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Determination of Phenolic Content, Biological Activity, and Enzyme Inhibitory Properties with Molecular Docking Studies of *Rumex nepalensis*, an Endemic Medicinal Plant

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Abstract This study was conducted to evaluate the phenolic content, antioxidant potential, and enzyme inhibitiory properties of *Rumex nepalensis* by *in vitro* spectrophotometric methods. The experiments demonstrated that glutathione S-transferase (GST), α-glycosidase (α-Gly), acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) enzymes were strongly inhibited by *R. nepalensis* extracts. The IC_{50} values for GST, α-Gly, AChE, and BChE enzyme inhibitions were calculated as 21.01 mg/mL, 34.65 mg/mL, 27.72 mg/mL, and 17.32 mg/mL, respectively. Also, effective antioxidant capacities of water and methanol extracts of *R. nepalensis* were determined by ABTS, CUPRAC, DPPH, and FRAP methods. Furthermore, quinic acid (15.61 mg/g), miquelianin (2.06 mg/g), quercitrin (1.97 mg/g), and protocatechuic acid (0.217 mg/g) were identified to be the major phenolic compounds of the plant extract according to the LC-MS/MS analysis. Finally, molecular docking studies were carried out to show the interactions of quinic acid, miquelianin, and quercitrin with AChE, BChE, GST, and α-Gly enzymes. Docking analysis indicated the possible roles of these phytochemicals in enzyme inhibitory activities.

Keywords: *biological activity, enzyme inhibition, molecular docking, phenolic compounds, Rumex*

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1. Introduction

Rumex genus contains high amounts of flavonoids, naphthalenes, anthraquinones, and phenolic compounds [1]. *Rumex* genus consists of around 250 plant species, which are mostly consumed as herbal drugs for the treatment of an array of human diseases [2,3]. Some parts of *Rumex* plants have been used in folk medicine. For example, underground parts prepared by decoctions have been claimed to be beneficial as tonic and cholagogue. Also, the fresh leaves are consumed as a vegetable which are also reported for use in the treatment of eczema for blood cleansing [4]. The roots of *Rumex nepalensis* have been used for the treatment of pain, tinea, bleeding, tumor, constipation, and inflammation in Chinese folk medicine. Likewise, the roots of *Rumex hastatus* possess medicinal importance against headache, fever, and cough. *Rumex nepalensis* and *Rumex hastatus* were reported to contain naphthalenes, anthraquinones, and flavonoids [5]. Due to the high contents of naphthalenes, stilbenoids, anthraquinones, polyphenols, and steroids, *Rumex* species have demonstrated several pharmacological attributes, such as antioxidant, antiviral, anti-inflammatory, antitumor, antibacterial, and antifungal properties [6]. A plenty of *Rumex* species have got medicinal value [7].

A great number of studies related to antioxidant activity and phenolic content of some cereal products, fruits, and vegetables have been reported [8]. Due to their aromatic and pharmacological properties, chemical compounds of the plants are used for several purposes [9]. These natural components, such as vitamins, anthocyanins, carotenoids, tannins, flavonoids, and volatile oils have been

extensively used in cosmetics. Some of them have wound-healing, antioxidant, anti-aging, and anti-inflammatory properties [10]. According to the report of World Health Organization, approximately 70% of the world population in developing countries are still consuming traditional medicines for their primary health care and about 25% of the current medicines are obtained from natural sources [11].

Bioactive phenolic compounds from plants have been reported as potent acetylcholinesterase inhibitors implicated to be used against Alzheimer's disease treatment [12]. AChE and BChE enzymes are known to be responsible for hydrolyzing the acetylcholine in the human body [13]. Acetylcholine is a nerve cell transmitter located at the lymph nodes in the internal organs motor systems, in the intersections of the nerve and muscles, and in the central nervous system. Many works demonstrated that acetylcholine has effects on the speed of individual neurons [14]. AChE takes charge of break up the acetylcholine in the brain synaptic cleft. Thus by the keeping of the acetylcholine for neurotransmission in the synaptic cleft, symptoms of Alzheimer's disease can be reduced or prevented [15]. Another enzyme α-glycosidase released from intestine cells hydrolyzes polysaccharides to the small glucose and fructose [16].

Despite numerous studies reporting antioxidant potentials and enzyme inhibitions of plants, to the best of our knowledge, there is no detailed study on investigating phenolic contents, antioxidant potential, and enzyme inhibition of *R. nepalensis*. Thus, the primary objectives of the present study are to determine the enzyme inhibition and antioxidant activity of *R. nepalensis.* Hence, we identified its phenolic contents by using the LC-MS/MS technique which led to the identification of antioxidant, antidiabetic, anti-Alzheimer's bioactive ingredients. Molecular docking and computational studies have also been applied to determine the possible role of the most detected phytochemicals on biological activities.

2. Materials and Methods

2.1. Plant Sample

R. nepalensis was collected from Bingöl, an Eastern city of Turkey, from an open *Quercus* forest at an altitude of 1650-1850 m, in May 2018. The plant species was subsequently identified and preserved as a herbarium sample by a plant taxonomist Dr. Omer Kılıç. The voucher specimen (5896) is deposited at the herbarium of the Yıldırımlı (Ankara) and at the Department of Park and Garden Plants of Bingol University, Turkey.

