total bond energy for all ligands depends on the esteric interactions, but the hydrogen bonds and the electrostatic interaction energy account for a lower share of the bond energy. MolDock score is one of the scoring functions in MVD software to estimate the binding affinity (the lowest energy binding) between protein and ligand. After completing the docking process, the MolDock score function is employed to rank each ligand that was attached during this process.

The docking results of our study showed that XDH with the lowest binding energy (-171 kJ/mol) is probably the main target protein of the BM and the main ligand, is luteolin rutinoside. The mechanism of ethanol-induced gastric ulcers is complex and multifaceted; ethanol damages mucosal cells by reducing mucus content, reducing blood flow, and secreting acid (in a mechanism similar to histamine). Due to the solubility of mucus in ethanol and exposing the mucosal tissue to the proteolytic and hydrolytic effects of hydrochloric acid and pepsin, ethanol penetrates the gastric mucosa and damages the membrane by dissolving its phospholipids. Ethanol also causes an imbalance in cellular antioxidant processes by increasing XDH activity. Free radicals in the form of superoxide and hydroxides released after ethanol metabolism lead to increased lipid peroxidation. As a result, significant changes occur at the cellular level, leading to membrane damage, cell death, and epithelial erosion [3]. Due to the presence of alcohol dehydrogenase and XDH in the stomach, ethanol absorbed in the stomach is catalyzed by alcohol dehydrogenase to acetaldehyde, which is then converted to free radicals by XDH. Free radicals increase the permeability and secretion of vasoactive mediators such as endothelin-1, leukotriene C4, and histamine. These vasoactive mediators aggravate gastric mucosal lesions by stimulating the cessation of blood flow to the mucosa, leading to bleeding, tissue necrosis, and eventual destruction of the mucosal barrier. Excessive production of free radicals by ethanol gavage is directly related to the penetration and activation of neutrophils. So, penetration and accumulation of neutrophils in the gastric mucosa, because of producing the free radicals, leads to damage to cellular components such as lipids, proteins, etc. [25, 26]. According to Raeesi et al., the protective effect of BM against ethanolic gastric ulcers in rats is partly due to its antioxidant properties and nitric oxide-accelerating effects [19].

In this study, we found that the main bioactive compound luteolin rutinoside interacts more with enzymes involved in the oxidative process such as XDH and SOD. In addition, the enzyme COX-2 is also a factor influenced by the flavonoid component of luteolin rutinoside. COX isoenzymes produce prostaglandins through the arachidonic acid pathway. COX-1 is considered a "housekeeping" enzyme and plays an important role in many physiological functions, such as cytoprotection of gastric mucosa, renal blood flow regulation, and platelet aggregation, while COX-2 predominates at the site of inflammation. However, some studies show that both COX-1 and COX-2 are involved in homeostasis and also act as a modulator of inflammation. One of the side effects of oral NSAIDs is their gastrointestinal side effects due to COX inhibition. To reduce these side effects, drugs that selectively inhibit COX-2 are preferred over classical, although, the use of this class of drugs increased the cardiovascular risk in patients [27]. Salman Aziz et al. reported significant inhibition of COX-2 isoenzyme expression level by using Oxyresveratrol (a herbal compound) in ethanol gastric

ulcer animal models [28]. Therefore, considering that COx-2 has an anti-inflammatory effect in ethanolic gastric ulcers, one of the target proteins of BM in the treatment of ethanolic gastric ulcers.

Insilico models facilitate the prediction of drug targets with access to abundant data, high-power screening, and bioinformatics algorithms. However, this method still makes limitations for researchers. In cases of unavailability of crystallographic structure or other reliable structures, the researcher must design an optimal model by using the amino acid sequence of the target protein (FASTA) (which is retrieved from Uniprot) in the Swiss Model (https://swissmodel.expasy.org). By online servers (such as Procheck (Saves.mbi.ucla.edu/)), the quality of the resulting 3D structure evaluates, validates, and optimizes. This process is time-consuming and largely unreliable. In our study, when the crystallographic structure was not available and a valid structure could not be obtained by modeling, the target protein was excluded from the study. Therefore, a limited number of target proteins include in this study. On the other hand, the results of Insilico studies can only be considered as a tool to predict the target protein and drug purposes, and the results must be re-examined by in vitro and/or in vivo studies. So, researchers can confirm our results through in vitro and in vivo studies.

CONCLUSION

The main bioactive compound of *Biebersteinia multifida* by the healing effect on gastric ulcers is flavonoid luteolin rutinoside. This bioactive compound interacts with the lowest binding energy to the enzymes xanthine oxidase, cyclooxygenase-2, and superoxide dismutase, respectively. In general, *Biebersteinia multifida*, due to its flavonoids such as luteolin rutinoside, affects the factors involved in ethanolic gastric ulcers and thus plays a role in healing gastric ulcers.

Abbreviation list: PUD: Peptic ulcer disease, NSAIDs: non-steroidal anti-inflammatory drugs, BM: *Biebersteinia multifida* , ROS: reactive oxygen species, MDA: Malondialdehyde, TNF-α: tumor necrosis factor-alpha, iNOS: induced nitric oxide synthase, NF-κB: nuclear factor kappa B COX-1: cyclooxygenase-1, COX-2: cyclooxygenase-2, XDH: Xanthine oxidase, MPO: myeloperoxidase, SOD: superoxide dismutase, MVD: Molegro Virtual Docker

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