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# *Alchemilla pseudocartalinica* Juz: Phytochemical Screening by UPLC-MS/MS, Molecular Docking, Anti-oxidant, Anti-diabetic, Anti-glaucoma, and Anti-Alzheimer Effects

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**Abstract:** *Alchemilla* species (Rosaceae) are popularly known as 'Lady's Mantle, Lion's claw' and are used for medicinal purposes as diuretic, laxative, tonic, and wound healing agents. Bioactivities and phenolic content of *Alchemilla pseudocartalinica* Juz. species have yet to be investigated. Our research focused on assessing the antioxidant characteristics of *A. pseudocartalinica* methanol (MEAP) and water extracts (WEAP), as well as their inhibitory effects on acetylcholinesterase (AChE), α-glycosidase (α-gly), and human carbonic anhydrase II (hCA II) enzymes. Additionally, we conducted chemical characterization using UPLC-MS/MS and investigated the correlation between major phenolic compounds and enzymes through molecular docking analysis. To assess the antioxidant activities of the MEAP and WEAP, six test systems were employed, including DPPH, ABTS, DMPD, FRAP, CUPRAC, and $Fe^{3+}$ reducing assays. The outcome showed that the methanol extract of the plant generally has stronger antioxidant activity. In addition, UPLC-MS/MS analysis indicated, miquelianin (44.095 mg/g), quinic acid (17.054 mg/g), and ellagic acid (6.492 mg/g) were significant in the methanol extract. A molecular docking study revealed a significant affinity for binding between the hCAII enzyme and quinic acid, miquelianin, and AChE/*α*-gly enzymes. *A. pseudocatalinica* methanol and water extracts have high antioxidant activity and good inhibition effect against AChE, *α*-glycosidase, and hCA II enzymes.

**Keywords:** *Alchemilla,* antioxidant activity; enzyme inhibition; UPLC-MS/MS; molecular docking. 

## 1. Introduction

The *Alchemilla* L. is a genus that demonstrated a spread in the northern parts of Turkey and the northern hemisphere worldwide and belongs to the Rosaceae family, a large family containing important herbaceous and woody species [1]. The genus *Alchemilla* L. is represented by more than 1000 species worldwide and 82 species in Turkey, 36 of which are endemic [1, 2].

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## Phytochemical consituents of *Alchemilla pseudocartalinica*

Phytochemical studies on some species of the genus *Alchemilla* have identified many compounds including phenolic acids, flavonoids, tannins, terpenes, as well as coumarins [3]. Studies on its pharmacological activities have reported that it has medicinal properties such as anti-inflammatory, antiseptic, sedative, hemostatic, wound-healing, expectorant, diuretic, astringent. Additionally, it is also employed in the treatment of atherosclerosis, diabetes and menorrhagia. Nevertheless, they have traditionally been used in the Turkish population for their medicinal properties due to their diuretic, laxative, tonics and wound-healing agents [4]. The genus *Alchemilla* is perennial, with a woody rhizome, palmate leaves and achene fruits [5].

Close inspection of the botanical characteristics of the *Alchemilla pseudocartalinica* Juz. species commonly known in Turkey under such names as 'Eagle's claw, hazelnut herb, keltat, order of the star' reveals that the flowering stems of the plant can grow up to 50 cm, and the leaves, which are toothed and glabrous, can grow up to 7 cm in width. It grows at altitudes of 1200-3000 m in moist meadows and rocky igneous slopes in continental and black sea climate [5]. Traditional medicine has documented the usage of the species *A. pseudocartalinica* primarily in the form of decoction or infusion for sleep disorders, bronchitis, colds, constipation, dyspepsia, diabetes, dysmenorrhea, and kidney and gynecological diseases, and in the ground form for eczema, hemorrhoids', and pain [6, 7].

Oxidative stress is associated with neurodegenerative, inflammatory, cardiovascular, immunological, endocrinological, and oncological diseases. Oxidative stress increases with the inability to control free radicals in the body [8]. It causes damage to the cell by reacting with bio-relevant molecules such as DNA/RNA and protein. Free radicals are unstable compounds that do not form pairs in their outermost atomic orbitals. Antioxidants neutralize free radicals by donating electrons to them and stabilize the unpaired electrons in their resonance structures. Thus, they prevent reactive oxygen species (ROS) from damaging the cell [9]. Natural or synthetic antioxidants are needed in the food industry to prevent oxidation in foods, in addition to prophylaxis. Since synthetic antioxidants are not preferred due to their negative effects, new natural herbal antioxidants have been searched for. Therefore, new herbal antioxidant sources that can direct the food industry are being sought [10].

Diabetes mellitus (DM) is a disorder characterized by metabolic and endocrine abnormalities occurring due to impaired insulin secretion. DM causes not only hyperglycemia but also problems in fat and protein metabolism [11]. DM has become an increasingly common disease due to lifestyle changes and economic development. According to the World Health Organization, there was a 5% increase in premature deaths due to diabetes between 2000 and 2016, and diabetes ranked ninth among causes of death in 2019 [12]. In diabetes, compounds that inhibit enzymes that are effective in the absorption and metabolism of glucose from the intestine takes a vital role in the treatment. α-Glycosidase is a pancreatic enzyme located in the small intestinal brush border that breaks down disaccharides and starch [13]. Thanks to the inhibition of $\alpha$-glycosidase enzymes, glucose absorption is delayed, and blood sugar regulation is provided [14]. Due to the adverse effects of many drugs used in the treatment of DM, medicinal plants and polyphenols to be obtained from them are being investigated to increase insulin sensitivity with fewer side effects. Since polyphenol compounds in plants can facilitate treatment with synergistic effects, plant extracts are also widely preferred by the public [15]. *A. pseudocartalinica* is also a plant preferred by the public in the treatment of diabetes and has not been previously investigated for its $\alpha$-glycosidase inhibitory effect [6].

hCA enzyme is a metalloenzyme containing zinc in its structure. It catalyzes the hydration of water and carbon dioxide that is reversible to proton in addition to bicarbonate, regulating pH [15, 16] and maintaining fluid balance in organs such as the stomach, eyes, and kidneys [17]. It is used in the treatment of peptic ulcers, osteoporosis, glaucoma, lung, kidney, and esophageal cancers by providing hCA II isozyme inhibition [17, 18]. hCA II inhibitors such as ethoxzolamide, methazolamide, and acetazolamide, dichlorphenamide are systemic medication used to treat glaucoma. However, when such drugs inhibit the localization of other CA isoforms outside the eye, they cause side effects such as paresthesia, fatigue, and anxiety. Therefore, it is desirable to find new inhibitors with fewer side effects, suitable for topical use and of natural origin [19]. Thus, we aim to determine the inhibition effect of *A. pseudocartalinica*, which can be used in the treatment of glaucoma, on hCA II enzyme and to fill the gap in the literature.

A neurodegenerative condition, Alzheimer's disease (AD) demonstrates pathological features such as neurofibrillary tangles formed by senile amyloid plaques and tau fibrils, synapse damage,

neuronal inflammation, and brain atrophy [17, 20]. The disease is characterized by clinical findings such as memory loss, decline in cognitive and mental functions such as forgetfulness, attention problems, speech difficulties, depression, sleep problems, and inability to adapt to daily life [19, 21]. The incidence and prevalence of AD increased by 148% and 161%, respectively, from 1990 to 2019, with 152 million people projected to suffer from AD by 2050 [22]. AD oxidative stress, acetylcholine (ACh) deficiency, inflammation and its presence are linked to the occurrence of the disease [23]. Current treatments aim to stop and reduce the progression of the disease with the use of AChE inhibitors, but these are not sufficient [24]. In addition, undesirable effects such as hepatotoxicity and gastrointestinal effects in existing drugs are aimed at finding natural products with fewer side effects [25, 26].

Chem-bioinformatics is a field that has been greatly influenced by technological and computational developments, and in the development of novel pharmaceuticals and bioactive compounds is on the rise. There are numerous programs available in chem-bioinformatics for the purpose of designing new drugs and active substances. Among these methods, molecular docking stands out as a significant approach. [27, 28]. Molecular docking is used as an essential tool for drug discovery by simulating the interactions between target and ligand. Molecular docking is a simulation method for predicting the stable complex structures that ligands and receptors will form as a result of their interactions [29, 30].

When previous studies were examined, it was understood that there was not enough research on the antioxidant, anticholinesterase, antiglaucoma and antidiabetic activities of *A. pseudocartalinica,* which is traditionally used among the public for medicinal purposes. Hence, our study aims to reveal the biological activities of *A. pseudocartalinica*, perform its chemical analysis, and reveal the relationship of major compounds with enzymes.