2.2. Plant Extraction

The water and methanol extracts of *R. nepalensis* leaves were prepared for antioxidant studies. For the water extraction, air-dried leaves of *R. nepalensis* (20 g) were powdered using a blender and was dissolved in 200 mL distilled water (1/10: w/v). The mixture was stirred at room temperature (25°C) for 24 h then was filtered using a filter paper. The same ratio and procedure were used for the methanol extract as well. The water extract was lyophilized at -50°C and 5 mm Hg in a lyophilizer (Labconco, Freezone 1 L). The methanol extract was evaporated using a rotary evaporator (Heidolph 94200, Bioblock Scientific).

2.3. Determination of Phenolic Compounds

Ultrahigh performance liquid chromatography (Shimadzu-Nexera) coupled with a tandem mass spectrometer (LCMS-8040 model) was used to determine the phenolic contents of *R. nepalensis*. The reversed-phase UHPLC was equipped with a SIL-30AC model autosampler, CTO-10ASvp model column oven, LC-30CE model binary pumps, and DGU-20A3R model degasser. Analytical column: RP-C18 Inertsil ODS-4 (100 mm×2.1 mm, 2 µm) and 120 EC-C18 models (150 mm×2.1 mm, 2.7 µm) were used. The column temperature was set to 40°C. As mobile phases methanol and acetonitrile were used. As mobile phase additives ammonium formate, acetic acid, ammonium acetate, and formic acid were used. The flow rate was 0.5 mL/min and the injection volume was 5 µL. For spectrometric detection ionization source (ESI) was used and for vanillin, daidzin, piceid, coumarin, and hesperidin ESI was operated in positive ionization mode while for other standards ESI was operated in negative mode. MS conditions were adjusted as follows; 3 L/min for nebulizing gas (N_2) flow, 15 L/min for drying gas (N_2) flow, 350°C for interface temperature, 250°C for DL temperature, and 400 °C for heat block temperature [17].

2.4. Antioxidant Activity

2.4.1. ABTS Assay

ABTS cation radical solution was obtained by the reaction of 2 mM ABTS solution with 2.45 mM $K_2S_2O_8$ solution for twelve hours. The absorbance of the control solution at 734 nm was adjusted to 0.9±0.1 with phosphate buffer (0.1 M, pH 7.4) dilution. $ABTS^{\bullet+}$ radical scavenging activity was determined by calculating the reduction in sample absorption [18].

2.4.2. DPPH Assay

DPPH free radical solution was obtained by mixing 1 mM DPPH with ethanol using a magnetic stirrer for 12 h at 25°C. The DPPH radical solution (1 mL) was added to 3 mL of the extracts and standard solutions (10–30 mg/mL), separately and left to incubation for 30 min at 25°C. The absorbance was measured at 517 nm. Decreasing absorbance demonstrates DPPH free radical scavenging capacity [19].

2.4.3. CUPRAC Assay

The CUPRAC method was applied as detailed in a previous study [20]. Briefly, the different concentrations (10-30 µg/mL) of samples were mixed with $CuCl_2$ solution (0.25 mL, 10 mM), neocuproine solution (0.25 mL, 7.5 mM), and CH_3COONH_4 buffer solution (0.25 mL, 1.0 M), respectively. Increasing absorbance at 450 nm demonstrates Cu^{2+} reducing for antioxidant capacity of a sample.

2.4.4. FRAP Assay

The mixtures including *R. nepalensis* extracts at different concentrations (10-30 µg/mL), phosphate buffer (2.5 mL, 0.2 M), and potassium ferricyanate (2.5 mL, 1%) were prepared to initialize the reaction. After incubation at 50°C for 20 min, trichloroacetic acid (2.5 mL, 10%), and $FeCl_3$ (0.5 mL, 0.1%) solutions were added to each mixture. Increasing absorbance at 700 nm demonstrates Fe^{3+} reducing for antioxidant capacity of a sample [20].

2.5. Enzyme Inhibition Potential

The inhibitory effect of different concentrations (20-100 µM) of *R. nepalensis* on GST enzyme was determined as detailed in a previous study [16]. The GST inhibition effect of *R. nepalensis* at different concentrations in a range of 20-100 µM was determined as detailed in our previous study [21]. The inhibitory activity of *R. nepalensis* on AChE and BChE enzymes were determined by using acetylthiocholine iodide (AChI) and butyrylcholine iodide (BChI) as substrates [22].

2.6. Molecular Docking

Molecular docking was performed to investigate the exact binding site of the ligand on the protein and its binding mechanism. Maestro Molecular Modeling platform (version 11.8) by Schrodinger, LLC model was applied in the molecular doking study [23,24]. High-resolution (1.55-2.10 Å) crystalline structures of acetylcholinesterase (PDB ID: 6O4W), butyrylcholinesterase (PDB ID: 6QAA), glutathione s-transferase (PDB ID: 3KTL), and α-glycosidase (PDB ID: 5NN8) enzymes were obtained from protein data bank at research collaboration for Structural Bioinformatics (RSCB) protein database (PDB) [25]. The structures of the most intensive phenolic of the *R. nepalensis* plant were downloaded from PubChem site in SDF file format as 3D structures (Figure 1). Molecular minimization of the ligands was prepared by applying the default parameters by the Lig prep module in Maestro. All proteins were prepared using the protein preparation wizard of Maestro software; at this stage, all water molecules were removed and polar hydrogen atoms were added. A grid box was formed around the active site of the proteins containing natural ligands. These studies were carried out according to the methods used in previous studies [26,27]. Each ligand was analyzed for docking score and energy, receptor binding affinity, and structure of all proteins, respectively. The docking studies were performed with the Glide docking module. The resulting receptor model, 2D & 3D interactions were visualized with Discovery Studio 2017 version [28].

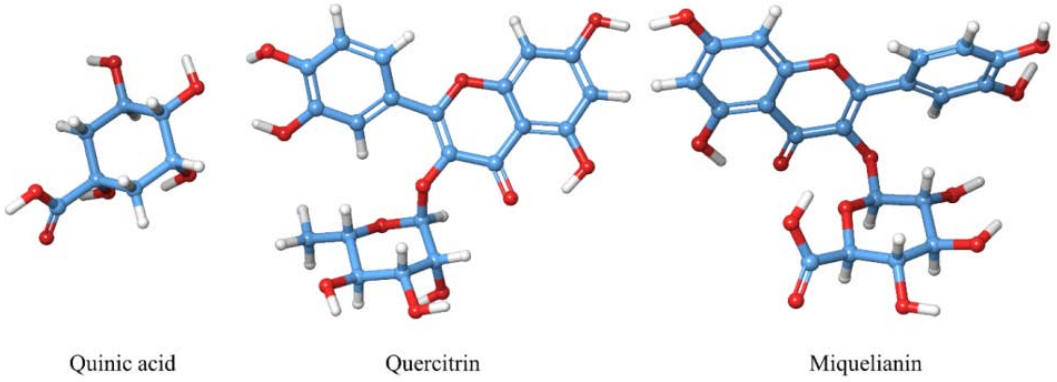

Figure 1. The structures of the ligands; the 3D views of quinic acid, quercitrin, and miquelianin

Table 1. LC-MS/MS parameters and phenolic compounds of *R. nepalensis*

No	Standards	*R. nepalensis* (mg/g extract)	RSD%[a]	RT[b]	M.I. (m/z)[c]	F.I. (m/z)[d]	r^{2}[e]	Linearity Range (mg/L)	*LOD/LOQ* (µg/L)[f]	Recovery (%)
1	Apigenin	0.005	1.17	38.2	268.8	151.0/149.0	0.998	0.05-2.5	1.3/2.0	0.9985
2	Naringenin	0.005	2.34	35.9	270.9	119.0	0.999	0.1-5	2.6/3.9	1.0062
3	Salicylic acid	0.009	1.48	21.8	137.2	65.0	0.999	0.1-5	6.0/8.3	0.9950
4	Luteolin	0.012	1.67	36.7	284.8	151.0/175.0	0.999	0.1-5	2.6/4.1	0.9952
5	Hesperidin	0.03	1.84	25.8	611.2	449.0	0.999	0.1-5	19.0/26.0	0.9967
6	Gallic acid	0.036	1.60	4.4	168.8	79.0	0.999	0.1-5	13.2/17.0	1.0010
7	Rutin	0.052	1.38	25.6	608.9	301.0	0.999	N.A.	15.7/22.7	0.9977
8	Quercetin	0.075	1.89	35.7	301.0	272.9	0.999	N.A.	15.5/19.0	0.9967
9	*p*-Coumaric acid	0.13	1.92	17.8	163.0	93.0	0.999	0.1-5	25.9/34.9	1.0049
10	Kaempferol	0.15	1.49	37.9	285.0	239.0	0.999	0.05-2.5	10.2/15.4	0.9992
11	Protocatechuic acid	0.217	1.43	6.8	152.8	108.0	0.957	0.1-5	21.9/38.6	0.9972
12	Quercitrin	1.966	2.24	29.8	447.0	301.0	0.999	0.1-5	4.8/6.4	0.9960
13	Quinic acid	15.61	0.69	3.0	190.8	93.0	0.996	0.1-5	25.7/33.3	1.0011
14	Miquelianin	2.06	1.31	24.1	477.0	150.9	0.999	0.05-2.5	10.6/14.7	0.9934

[a] Relative standard deviation, [b] Retention time, [c] Molecular ions of the standard analytes (m/z ratio), [d] Fragment ions, [e] Coefficient of determination, [f] Limit of detection/quantification.