## 2. Materials and Methods

### *2.1. Plant Material*

The aerial parts of *A. pseudocartalinica* Juz. were obtained from Erzurum Köşk village, 40º 5’ 21” N 41º 25’ 36” E, at an altitude of 1950 m on 24 June 2018 by Dr. Leyla Güven. The specimens were identified by Prof Dr. Yusuf Kaya. Herbarium samples were kept in the Biodiversity Application and Research Centre of Atatürk University with herbarium number AUEF 1391 (Figure S1).

### *2.2. Extraction Process*

The aerial part of *A. pseudocartalinica* was dried and powdered in a dry and sun-free environment. Then 500 mL of methanol was supplied to 20g of powdered plant and kept overnight. After maceration, it was extracted at 25°C for 24 hours. The extract was filtered, and evaporation of the filtrate was observed under low pressure at 40°C at 120 rpm (Heidolph VV2000, Schwabach, Germany). The resulting extract had a yield of 23.56%. Storage of the extract was conducted at -18°C until the day of the experiment [31].To obtain the water extract of the aerial part of *A. pseudocartalinica*, boiled water of about 500 mL was added to 20g of powdered plant and extracted at 50°C for 2 hours. After extraction, it was filtered while hot, and the filtrate obtained was dried in a lyophilizer. The yield of the obtained extract was 9.4%. Storage of the extract was handled at -18°C till the day of the experiment [15].

### *2.3. Antioxidant Assays*

#### *2.3.1. $Cu^{2+}$ -$Cu^{+}$ Reduction Capacity (CUPRAC Method)*

$Cu^{2+}$ reducing capacities of *A. pseudocartalinica* extracts were investigated by a slight modification of the Gülçin method [32]. For this purpose, *A. pseudocartalinica* extracts and standards prepared at different concentrations (15-45 µL) were pipetted into tubes, and 10 mM, 500 µL $CuCl_2$ solution, $7.5x10^{-3}$ M and 500 µL neocuproine solution and 500 µL buffer solution were added, respectively. Distilled water was added to the tubes so that the total volume was 4 mL. Three repetitions were made at three different concentrations and prepared for measurement. After incubation in a dark environment for thirty minute, the measurement of absorbance values produced the result of 450nm against the blind and recorded [33].

#### *2.3.2. $Fe^{3+}$ - $Fe^{2+}$ Reduction Capacity*

$Fe^{3+}$ - $Fe^{2+}$ reduction capacity estimation was performed by making a minor adjustment to the approach used by Oyaizu [8, 10, 34]. In the experiment, stock solution and standard solutions (15-45 µL) were added to test tubes at 1 mg/mL concentration, and the completion of the volume was done to 750 µL with distilled water. 1 mL phosphate buffer and 1 mL 1% [$K_3Fe(CN)_6$] were added in each tube and incubated at 50 °C for half an hour. After incubation, 1 mL TCA and 250 µL $FeCl_3$ were put in the tubes and thoroughly mixed using vortex. Three repetitions were performed at three different concentrations. At the end, the values of absorbance were measured and recorded at 700 nm against the blind [35].

#### *2.3.3. FRAP Reduction Capacity*

FRAP reducing capacities of *A. pseudocartalinica* extracts and standard solutions were determined based on the method developed by Benzie and Strain [36]. 15-45 µL of *A. pseudocartalinica* extracts were put in 500 µL of buffer solution. 2250 µL $FeCl_3$ solution and 2250 µL FRAP solution were added to each tube. The tubes were mixed thoroughly with the help of a vortex and kept in the dark for half an hour, after which the absorbance values were measured at 593 nm [36].

#### *2.3.4. 1,1-Diphenyl 2-picrylhydrazyl Radical ($DPPH^{\cdot}$) Reduction Capacity*

The estimation of DPPH- activity was performed according to the modified Blois method [37, 38]. Stock solutions and standard solutions prepared at varying concentrations (15-45 µL) were put in the test tubes. Their volumes were adjusted to 2 mL using ethanol. Then, 500 µL of 1 mM DPPH free radical solution was added to the sample tubes and mixed thoroughly with the help of a vortex. Only 2 mL ethanol and 0.5 mL DPPH radical solution were added to the control tubes. After keeping for thirty minutes in the dark at room temperature, the absorbance values were read against the blind at 517 nm [39].

#### *2.3.5. 2,2-Azino-bis(3-ethylbenzo-thiazolin-6-sulphonic acid) Radical ($ABTS^{\cdot+}$) Scavenging Ability*

$ABTS^{\cdot+}$ scavenging activity determination was performed according to the modified method of Re et al. [11, 40]. Initially, *A. pseudocartalinica* extracts and solutions of standard characteristics were transferred to tubes at varying concentrations (15-45 µL), and the volumes were topped up with ethanol. To generate ABTS radicals, 2.45 mM persulfate solution and 2mM ABTS solution were mixed. The absorbance of $ABTS^{\cdot+}$ radical was then adjusted to 0.750±0.030 nm at 734 nm. Then, $ABTS^{\cdot+}$ radical solution was put in all tubes and vortexed. At the end of the 30-minutes incubation period, the absorbance was recorded at 734 nm against ethanol-derived blind [35].

*2.3.6. N,N′-Dimethyl-p-phenylenediamine dihydrochloride Radical ($DMPD^{\cdot+}$) Scavenging Capacity*

$DMPD^{\cdot+}$ activity was determined by modifying the approach adopted by Fogliano et al. [41, 42]. Different concentrations (15-45 µL) of *A. pseudocartalinica* extracts and standard solutions were adjusted to 0.5 mL using distilled water. $DMPD^{\cdot+}$ radical cation solution was prepared by adding 0.2 mL of 0.05 M $FeCl_3$ solution. All tubes were treated with 1 mL of radical $DMPD^{\cdot+}$, and after incubation for 20 min, measurements were taken against the blind at 505 nm.

*2.4. Enzyme Inhibition Assays*

*2.4.1. Determination of AChE Inhibitory Effects*

The effects of AChE on the inhibition were determined spectrophotometrically by a modified version of the Ellman method [43, 44]. Acetylcholine iodate (AChI) and 5,5'-dithio-bis(2-nitro-benzoic) acid (DTNB) are the substrates utilized in the enzymatic reaction. 100 M, 50 µL Tris/HCl buffer (pH 8.0) was combined with 390 µL sample and 10 µL AChE and kept in a dark environment for 10 minutes at room temperature. Then, 25 µL of each solution containing the substrates were added. In the mixture, DTNB reacts with the breakdown product thiocholine, resulting in the formation of 5-thio-2-nitrobenzoic acid with a yellow color. The records showed absorbance of the yellow color at a wavelength of 412 nm [45].

*2.4.2. Determination of α-Glycosidase Inhibitory Effects*

The *α*-glycosidase enzyme inhibitory effect of *A. pseudocartalinica* plant extracts was performed using *p*-nitrophenyl-D-glycopyranoside (p-NPG) substrate according to the method developed by Tao et al. [46]. Firstly, 50 µL phosphate buffer (pH 7.4), 10 µL enzyme solution (0.15 U/mL) and 20-100 µL sample were mixed. The substrate was then added and incubated at ambient temperature to start the reaction. Measurement of absorbances spectrophotometrically revealed the result of 405 nm [47].

*2.4.3. Determination of hCA II Isoenzyme Inhibitory Effect*

hCA II enzyme inhibitory effect assay of the extracts obtained using *A. pseudocartalinica* plant was performed by utilizing the method of Senol et al. [17]. hCA II isoenzyme was obtained by centrifugation of human erythrocytes and purification of the resulting serum using Sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatography technique. The eluates obtained were recorded at 280 nm by controlling the spectrophotometer. In the enzyme inhibitory effect assay, *p*-nitrophenolate was used as substrate. The absorbance differences of *p*-nitrophenolate ion formed as a result of the reaction in 3 minutes were determined by spectrophotometric measurements at 348 nm [11].

*2.5. Determination of Total Phenolic and Flavonoid Contents*

The plant sample's total phenolic contents were established by a modification of the Singleton and Rossi method [48]. For the purpose of identifying the total phenolic content, 0.5 mL plant extract, 1 mL Folin-Ciocalteu reagent, and 0.5 mL 1% $Na_2CO_3$ were mixed, and the mixture was vortexed. After two hours at ambient temperature and in a dark environment, measurement of absorbance produced the result of 760 nm. The amount of phenol in one gram of *A. pseudocartalinica* methanol and water extracts was recorded as mg gallic acid equivalent (GAE). The determination of total flavonoid content was performed according to the method of Gülçin et al. 1.5 mL ethanol, 0.5 µg/mL plant extracts, 10% 1.5 mL $Al(NO_3)_3$, 0.5 mL, 1 M $CH_3COOK$, and 2.3 mL distilled water were mixed. The absorbances of the mixtures were recorded at 415 nm following incubation for thirty minutes at ambient temperature. Quercetin was utilized as a standard. The amounts of flavonoid were calculated as milligram quercetin equivalent (QE) per gram of extracts [49].