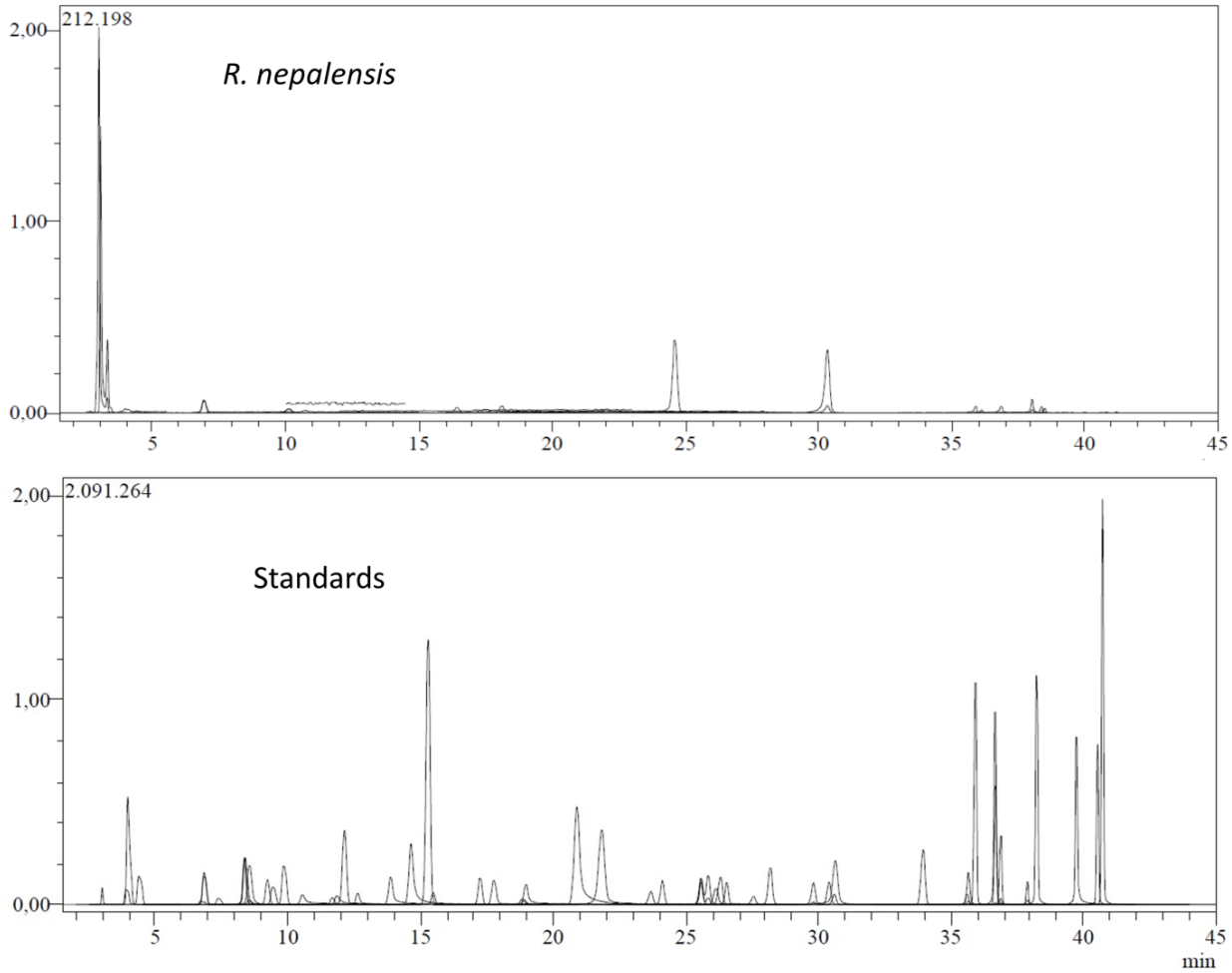

Figure 2. LC-MS/MS chromatograms of *R. nepalensis* (1-gallic acid, 2-catechin, 3-caffeic acid, 4-p-coumaric acid, 5- trans-ferulic acid, 6-o- coumaric acid,7-quercetin) and standards

3. Results and Discussion

3.1. LC–MS/MS Analysis

R. nepalensis plant has been investigated as a potent metabolic enzyme inhibitor for a new therapeutic approach against diabetes mellitus and Alzheimer's disease. Phenolic compounds of the *R. nepalensis* extracts were identified and quantified by a developed LC-MS/MS method as given in Table 1 and Figure 2. Method validation parameters such as the accuracy (recovery), precision (repeatability) were studied for standard uncertainties of each analyte according to EURACHEM guide [29].

Quinic acid (15.61 mg/g), miquelianin (2.06 mg/g), quercitrin (1.966 mg/g), protocatechuic acid (0.217 mg/g), kaempferol (0.15 mg/g), and *p*-coumaric acid (0.13 mg/g) were found to be the most intensive phenolics compounds in *R. nepalensis*, as shown in Table 1. Also, apigenin (0.005 mg/g), naringenin (0.005 mg/g), salicylic acid (0.009 mg/g), luteolin (0.012 mg/g), gallic acid (0.036 mg/g), hesperidin (0.03 mg/g), rutin (0.052 mg/g), and quercetin (0.075) compounds are the other compounds determined in *R. nepalensis* extract. However, the rest of analytes used as standards were not detected in the plant extract.

The association of the most intensive phenolic compounds (quinic acid, miquelianin, and quercitrin) of *R. nepalensis* with various biological properties and their metabolic functions in the treatment of many diseases have been described in previous studies. Quinic acid derivatives demonstrated favorable effects on human health via their anti-inflammatory, hepatoprotective, antioxidant, anti-hepatitis B virus, and anti-HIV properties [30,31]. A former study revealed the efficacy of miquelianin as promising antidiabetic, wound healing, and antioxidant agents [32]. Another study proved that quercitrin significantly prevents inflammation [33].

Phenolic compounds have many biological attributes, such as antioxidants, antiviral, and antimutagenic properties. Plant phenolic compounds have several health benefits which have been correlated with their antioxidant properties [34,35].

3.2. Antioxidant Activity

Antioxidants are considered as important factors for preventing some serious diseases such as cancer, brain dysfunction, cardiovascular diseases, and cataracts [36]. Free radicals, such as reactive nitrogen species (RNS) and reactive oxygen species (ROS), the hallmarks of AD pathology, trigger an imbalance between antioxidant mechanisms and free radical production [37]. The levels of free radicals and antioxidants are balanced by the human body [38].

In the present study, the water and methanol extracts of *R. nepalensis* leaves were examined by four *in vitro* antioxidant methods. ABTS (2,2-azino-bis 3-ethylbenzothiazloine-6-sulphonic acid) and DPPH (1,1-diphenyl-2-picrylhydrazyl) methods were used for measuring the radical scavenging capacity whereas CUPRAC (cupric ion reducing antioxidant capacity) and FRAP (ferric ion reducing antioxidant power) methods were used for measuring the reducing power antioxidant activity.

ABTS and DPPH radical scavenging properties of *R. nepalensis* extracts were compared with standard antioxidants. BHA (butylated hydroxyanisole), BHT (Butylated hydroxytoluene), α-tocopherol, and ascorbic acid were the standard references of the present study. In the radical scavenging methods, decreasing absorbance indicates radical scavenging potential. According to the measurements, WER (water extract of *R. nepalensis*) and MER (methanol extract of *R. nepalensis*) showed quite low free radical scavenging activity in the DPPH method, while both extracts revealed effective cation radical scavenging activity in the ABTS method (Figure 3).

ABTS radical scavenging percentage values of the extracts and standards at the same concentration (30 µg/mL) for the ABTS radicals were found as follows: WER (53.6±8.7 %), MER (51.9±7.8 %), BHA (61.4±6.9 %), BHT (27.9±9.8 %), ascorbic acid (39.8±7.1 %), and α-tocopherol (54.1±10.7 %) as shown in Figure 3a. According to the ABTS cation radical scavenging method, the IC_{50} values (the concentration of a sample to scavenge 50% of radicals) of *R. nepalensis* extracts and standards were ordered as: BHA (16.3±6.9 µg/mL) < WER (18.2±5.3 µg/mL) < MER (18.5±3.5 µg/mL) < α-tocopherol (25.5±10.8 µg/mL) < ascorbic acid (26.8±7.3 µg/mL) < BHT (34.3±9.5 µg/mL) (Figure 3b). The water and methanol extracts showed close ABTS cation radical scavenging amounts. A former study reported close amounts of ABTS cation radical scavenging IC_{50} values for chloroform (10.24 µg/mL) and ethyl acetate (8.70 µg/mL) extracts of *R. nepalensis* [39].

In DPPH method, the IC_{50} values of standards were as follows: BHA (10.9±4.8 µg/mL), BHT (12.9±6.0 µg/mL), ascorbic acid (11.1±6.2 µg/mL), and α-tocopherol (11.3±6.7 µg/mL). The IC_{50} values of WER and MER were not significant. The lower IC_{50} means the higher radical scavenging activity for both $ABTS^{\bullet+}$ and DPPH radical scavenging assays. The DPPH scavenging percentages of *R. nepalensis* extracts and standards decreased as ascorbic acid (91.9±0.6 %) > α-tocopherol (90.2±3.5 %) > BHA (88.9±1.8 %) > BHT (75.6±7.6 %) > MER (2.6±2.4 %) > WER (1.5±1.3 %).

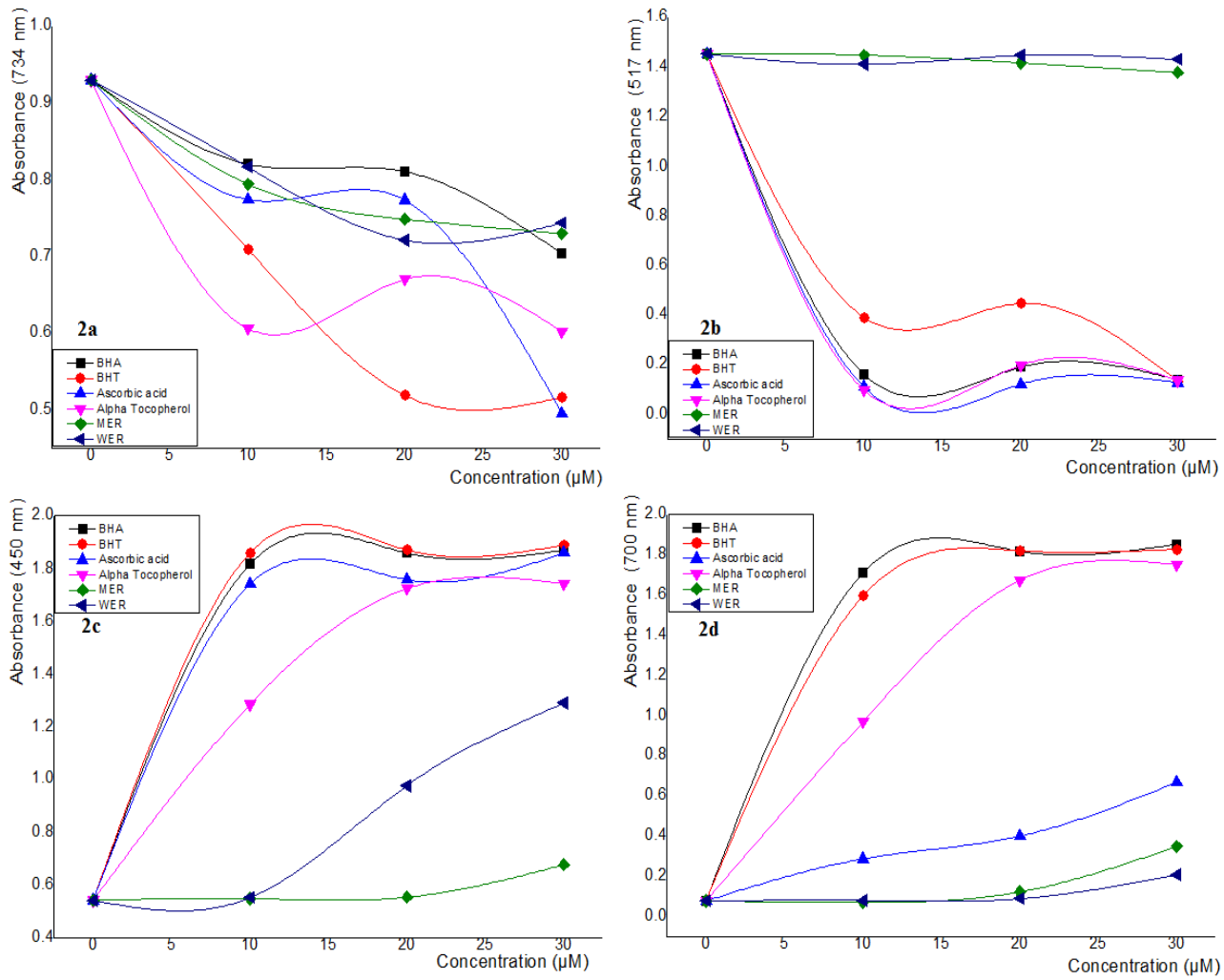

Figure 3. Antioxidant activities of *R. nepalensis* and standards for ABTS, DPPH, CUPRAC, and FRAP methods