### *2.6. Phytochemical Analysis by UPLC-MS/MS*

#### *2.6.1. Preparation of Samples*

In this experiment, a 100 mg quantity of methanol extract derived from *A. pseudocartalinica* is dispersed in a volumetric flask containing 5 mL of a mixture of ethanol and water in a 50:50 ratio (v/v). Subsequently, 1 mL of this prepared solution is transferred into another volumetric flask with a volume of 5 mL. To this flask, 100 µL of methanol extract from *A. pseudocartalinica* is added, and the volume is adjusted using the (v/v 50:50) water-ethanol mixture. From this final mixture, a 1.5 mL portion is transferred into a flask equipped with a cover. Then, 10 µL of the specimen is introduced into the UPLC-MS/MS instrument. It is important to note that throughout the entire essay, the specimens in the autosampler are maintained at a temperature of 15°C.

#### *2.6.2. Test Solution for Mass Spectrometer (UPLC-MS/MS) and Chromatography Conditions*

In this study, the analysis utilized an analytical strategy that was consistent with the latest research findings. To determine the precise composition of bioactive phytochemicals in the methanol extract of *A. pseudocartalinica*, a previously established and validated UPLC-MS/MS method was employed. The phytochemical analysis of the methanol extract of *A. pseudocartalinica* was carried out using the UPLC-MS/MS method, which had been previously developed and validated [50]. The validation data for the UPLC-MS/MS analytical method can be accessed in Table S1 of the supporting information file. The elution gradient consisted of two eluents: eluent A, which comprised water, 5 mM ammonium formate, and 0.1% formic acid, and eluent B, which consisted of methanol, 5 mM ammonium formate, and 0.1% formic acid. The elution profile followed a gradient pattern: starting from 20% B, it increased to 100% B over a period of 25 minutes, remained at 100% B for the next 10 minutes, and finally decreased to 20% B within 10 minutes [50].

### *2.7. Molecular Docking Studies*

In this study, the quinic acid, miquelianin, and ellagic acid interactions, which constitute an important part of the phytochemical capacity of *A. pseudocartalinica* methanol extract determined by LC-MS-MS method, with hCA II, AChE, and *α*-glycosidase enzymes were determined using molecular docking method. To this end, in the first phase of molecular docking studies, molecular structures were formed using SMILES codes from the Drugbank internet database, and Utilizing Maestro's LigPrep software, optimization studies were undertaken. (S. Schrödinger Edition 2020-3: LigPrep, LLC, New York, NY, 2020). Enzyme structures were taken from the RCSB Protein Database website (PDB identifiers; hCA II: 5AML, AChE: 4TVK, *α*-glycosidase: 3L4Y). The potential interactions of molecules with each enzyme and their binding affinities were established using the molecular docking software called the Schrödinger Molecular Modeling Suite (Maestro Version 12.5.139, Mmshare Version 5.1.139 docking software)

### *2.8. Examining Data Statistically*

Statistical analyses were conducted using the SPSS 25.0 (IBM Corp., Armonk, N.Y., USA) software package. There were descriptive statistical analysis carried out. reporting the mean and standard deviation (mean±SD). Statistical significance was determined at a 95% confidence interval, with p values less than 0.05 considered significant. To assess the impact of positive controls and MEAP and WEAP on the parameters, one-way ANOVA and two-way (2x2) analysis of variance (ANOVA two-way between subjects) tests were employed. Additionally, the antioxidant capacities ($Cu^{3+}$ reducing, $Fe^{3+}$ reducing, FRAP) were evaluated at 15- 45 µg/mL doses. using a one-way ANOVA test followed by a post-hoc Tukey test to identify any significant differences. The analysis employed in this study was a two-way (2x2) ANOVA, which aimed to determine i-group differences in $Cu^{3+}$ reducing, $Fe^{3+}$ reducing, and FRAP, ii-dose differences at 15- 45 µg/mL, and iii- groupxdose interactions. By utilizing this approach, the study investigated the effects of two factors (group and dose) on a single variable ($Cu^{3+}$ reducing, $Fe^{3+}$ reducing, FRAP), thereby avoiding the need for separate one-way

ANOVA analyses that assess the impact of each factor individually. There are several justifications for these assertions. Firstly, repeating the same test multiple times can result in an elevated type 1 error. Secondly, two-way factor analysis allows for the examination of interactions between two factors that have an impact on each other, but are not observable in isolation. Lastly, opting for a single variance analysis instead of conducting two separate tests adds attractiveness and logical coherence to this method [51, 52].

## 3. Results and Discussion

Upon the investigating the literature through WoS, we were not able to come across any previously made biological or analytical studies on *A. pseudocartalinica*. With our study, this species was first examined in detail in terms of its biological activities, its phytochemical profile was explained, and the binding affinities of major phytochemical substances with enzymes were determined by molecular docking studies.

### *3.1. Antioxidant Potential*

Phytochemical substances in plants are essential for plants to maintain their vitality and continue their generation [53]. The fact that secondary metabolites in plants are effective against oxidative damage increases the interest in them more and more every day. Since reactive oxygen species (ROS) damage cells, they disrupt the balance of the body and cause many conditions such as diabetes, cancer, Alzheimer's and Parkinson's, and many more [38, 54]. Secondary metabolites such as phenolic acid, flavonoids, and tannins found in plants serve as antioxidant compounds [55].

Oxidative stress can manifest itself in many different ways in the body, such as metabolic activities, UV, environmental factors, and exposure to heavy metals. Different oxidative damages should be eliminated in cells by different methods. In studies, it is of vital importance to determine the antioxidant activities of plants, foods, or chemical substances used for medicinal purposes by different methods [38, 56].

Therefore, the antioxidant activity of plant extracts was determined by six individual methods in our study. The potent antioxidant activity of the extracts was compared with synthetic antioxidants. The present findings showed that *A. pseudocartalinica* extracts were better than synthetic preservatives BHA or BHT in some antioxidant assays such as DPPH, $Fe^{3+}$ reducing, and $Fe^{3+}$-TPTZ reducing. *A. pseudocartalinica* showed significantly better ($p< 0.05$) antioxidant activity in all methods used in our study.

Medicinal plants are used in foods to prevent oxidation and in various supplements due to their positive effects on health. There are many methods to determine antioxidant properties. In our study, methanol and water extracts of *A. pseudocartalinica* were tested by six different antioxidant activity determination methods.

As shown in Figure 1A and Table 1, MEAP and WEAP have an effective $Fe^{3+}$-reducing ability. The $Fe^{3+}$-reducing potential of the methanol and water extracts of *A. pseudocartalinica* and positive controls were established by conducting measurements of the absorbance values at 700 nm at 15, 30, and 45 µg/mL concentrations, and the ranking of absorbance values at 30 µg/mL were found as BHT (2.018), α-Tocopherol (1.895), Trolox (1.545), MEAP (1.308), BHA (1.257), WEAP (1.182). The $Fe^{3+}$ reduction effect of MEAP extract was higher than that of BHA used as the positive control.

The reduction capacity of copper ions ($Cu^{2+}$) at 15, 30, and 45 µg/mL concentrations of MEAP and WEAP was measured at 450 nm, and it was observed that the reduction capacity of $Cu^{2+}$ ions increased proportionally with concentration. From the absorbance values shown in detail in Figure 1B and Table 1, the absorbance values at 30 µg/mL were compared and ranked as follows: BHT (2.912), Trolox (2.323), BHA (1.800), *α*-Tocopherol (1.139), MEAP (0.650)> WEAP (0.430). Standard positive controls have higher antioxidant activity than MEAP and WEAP.

The $Fe^{3+}$-TPTZ reducing effects of *Alchemilla* methanol and water extracts and positive controls were also found to be dose-dependent (15-45 µg/mL) and are shown in Figure 1C and Table 1. The $Fe^{3+}$-TPTZ reducing ability of MEAP and WEAP and positive controls were ranked as: BHT, (2.089),

α-Tocopherol (1.995), Trolox (1.755), MEAP (1.184), BHA (0.884), WEAP (0.720). The $Fe^{3+}$-TPTZ reducing the effect of MEAP extract was higher than that of BHA used as the positive control.