CUPRAC and FRAP methods were used to determine the reducing antioxidant activities of *R. nepalensis* extracts. Increasing absorbance of a sample was proportionate to the increase of its reducing antioxidant potential. Antioxidants cause the reduction of ferric (Fe^{3+}) ions to the ferrous (Fe^{2+}) ions as well as the reduction of cupric (Cu^{2+}) ions to the cuprous (Cu^{+}) ions due to their reducing capacities. As seen in Figure 3d, reducing levels of the WER and MER were very weak in the FRAP method. The ferric ions reducing antioxidant capacities at 30 µg/mL concentration were decreased as: α-Tocopherol > BHT > BHA > ascorbic acid > MER > WER. In the CUPRAC method, MER showed weak reducing activity, while WER showed moderate activity compared to the standards. The cupric ions reducing capacities at 30 µg/mL concentration ordered as follows: BHA > ascorbic acid > BHT > α-Tocopherol > WER > MER (Figure 3c). Notable differences were calculated for WER and MER in CUPRAC method while some minor differences were observed in other three techniques minor differences were observed.

3.3. Enzyme Inhibition

In the present study, glutathione S-transferase (GST), AChE, BChE, and α-glycosidase enzymes were adequately inhibited by the *R. nepalensis* extracts. The IC_{50} values for *R. nepalensis* methanol extract on used enzymes were calculated as 21.01 mM for GST, 27.72 mM for AChE, 17.32 mM for BChE, and 34.65 mM for α-Gly. The results showed that α-Gly, AChE, BChE, and GST were very efficiently inhibited by *R. nepalensis* methanol extracts.

AChE acts as a predominant enzyme while BChE has been identified as a regulator of acetylcholine levels with a secondary role in the human brain [40]. Cholinesterase enzymes catalyze the hydrolysis of the neurotransmitter acetylcholine and butyrylcholine. Many studies examined the effects of glutathione S-transferase isoenzymes. Glutathione S-transferase is a multifunctional enzyme catalyzing the metabolic pathway for detoxification. This enzyme is used as a catalyst in the first step in forming mercapturic acid, which is the water-soluble end product [41]. Catalyzed by GST in this first step, glutathione (GSH) binds the endogenous and exogenous hydrophobic electrophiles [42].

Alpha-glucosidase inhibitors (α-GIs) have great importance for controlling hyperglycemia and type-2 diabetes mellitus [43]. α-Glycosidase is an enzyme that is particularly important for studying enzyme inhibitory activity and it is also performs lag glucose absorption. The liberalization of the glucose molecule from complex carbohydrate compounds results in decreased postprandial plasma blood glucose and suppression of postprandial hyperglycemia [44]. In another study, it is reported that caffeic acid phenethyl ester (CAPE) effectively inhibited by AChE, BChE, and GST enzymes. These data may explain the beneficial effects of such compounds on human health and also enable researchers and drug designers to focus on them.

3.4. Molecular Modeling and Docking Studies

Nowadays, *in silico* ADME studies are used in drug production to select the most promising compounds and to minimize the risk of drug attrition [45,46]. With these studies, an equilibrium between pharmacodynamic and pharmacokinetic properties can be determined as preliminary information. Several parameters such as molecular properties, drug solubility, cell permeability, human intestinal absorption, polar surface area, and drug similarity scores are investigated with virtual scanning methods used on small molecules [47]. The molecular weight and LogP of an existing oral drug selected by the 5 rules of Lipinski [48] should not be more than 500 and 5, respectively. Also, the number of hydrogen bond acceptors should be less than 10 and the number of hydrogen bond donors should be less than 5.

Online servers such as Molinspiration and SwissADME softwares were employed to check the chemo-informatics and biological properties of the major compounds of the plant extract as ligand molecules. The results showed that the compounds are compatible with MW 192.17 - 478.36 g/mol, LogP values according to the Lipinski rule with -1.69 to 1.60 and HBA 5-8 (Table 2). Among all the values, quinic acid appears to have better permeability than the other molecules.

Molecular docking is a reliable calculation technique that can be used to examine the binding mechanism between the ligand-receptor and to predict possible binding modes, and the results can be experimentally controlled [49,50]. In this study consisting of three different phenolic compounds with four enzyme sets, totally twelve promising docking results were obtained. These ligands were placed in the catalytic active region of the enzyme and the docking results were analyzed based on binding affinities and interaction modes. Glide scores demonstrated that quercitrin exhibited good binding affinity with the AChE enzyme and exhibited excellent binding affinities with AChE and BChE enzymes, as seen in Table 3. Miquelianin achieved a good binding score with all enzymes. However, the binding affinity score of quinic acid the smallest molecular structure was slightly lower than the others.

Table 2. Physicochemical and lipophilicity of the major compounds of *R. nepalensis* by using SwissADME software

Compounds	Lipophilicity consensus logP	MW[a] (g/mol)	Heavy atoms	Aromatic heavy atoms	Rot. bond	H-bond acc.	H-bond don.	MR[b]	TPSA[c] (A^2)	% ABS[d]
Quinic acid	-1.69	192.17	13	0	1	6	5	40.11	118.22	68.29
Quercitrin	1.60	448.38	32	16	3	11	7	109.00	190.28	43.45
Miquelianin	1.13	478.36	34	16	4	13	8	110.77	227.58	33.10

[a] molecular weight, [b] molar refractivity, [c] topological polar surface area, [d] percentage of absorption.

Table 3. Best-binding affinity scores (kcal/mol) of major phenolic compounds of *R. nepalensis* in the catalytic sites of the enzymes

	AChE (PDB:6O4W)	**α-Gly** (PDB: 5NN8)	**GST** (PDB:3KTL)	**BChE** (PDB: 6QAA)
Quinic acid	-5.988	-6.266	-6.512	-5.251
Quercitrin	-7.604	-5.853	-6.561	-8.495
Miquelianin	-7.109	-5.808	-7.617	-7.912

After the best exposure selection in all ligand-enzyme docking, the binding modes were analyzed to understand the inhibition mechanisms. Figure 4 shows the 3D and 2D interactions of quercitrin-AChE docking. The Glide score was calculated as -7.604 cal/mol in the binding affinity of quercitrin with AChE. Here in the binding mechanism; Conventional hydrogen bond on the TYR-124 (2.54 Å) in the middle phenyl ring, TRP-286 (4.20 Å) Pi-Pi stacked on the phenyl ring in the middle, SER-293 (2.35 Å) carbon-hydrogen bond on branched six rings, GLU-292 (2.68 Å) carbon hydrogen bond on oxygen-bonded to a branched six ring are samples. The other interactions and the 3D view of the hydrogen bonds donor/acceptor surface on the receptor are shown in Figure 4.

Figure 5 shows the 3D and 2D interactions and binding affinity of quinic acid with α-Gly. The Glide score of quinic acid was calculated as -6.266 cal/mol in the binding affinity of quinic acid with α-Gly. The obtained results of quinic acid were close to the previous studies [51,52]. Here in the binding mechanism; ASP-616 (2.65 Å) first hydroxyl-bonded on the six ring carbon-hydrogen bond, ASP-404 (1.86 Å) second hydrogen hydroxy-bonded on the six rings conventional hydrogen bond and HIS-674 (2.49 Å) hydrogen-bonded on the six ring hydrogen carbon bond are samples. Figure 5 shows the 3D view of the SAS surface on the receptor and 2D view of other interactions.

Figure 6 represents the 3D and 2D interactions of miquelianin-GST docking study. The Glide score was calculated as -7.617 cal/mol in miquelianin-GST binding affinity. The obtained results were close to a previous study [53]. The binding mechanism; LEU-107 (2.26 Å) hydroxyl-bonded in middle phenyl ring conventional hydrogen bond, PHE-222 (4.74 Å) and PHE-220 (5.74 Å) middle six ring bonded to the center Pi-Pi stacked, VAL-111 (5.46 Å) middle six rings bonded to the center pi-alkyl, Hydroxyl-bonded VAL-55 (1.67 Å) in the branched six rings, oxygen-bonded GLN-54 (2.67 Å), carboxyl-bonded ARG-15 (1.97 Å) conventional hydrogen bond are the other bonds.

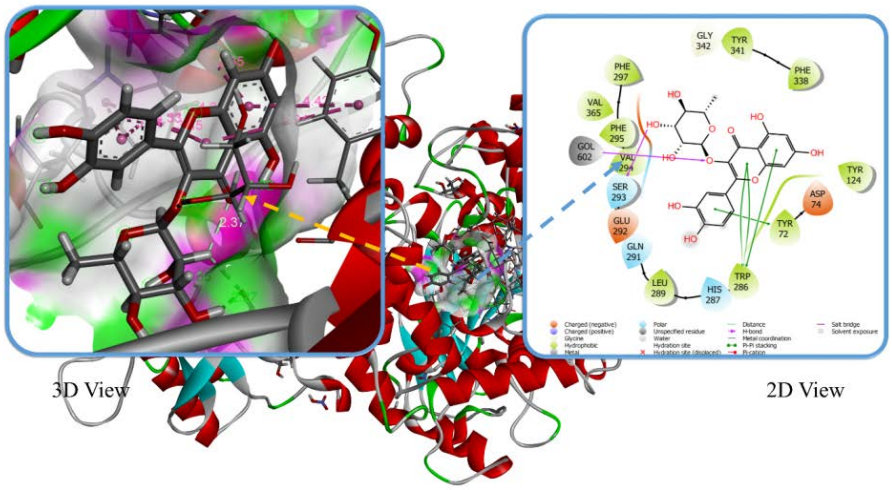

Figure 4. The interaction mode between quercitrin - AChE with enzyme, 3D view of the hydrogen bonds donor/acceptor surface on the receptor and 2D view of the interactions

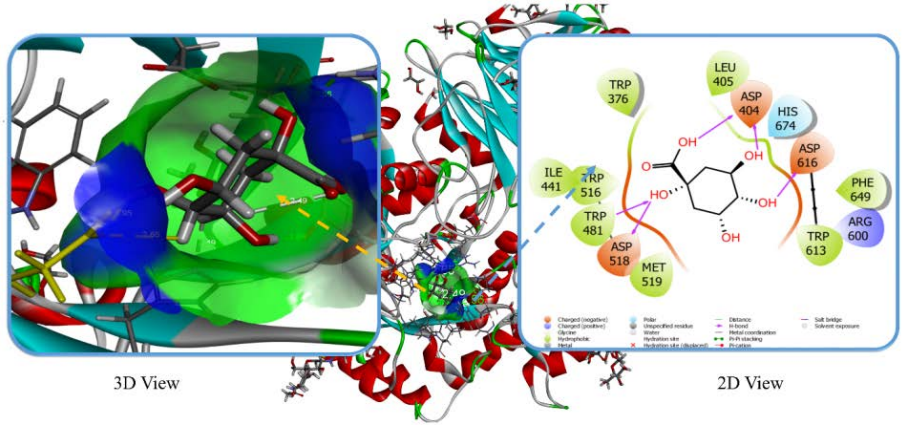

Figure 5. The interaction mode between quinic acid - α-Gly with enzymes, 3D view of the SAS surface on the receptor, and 2D view of the interactions