**Table 1.** $Fe^{3+}$, $Cu^{2+}$, $Fe^{3+}$-TPTZ ions reduction abilities of *A. pseudocartalinica* extracts and standards at 15- 45 µg/mL

| **Antioxidants** | **$Fe^{3+}$ reducing ability** | | | |
|---|---|---|---|---|
| | 15 µg/mL | 30 µg/mL | 45 µg/mL | **$r^2$** |
| **BHA** | 1.146±0.050 | 1.257±0.090 | 1.476±0.060 | 0.9523 |
| ***α*-Tocopherol** | 1.860±0.010 | 1.895±0.010 | 1.899±0.010 | 0.9402 |
| **BHT** | 1.912±0.020 | 2.018±0.030 | 2.160±0.070 | 0.9466 |
| **Trolox** | 1.039±0.030 | 1.545±0.020 | 2.033±0.020 | 0.9966 |
| **MEAP** | 0.853±0.070 [ac] | 1.308±0.060 [ac] | 1.594±0.010 [ac] | 0.9997 |
| **WEAP** | 0.857±0.060 [bd] | 1.182±0.090 [bd] | 1.413±0.090 [bd] | 0.9955 |
| | **$Cu^{2+}$ reducing (CUPRAC) ability** | | | |
| | 15 µg/mL | 30 µg/mL | 45 µg/mL | **$r^2$** |
| **BHA** | 2.513±0.020 | 2.912±0.160 | 2.969±0.110 | 0.9742 |
| ***α*-Tocopherol** | 0.626±0.020 | 1.139±0.090 | 1.831±0.050 | 0.9980 |
| **BHT** | 1.135±0.050 | 1.800±0.010 | 2.473±0.020 | 0.9969 |
| **Trolox** | 1.194±0.050 | 2.323±0.050 | 2.672±0.050 | 0.9935 |
| **MEAP** | 0.339±0.090 [ac] | 0.650±0.030 [ac] | 0.993±0.130 [ac] | 0.9993 |
| **WEAP** | 0.214±0.030 [bd] | 0.430±0.003 [bd] | 0.625±0.004 [bd] | 0.9992 |
| | **$Fe^{3+}$-TPTZ reducing (FRAP) ability** | | | |
| | 15 µg/mL | 30 µg/mL | 45 µg/mL | **$r^2$** |
| **BHA** | 0.494±0.060 | 0.884±0.120 | 1.111±0.070 | 0.9905 |
| ***α*-Tocopherol** | 1.696±0.070 | 1.995±0.020 | 2.052±0.020 | 0.9807 |
| **BHT** | 1.936±0.080 | 2.089±0.030 | 2.146±0.002 | 0.9581 |
| **Trolox** | 1.084±0.020 | 1.755±0.090 | 2.051±0.080 | 0.9990 |
| **MEAP** | 0.747±0.030 [ac] | 1.184±0.010 [ac] | 1.497±0.020 [ac] | 0.9999 |
| **WEAP** | 0.486±0.010 [bd] | 0.720±0.030 [bd] | 0.976±0.010 [bd] | 0.9997 |

[a, b, c, d] demonstrate major differences in post-hoc comparisons between varying groups ($p<0.05$). [a:] MEAP vs *α*-Toc., [b:] WEAP vs *α*-Toc., [c:] MEAP vs Tro., [d:] WEAP vs Tro. (Toc.: *α*-Tocopherol, Tro.: Trolox, BHA: butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), MEAP: *A. pseudocartalinica* methanol extract, WEAP: *A. pseudocartalinica* water extract)

The effects of *Alchemilla* extracts of dose (15-45 µg/mL), groups (antioxidant samples), and the interaction of dose x group interaction were analysed by the Two-Way ANOVA test (Table 2).

**Table 2.** The dose, group, and dose x group interaction effects

| | **p** | | | | | |
|---|---|---|---|---|---|---|
| | **Two-Way ANOVA testing** | | | **One-way ANOVA post-hoc testing** | | |
| **Reducing assays** | **Groups (Antioxidant samples)** | **Doses µg/mL (15, 30, 45)** | **Group x Dose interaction** | **Dose µg/mL 1 (15) vs Dose 2 (30)** | **Dose µg/mL 1 (15) vs Dose 3 (45)** | **Dose µg/mL 2 (30) vs Dose 3 (45)** |
| **CUPRAC** | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| **$Fe^{3+}$ reducing** | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| **FRAP** | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |

p values were given for Two-Way (2 x 2) ANOVA analysis test statistics and post-hoc Tukey test following One-Way ANOVA test for conducting pairwise comparisons at varying doses

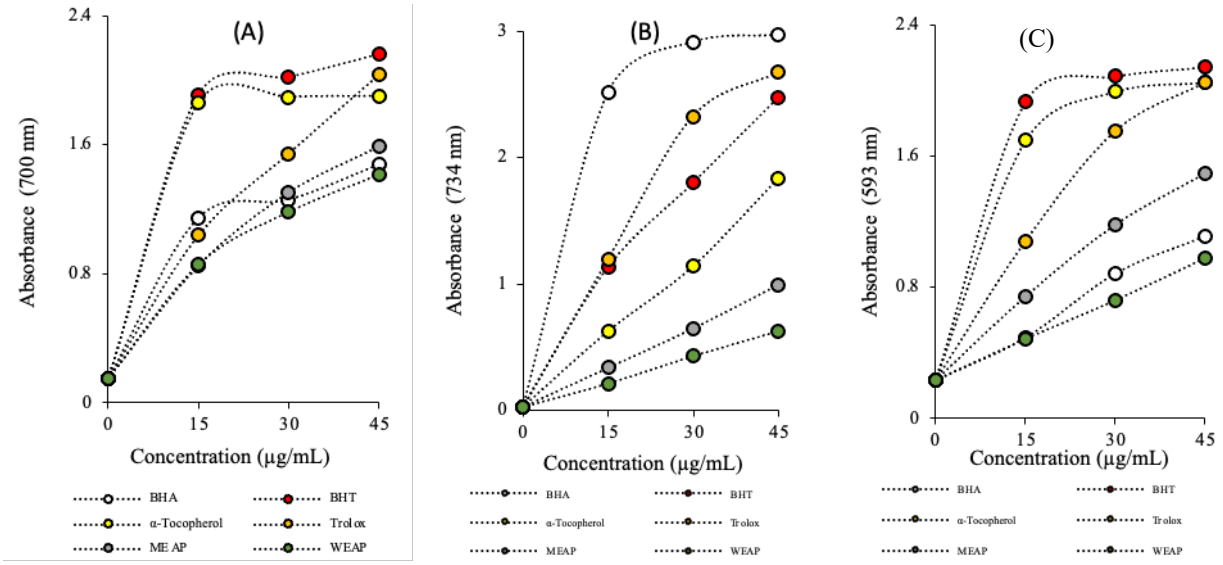



**Figure. 1. A.** $Fe^{3+}$ reducing capability, **B.** $Cu^{2+}$ reducing capability and **C.** $Fe^{3+}$-TPTZ reducing capability of MEAP, WEAP, and standards. MEAP: *A. pseudocartalinica* methanol extract, WEAP: *A. pseudocartalinica* water extract

When previous studies were examined, it was reported that *A. vulgaris* CUPRAC was 216.14 mg Trolox equivalent/g and FRAP was 7899.45 mg ascorbic acid equivalent/g in $Cu^{+2}$ and $Fe^{+3}$ reduction experiments of different *Alchemilla* species [57].

The DPPH free radical scavenging ability of *A. pseudocartalinica* extracts and positive antioxidants was determined by calculating $IC_{50}$ values, and the radical scavenging ability improved with increasing concentration (Figure 2D and Table 3). In DPPH$^{\bullet}$ radical scavenging assay, the decreasing $IC_{50}$ values of both extracts and standard antioxidants are listed as follows: 69.32 µg/mL ($r^2$: 0.9966) for WEAP, 21.00 µg/mL ($r^2$: 0.9668) for BHT, 18.24 µg/mL ($r^2$: 0.9899) for MEAP, 9.63 µg/mL ($r^2$: 0.9947) for Trolox, 9.00 µg/mL ($r^2$: 0.9399) for BHA, and 5.92 µg/mL ($r^2$: 0.9770) for *α*-Tocopherol. The MEAP sample was also noted for a higher radical scavenging property than the standard antioxidant BHT.