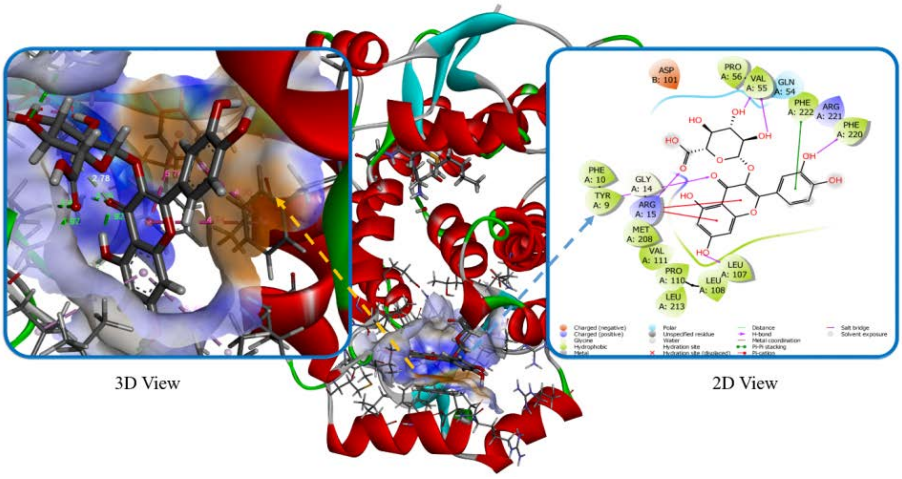

Figure 6. The interaction mode between miquelianin -GST enzyme, 3D view of the aromatic surface on the receptor, and 2D view of the interactions

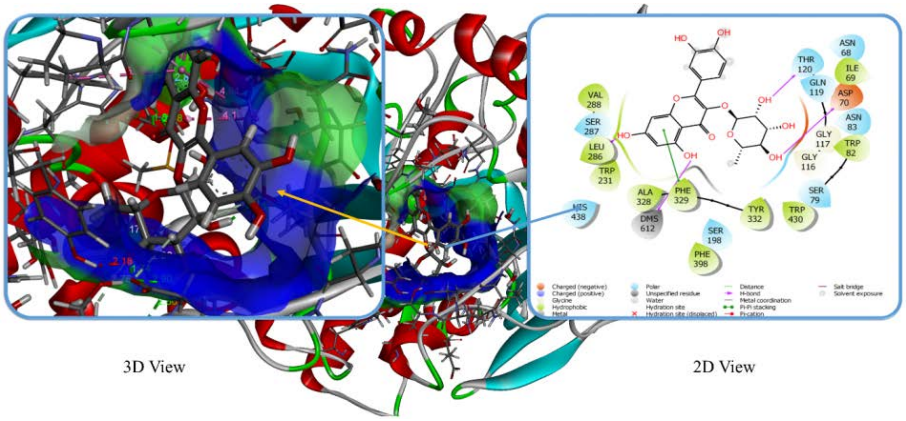

Figure 7. The interaction mode between quercitrin-BChE enzyme, 3D view of the SAS surface on the receptor d) 2D view of the interactions

In Figure 7, the 3D and 2D interactions are given as a result of the quercitrin-BChE docking study. The Glide score was calculated as -8.495 cal/mol in the highest affinity for quercitrin-BChE. In the binding mechanism; GLY 116 (4.61 Å) in the middle of the center of the phenyl ring amide-Pi stacked, in the middle phenyl ring, oxygen-bonded LEU 286 (2.00 Å) and hydroxyl-bonded SER 198 (2.89 Å) conventional hydrogen bond, PHE 329 (5.26 Å) and TRP 231 (5.67 Å), bonded to the center of the phenyl ring in the middle Pi-Pi T-shaped, TR-82 (4.17 Å) carbon-bonded in the branched hex ring Pi-alkyl, THR 120 (2.89 Å) carbon-bonded in a branched six ring carbon-hydrogen bond are the samples. Quercetin proved to be an effective inhibitor that binds with the enzyme structure by many chemical bonds [54,55,56].

4. Conclusion

The present study investigates the phytochemical contents, biological activities, molecular insertion, and computational insights of the endemic plant, *R. nepalensis*. The results demonstrated the potent antioxidant and enzyme inhibitory activities of *R. nepalensis* extracts against glutathione S-transferase, α-glycosidase, acetylcholinesterase, and butyrylcholinesterase enzymes. The docking study revealed the highest affinity energy of quercitrin for AChE and BChE, quinic acid for α-Gly, and miquelianin for GST, respectively. Since the molecular size of quinic acid is smaller than the other reference compounds, the surface fully complies with oral absorption and with Lipinski rule. Therefore, the inhibition effect against AChE is more significant than the other inhibition values. Altogether, this plant extracts could be exploited as a novel source of the phenolic ingredient, antioxidants, and inhibitors of diabetes mellitus and Alzheimer's disease related enzymes for pharmaceutical industries.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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