The second radical scavenging assay evaluated in this study was DMPD$^{\bullet+}$ scavenging activity. As shown in Table 3 and Figure 2E, unlike other antioxidant activity assays, WEAP extract was found to possess a superior ability as radical scavenging than MEAP extract. The activity of radical scavenging of the extracts and standard antioxidants increased significantly depending on different concentrations (15, 30, 45 µg/mL). The $IC_{50}$ values of DMPD$^{\bullet+}$ removal ability of *A. pseudocartalinica* extracts were calculated as follows: 86.64 µg/mL ($r^2$: 0.9974) for MEAP, 53.32 µg/mL ($r^2$: 0.9993) for WEAP; $IC_{50}$ values of standard antioxidants were calculated as 43.32 µg/mL ($r^2$: 0.9993) for BHA, and 23.90 µg/mL ($r^2$: 0.9349) for Trolox. Standard positive controls BHA and Trolox have higher antioxidant activity than MEAP and WEAP. These results clearly demonstrated that both *A. pseudocartalinica* extracts were able to transfer hydrogen atoms to DMPD radicals and decolorize the radical solution.

Lastly, we determined ABTS radical scavenging activity in *Alchemilla* extracts as an antioxidant test in our study. According to the calculated $IC_{50}$ values, plant extracts have an effective activity of radical scavenging. BHA, BHT and Trolox had the same $IC_{50}$ values and were ranked as follows: 7.71 µg/mL ($r^2$: 0.9330), MEAP 9.81 µg/mL ($r^2$: 0.9968) and WEAP 11.82 µg/mL ($r^2$: 0.9921) (Figure 2F, and Table 3). Standard positive controls have higher antioxidant activity than MEAP and WEAP. Examination of the ranking shows that MEAP and WEAP have effective antioxidant activity.

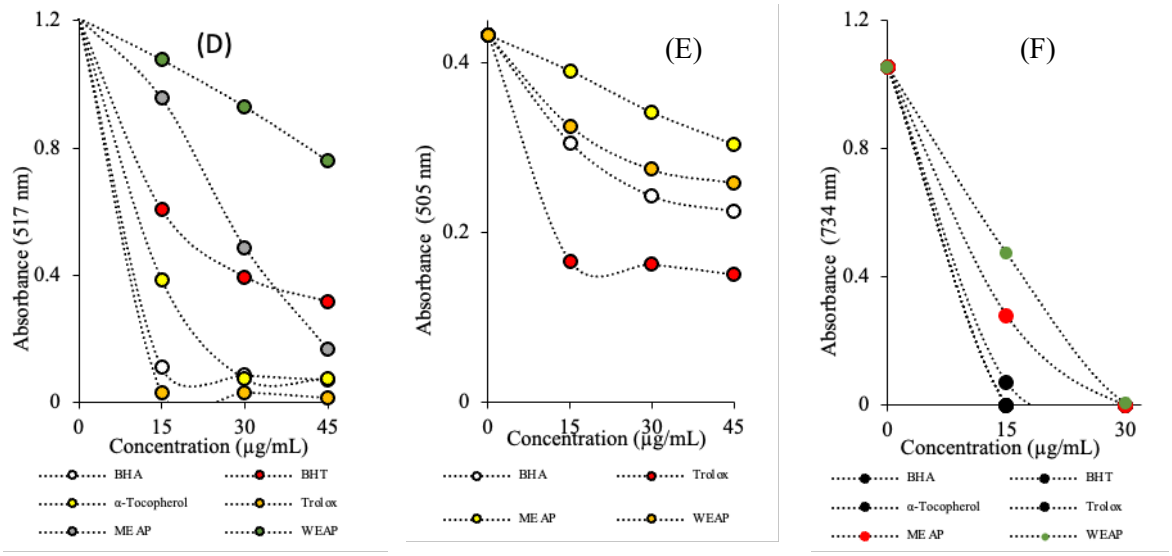



**Figure 2.** Radical scavenging impacts of *A. pseudocartalinica* extracts and standards **D**. DPPH$^{\bullet}$ **E**. DMPD$^{\bullet+}$, **F**. ABTS$^{\bullet+}$ scavenging capacities *MEAP: *A. pseudocartalinica* methanol extract, *WEAP: *A. pseudocartalinica* water extract.

**Table 3.** The $IC_{50}$ (µg/mL) values of *A. pseudocartalinica* extracts and standard antioxidants. for DPPH$^{\bullet}$, ABTS$^{\bullet+}$, and DMPD$^{\bullet+}$ scavenging

| **Antioxidant samples** | **DPPH$^{\bullet}$ scavenging ability** | | **ABTS$^{\bullet+}$ scavenging ability** | | **DMPD$^{\bullet+}$ scavenging ability** | |
|---|---|---|---|---|---|---|
| | **$IC_{50}$** | **$r^2$** | **$IC_{50}$** | **$r^2$** | **$IC_{50}$** | **$r^2$** |
| **Trolox** | 9.63 | 0.9947 | 7.71 | 0.9330 | 23.90 | 0.9349 |
| ***α*-Tocopherol** | 5.92 | 0.9770 | 8.1 | 0.9550 | - | - |
| **BHT** | 21.0 | 0.9668 | 7.71 | 0.9330 | - | - |
| **BHA** | 9.0 | 0.9399 | 7.71 | 0.9330 | 43.32 | 0.9993 |
| **MEAP** | 18.24 | 0.9899 | 9.81 | 0.9968 | 86.64 | 0.9974 |
| **WEAP** | 69.32 | 0.9966 | 11.82 | 0.9921 | 53.32 | 0.9993 |

In our study, the antioxidant level was determined by six different methods. To the best of our knowledge, the same units were specified in a small number of studies in literature. Antioxidant levels presented in different units make comparison difficult. In addition, some antioxidant activity experiments performed with the same methods and units provided the opportunity to compare the biological activities of *A. pseudocartalinica* methanol and water extracts investigated with other species. For instance, our present study demonstrates that the $IC_{50}$ value of DPPH radical scavenging ability of *A. pseudocartalinica* methanol extract is 18.24 µg/mL and the $IC_{50}$ value of ABTS radical scavenging ability is 9.81 µg/mL (Table 3). However, it was reported in a study that the $IC_{50}$ values of *Alchemilla vulgaris* aerial parts were 5.96 µg/mL (DPPH) and 14.80 µg/mL (ABTS), respectively. BHT, catechin and ellagic acid were used as positive controls, and their $IC_{50}$'s at DPPH were reported as 26.25, 7.52 and 3.54 µg/mL, respectively. In addition, the ABTS radical extinguishing abilities of standard antioxidants are seen to be 44.67, 5.97, 8.14 µg/mL in the same order [58].

In other studies, too, DPPH and ABTS radical scavenging assays were performed in general, and the experimental results reported with $IC_{50}$ were analyzed. For instance, when the literature searched, $IC_{50}$ value of methanol extract of aerial part of *A. acutiloba* against DPPH radicals is 18.69 µg/mL. It was established as 4.90µg/mL for Ascorbic acid against DPPH radicals and 6.17µg/mL against ABTS radicals. Also, Trolox demonstrated $IC_{50}$ value of 3.07µg/mL against ABTS radicals [59]. $IC_{50}$ value of *A. alpina* towards DPPH radicals [60]. *A. bulgarica* and *A. ymaros* had $IC_{50}$ values of 75.63 and 46.03 µg/mL towards DPPH radicals, respectively [61].

*3.2. Enzyme Inhibition Studies*

In our study, the inhibition effects of *Alchemilla pseudocartalinica* methanol and water extracts against *α*-glycosidase, AChE and hCA II enzymes, $IC_{50}$ values calculated by drawing graphs are shown in Table 4. The effect of *Alchemilla* species against diabetes is known [62]. However, to our knowledge, a study in which the antidiabetic effect of *A. pseudocartalinica* species has been established is non-existent. In our study, the inhibition effect of *A. pseudocartalinica* methanol and water extracts against the *α*-glycosidase enzyme, a digestive enzyme, was investigated. The $IC_{50}$ values were calculated from the graph obtained based on the study as 8.59 µg/mL ($r^2$: 0.9930) for MEAP, 41.19 µg/mL ($r^2$: 0.9934) for WEAP, and 14.72 µg/mL ($r^2$: 0.9922) for acarbose, employed as positive control (Table 4). The outcome shows that the methanol extract has a more effective anti-diabetic potential than the water extract.

The ever-increasing elderly population and the high incidence of Alzheimer's brought with it make it necessary to find alternative, new resources against this disease. The use of plant-derived galantamine in previous studies [63] has been encouraging to find more effective natural compounds with fewer side effects [64]. In our study, the inhibition effect of *A. pseudocartalinica* methanol and water extracts against the AChE enzyme was shown by calculating the $IC_{50}$ value. According to the results, MEAP and WEAP have 2.57 µg/mL ($r^2$: 0.9903), and 2.35 µg/mL ($r^2$: 0.9959), respectively (Table 4). The results give hope for a new alternative product. However, this hypothesis should be supported by future *in vivo* and *in vitro* studies.

hCA enzyme, which takes part in many metabolic activities of our body, adjusts pH, provides bone resorption, and balances body internal pressure, can cause such conditions glaucoma, epilepsy, obesity, and cancer when it works excessively [65, 66]. Systemic carbonic anhydrase inhibitor drugs have side effects such as decreased libido, metallic taste, depression, fatigue, gastrointestinal problems, metabolic acidosis, kidney stones, and transient myopia. For this reason, topical products are preferred more. In addition, it is necessary to add economical and more effective natural products to hCA II inhibitor drugs [67]. In our study, it was shown with the calculated $IC_{50}$ values that extracts of methanol and water from *A. pseudocartalinica* had effective hCA II inhibition effects. According to these results, MEAP and WEAP had $IC_{50}$ values of 6.03 µg/mL ($r^2$: 0.9772), 15.40 µg/mL ($r^2$: 0.9671), respectively, whereas positive control of Acetazolamide exhibited $IC_{50}$ value of 1.85 µg/mL ($r^2$: 0.9825) (Table 4).

The results of our anticholinesterase, anti-diabetic, and antiglaucoma activity experiments on methanol and water extracts of *A. pseudocartalinica* species are given as $IC_{50}$. Likewise, the existing studies in the literature were given in different units, making it difficult to compare. For instance, the $IC_{50}$ inhibition value of *A. vulgaris* extracts was calculated as 5.17 mg Galantamine equivalent per g extract against cholinergic AChE enzyme [57]. In another study, it was reported that 96.50% AChE inhibition at 3 mg/mL, positive control acarbose 99.98% AChE inhibition at 1.5 mg/mL [68]. A recent study established that *A. barbatiflora* methanol and water extracts reduced 95.36 and 96.73% *α*-glycosidase activity. This values was calculated as 95.36% *α*-glycosidase activity reducing at 250 µg/mL [62].

**Table 4.** *Alchemilla* extracts' inhibitory values against *α*-Gly, AChE, and hCA II enzymes

| | **$IC_{50}$ (µg/mL)** | | | | | |
|---|---|---|---|---|---|---|
| **Samples** | **hCA II** | **$r^2$** | **AChE** | **$r^2$** | ***α*-Gly** | **$r^2$** |

Phytochemical consituents of *Alchemilla pseudocartalinica*

| | | | | | | |
|---|---|---|---|---|---|---|
| **MEAP** | 6.03 | 0.9772 | 2.57 | 0.9903 | 8.59 | 0.9930 |
| **WEAP** | 15.40 | 0.9671 | 2.35 | 0.9959 | 41.19 | 0.9934 |
| **Acetazolamide**[i] | 1.85 | 0.9825 | - | - | - | - |
| **Tacrine**[ii] | - | - | 1.183 | 0.9706 | - | - |
| **Acarbose**[iii] | - | - | - | - | 14.72 | 0.9922 |

MEAP: *A. pseudocartalinica* methanol extract, *WEAP: A. pseudocartalinica* water extract [i]Acetazolamide (AZA) acts as standard for CA II inhibition, [ii]Tacrine was employed to act as positive control for the AChE enzyme[26], [iii]Acarbose was adopted as positive control for *α*-glycosidase[45]

### *3.3. Total Phenolic and Flavonoid Contents*

The overall quantity of phenolic compounds present in the extracts of *Alchemilla* was calculated using the reference phenolic compound gallic acid. The equation was established using the standard curve generated from the graph [y=0.002x ($r^2$: 0.9983)]. The total phenolic compound- quantity in the extracts were calculated as gallic acid equivalents (GAE) using the equation y=0.002x. The 760 nm absorbance values are MEAP 0.223 and WEAP 0.185, respectively. When the absorbance values are put into their places in the equation, the total phenolic amounts of MEAP and WEAP are 111.50 mg/g and 92.50 mg/g extract as gallic acid equivalents (GAE).

The calculation of the overall quantity of flavonoids in *Alchemilla* extracts was performed using quercetin as a reference. The equation was calculated from the standard curve generated from the graph [y=0.0061x ($r^2$: 0.9980)]. The total amounts of flavonoids in the extracts were established as quercetin equivalents (QE) using the equation y=0.0061x. MEAP and WEAP were measured as absorbance 0.889 and 0.145 at 415 nm. From the absorbances substituted in the equation, 145.74 mg/g quercetin equivalent (QE) extract and 23.77 mg/g QE extract were calculated to have flavonoids, respectively.

When the studies on *Alchemilla* species are examined, it is observed that the species contain varying amounts of total phenolic and flavonoid substances. In some studies, *Alchemilla* species are observed to have lower total phenolic and flavonoid content than the *A. pseudocartalinica* plant. For example, it was observed that *A. vulgaris* contains 61.12 mg GAEs/g extract phenolic, 1.07 mg QEs/g extract flavonoid [69], *A. mollis* contains 3.53 mg extract GAEs/g phenolic, 2.12 mg QEs/g extract flavonoid [70]. On the other hand, some studies showed that *A. pseudocartalinica* had lower total phenolic and flavonoid content. For instance, in one study, it was reported that total phenolic 558 mg GAEs/g extract and total flavonoid 13.30 mg rutin equivalent/g extract [58] contained in methanol extract of *A. vulgaris* aerial part; *A. acutiloba* contained total phenolic 279.82 mg GAEs/g extract, total flavonoid 39.15 mg caffeic acid equivalent/g extract [59]; and *A. mollis* contained total phenolic 122.93 mg GAEs/g, total flavonoid 50.63 mg catechin equivalent/g [55]. The fact that the same species of plants show different effects and have different contents is due to the difference in the environment, climate, and stress conditions in which the plant grows [71].

The estimation of total phenolic and total flavonoid amounts can enable us to make predictions about the antioxidant activity of plants [72]. There is much evidence that natural antioxidants derived from medicinal plants can prevent and control the development of endocrinological, neurological, and oncological conditions among which are cancer, diabetes, Alzheimer's, and Parkinson's [45, 58].

### *3.4. UPLC-MS/MS Results*

In our study, 50 phenolic and three non-phenolic substances of *A. pseudocartalinica* methanol extract were analyzed according to the study of Yılmaz, which was previously validated and verified [50]. The chromatograms of the standard substances are shown in Figure 3A, and the phytochemical profile of *A. pseudocartalinica* methanol extract is presented in Figure 3B. In Table S1, the data obtained as a result of the validation and the number of substances in the extract are indicated with numerical data. The results are shown in mg analytes per gram dry extract. According to this, the methanol extract of the *Alchemilla* plant has a chemical profile from more to less as follows: miquelianin 44.095 mg/g, quinic acid 17.054 mg/g, ellagic acid 6.492 mg/g, isoquercitrin 2.026 mg/g, tannic acid 0.772 mg/g, astragalin 0.772 mg/g, gallic acid 0.553 mg/g, cynaroside 0.538 mg/g, gentisic acid 0.479 mg/g, rutin 0.455 mg/g, chlorogenic acid 0.421 mg/g, p-coumaric acid 0.378 mg/g, catechin 0.282 mg/g, nicotiflorin 0.241 mg/g, acacetin 0.174 mg/g, protocatechuic acid 0.171 mg/g, hesperidin 0.151 mg/g, cosmosiin 0.145 mg/g, quercetin 0.111 mg/g, caffeic acid 0.067 mg/g, chrysin 0.064 mg/g, quercitrin 0.036 mg/g, naringenin 0.02 mg/g, luteolin 0.016 mg/g, kaempferol 0.014 mg/g, protocatechuic aldehyde 0.013 mg/g, apigenin 0.013 mg/g, salicylic acid 0.012 mg/g, hesperetin 0.008 mg/g. According to the results of UPLC-MS/MS, the overall amount of total phenolic and non-phenolic substances was 75.573 mg/g. In addition, it was determined that 29 of 53 phenolic/non-phenolic compounds were analyzed in our plant extract. (Figure 3B, and Table S1)

Phytochemical consituents of *Alchemilla pseudocartalinica*

Methanol and water extract rich in polyphenols showed high activity in various biological activity tests. Especially quinic acid, miquelianin (quercetin 3-*O*-glucuronide), and ellagic acid increased the activity. Quinic acid, miquelianin, and ellagic acid have been reported to be effective in many studies investigating their antioxidant effects and in various antioxidant activity tests [73-75].

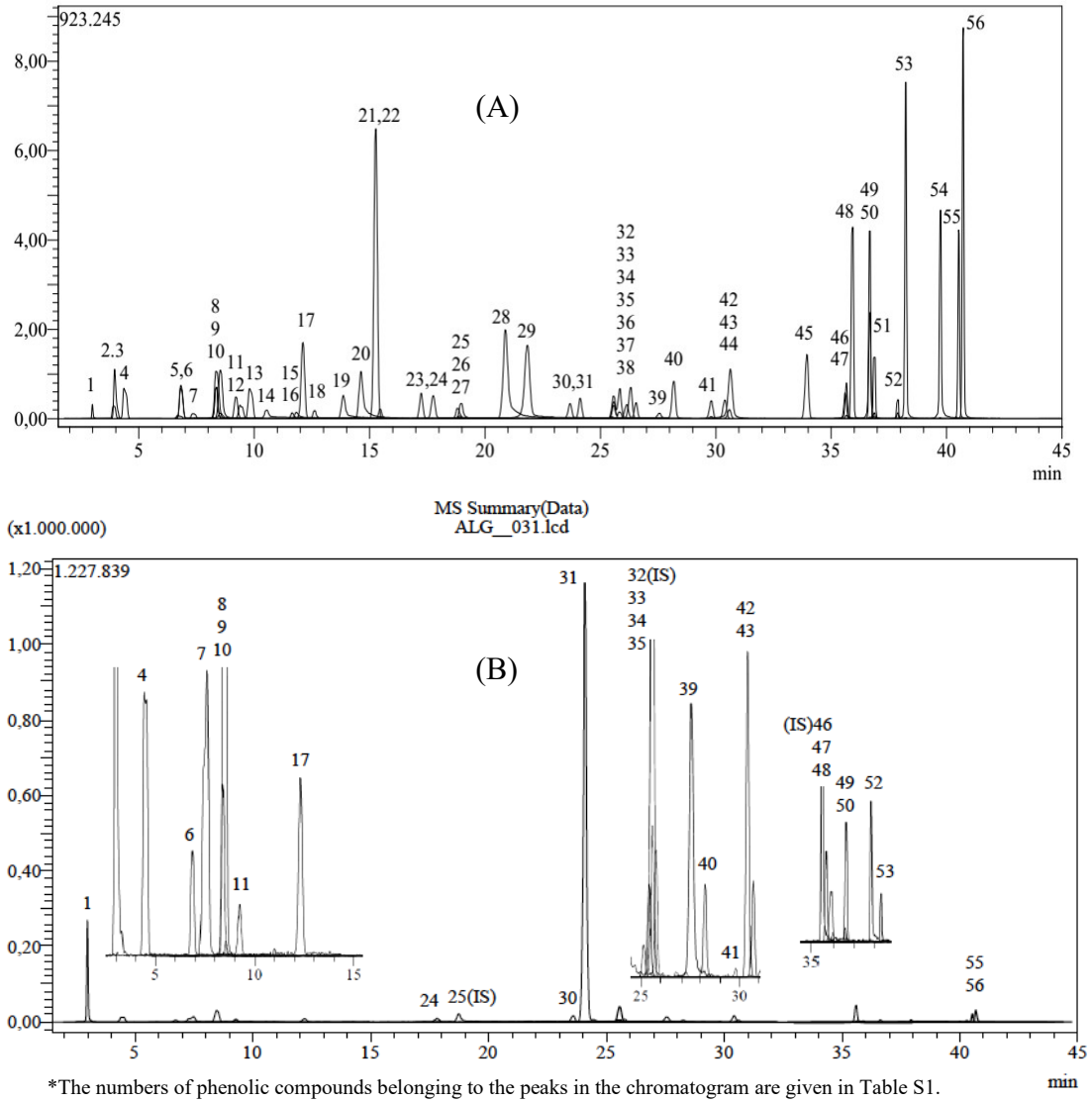



**Figure 3.** (A) Chromatogram showing the standard phenolic compounds examined by the developed UPLC-MS/MS method, (B) Chromatogram of the MEAP analyzed by the developed UPLC-MS/MS method

Species belonging to the genus *Alchemilla* are used for many purposes in traditional medicine. For this reason, there are many phytochemical analysis studies available. When the studies are analyzed, it is observed that *Alchemilla* species are rich in tannins, flavonoids, phenolic acids, terpenes, and fatty acids [76]. The phytochemical profile of *A. pseudocartalinica* was determined by us first.

In the present analysis of UPLC-MS/MS, to our knowledge, acacetin, chrysin, hesperidin and protocatechuic aldehyde were reported for the first time in an *Alchemilla* species. In a previous HPLC analysis study, it was reported that in the aerial parts of the plants, *A. mollis* contained 3.9 mg/g miquelianin and 15.6 mg/g ellagic acid, while *A. persica* contained 3.4 mg/g of miquelianin and 12.3 mg/g of ellagic acid [77]. The active ingredients in the study were also present in *A. pseudocartalinica* in our study. In another study, luteolin-7-*O*-*β*-glycoside 903.94 µg/g, catechin 704.55 µg/g, quercitrin-hexoside 424.27 µg/g, and quinic acid 284.56 µg/g were reported in *A. vulgaris* methanol extracts [57].

*3.5. Molecular Docking Results*

The molecular docking simulation program Schrödinger 2020-3 (S. Schrödinger Release 2020-3: Maestro, LLC, New York, NY 2021) was used to predict the interactions of quinic acid, miquelianin, and ellagic acid, which were found to constitute an important part of the phytochemical content of *A. pseudocartalinica*, with hCA II, AChE, α-glycosidase enzymes. Table 6 shows the results of simulations on molecular docking. In addition, two-dimensional (2D) ligand interactions of the most active compounds and compounds used as standards on the enzymes studied are given in Figures (4-6).

The results of molecular docking studies between hCA II isoenzyme and major substances showed that the structures had excellent binding scores compared to the acetazolamide used as standard. Quinic acid (-10.085) has the highest binding affinity on the hCA II enzyme compared to other structures and acetazolamide (-5.927). As shown in Figure 4, the interaction between quinic acid and the active site of the hCA II enzyme can be characterized by polar and non-polar interactions and hydrogen bonds observed between the structure's hydroxyl groups and the amino groups Asn 67, Gln 92 (Table 6 and Figure 4).

According to the results of molecular docking studies of the major substances in *A. pseudocartalinica* extract with AChE enzyme, miquelianin (-13.204) showed that it had a very good binding score compared to tacrine (-12.968) and other structures. As shown in Figure 5, the interaction between miquelianin and the active site of the AChE enzyme can be characterized by polar and non-polar interactions, hydrogen bonds between amino acid GLH166 and the hydroxyl group of the structure, and pi-pi stacking interactions between amino acids PHE330 and TRP84. (Table 6, and Figure 5).

The results of the molecular docking study with *α*-glycosidase enzyme demonstrated that miquelianin (-8.673) had the best binding score compared to other major substances, although it was not very efficient compared to the reference compound acarbose (-16.526). This interaction can be characterized by polar and non-polar interactions and hydrogen bonding with ASP 542 and pi-pi stacking interactions between PHE450 (Table 6, and Figure 6).

**Table 6.** Scores of docking and binding energies belonging to the compounds for hCA II enzymes, AChE, and α-glycosidase

| | **Docking Score** | **XP Gscore** | **Glide Gscore** | **Glide Emodel** |
|---|---|---|---|---|
| **hCA II (PDB: 5AML)** | | | | |
| Ellagic acid | -5.538 | -5.601 | -5.601 | -33.213 |
| Quinic acid | **-10.085** | **-10.085** | **-10.085** | **-39.184** |
| Miquelianin | -8.936 | -8.964 | -8.964 | -48.203 |
| Acetazolamide | -5.927 | -6.849 | -6.849 | -50.340 |
| **AChE (PDB: 4TVK)** | | | | |
| Ellagic acid | -8.554 | -8.617 | -8.617 | -42.008 |
| Quinic acid | -7.718 | -7.718 | -7.718 | -37.449 |
| Miquelianin | **-13.204** | **-13.232** | **-13.232** | **-46.515** |
| Tacrine | -12.968 | -12.968 | -12.968 | -59.064 |
| **α-Glycosidase (PDB: 3L4Y)** | | | | |
| Ellagic acid | -5.754 | -5.816 | -5.816 | -40.019 |
| Quinic acid | -6.366 | -6.366 | -6.366 | -35.898 |
| Miquelianin | **-8.673** | **-8.702** | **-8.702** | **-48.726** |
| Acarbose | -16.526 | -16.854 | -16.854 | -98.119 |

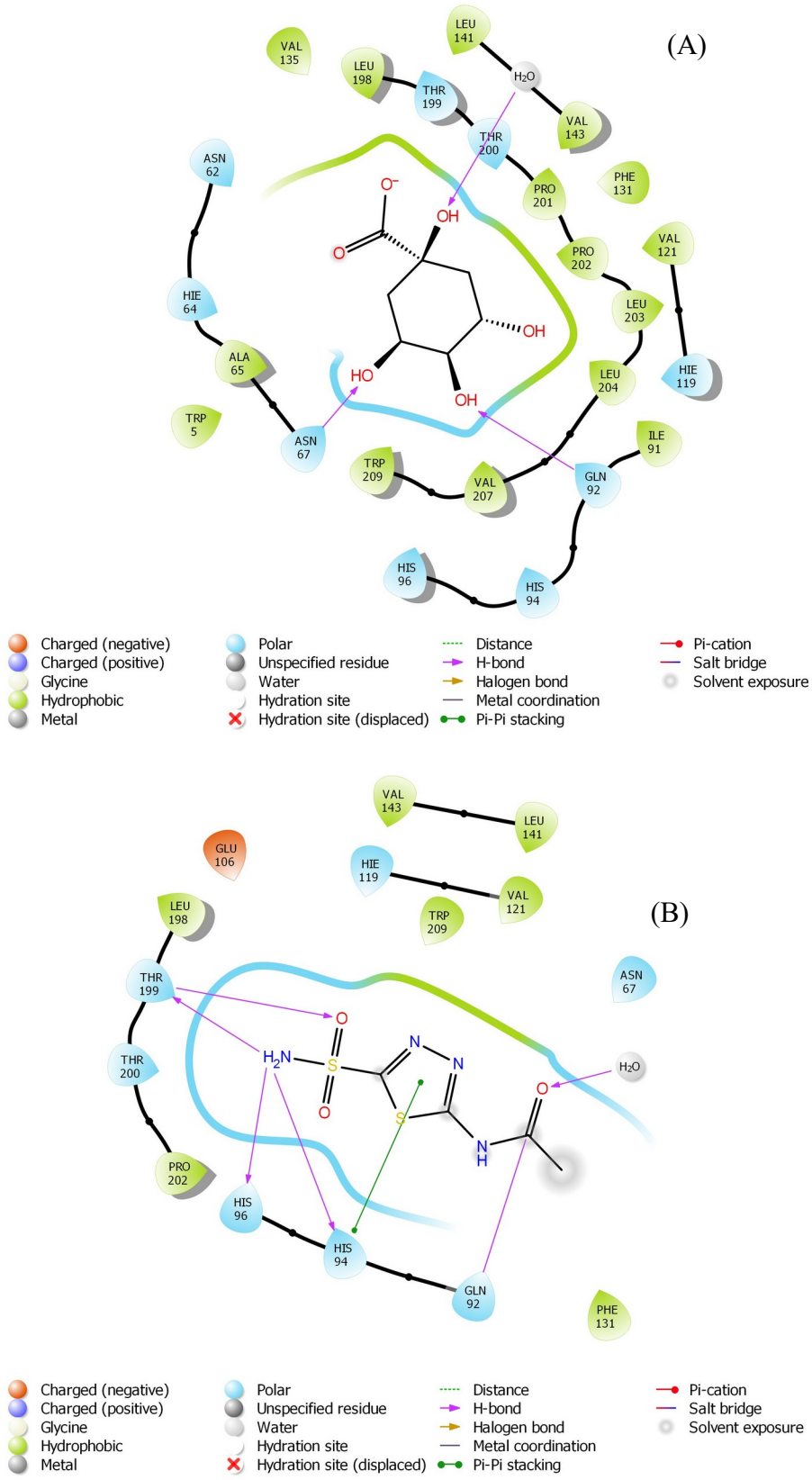
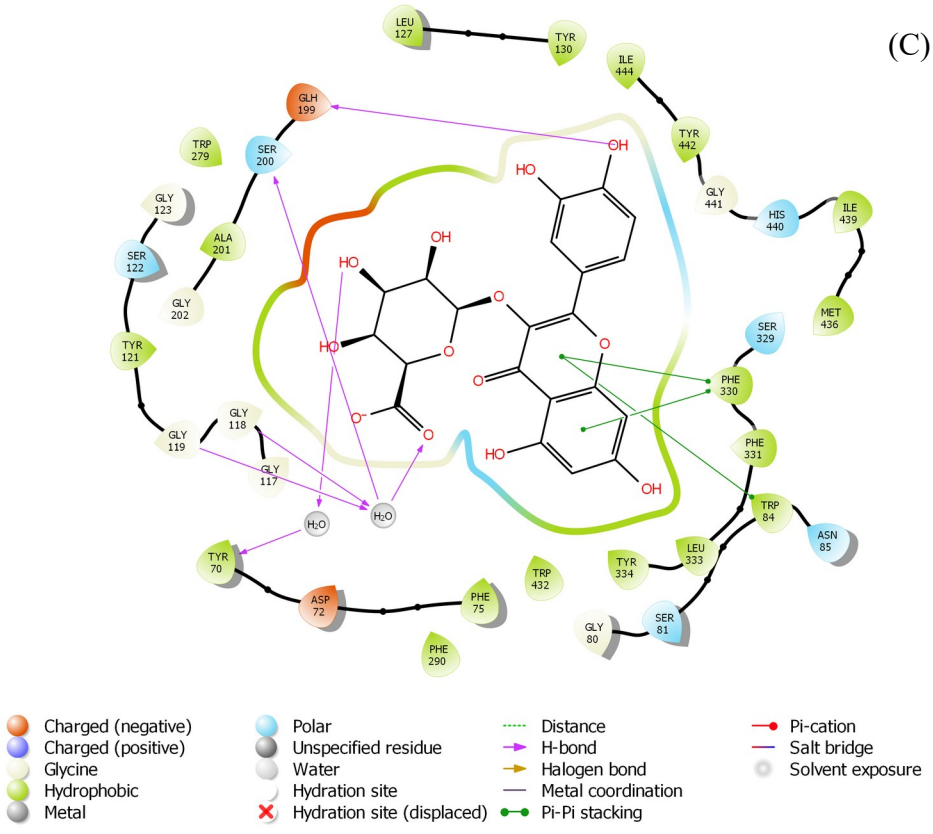



**Figure 4.** Two-dimensional (2D) ligand interactions of quinic acid with hCA II isozyme (A), acetazolamide with hCA II isozyme (B)

(C)




(D)

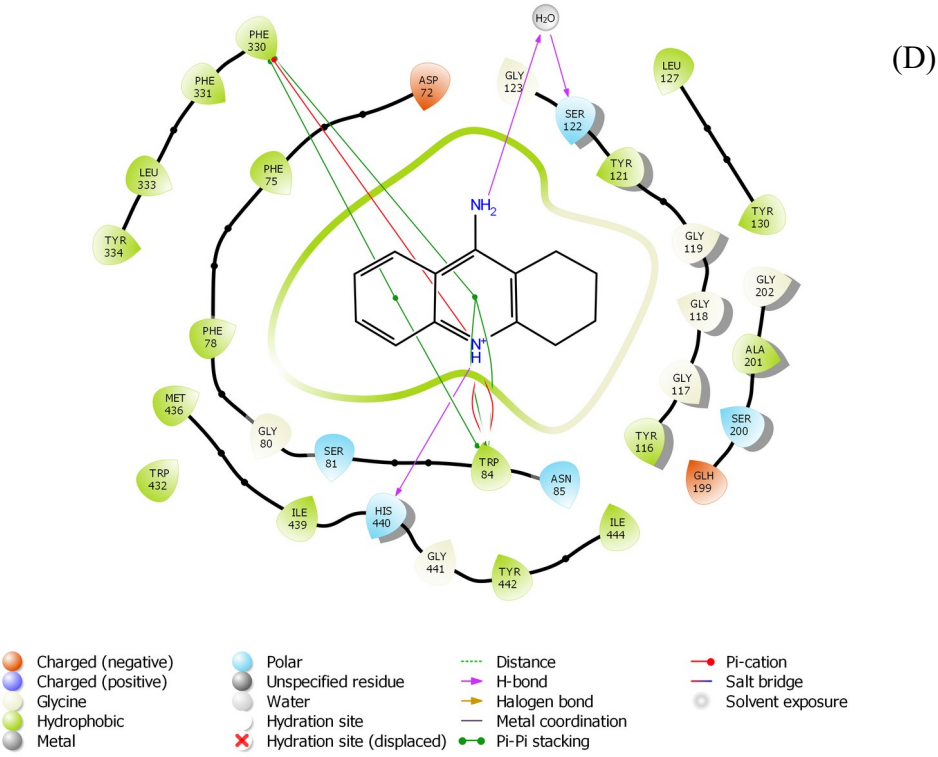



**Figure 5.** Two-dimensional (2D) ligand interactions of miquelianin with AChE enzyme (C), tacrine with AChE enzyme (D)

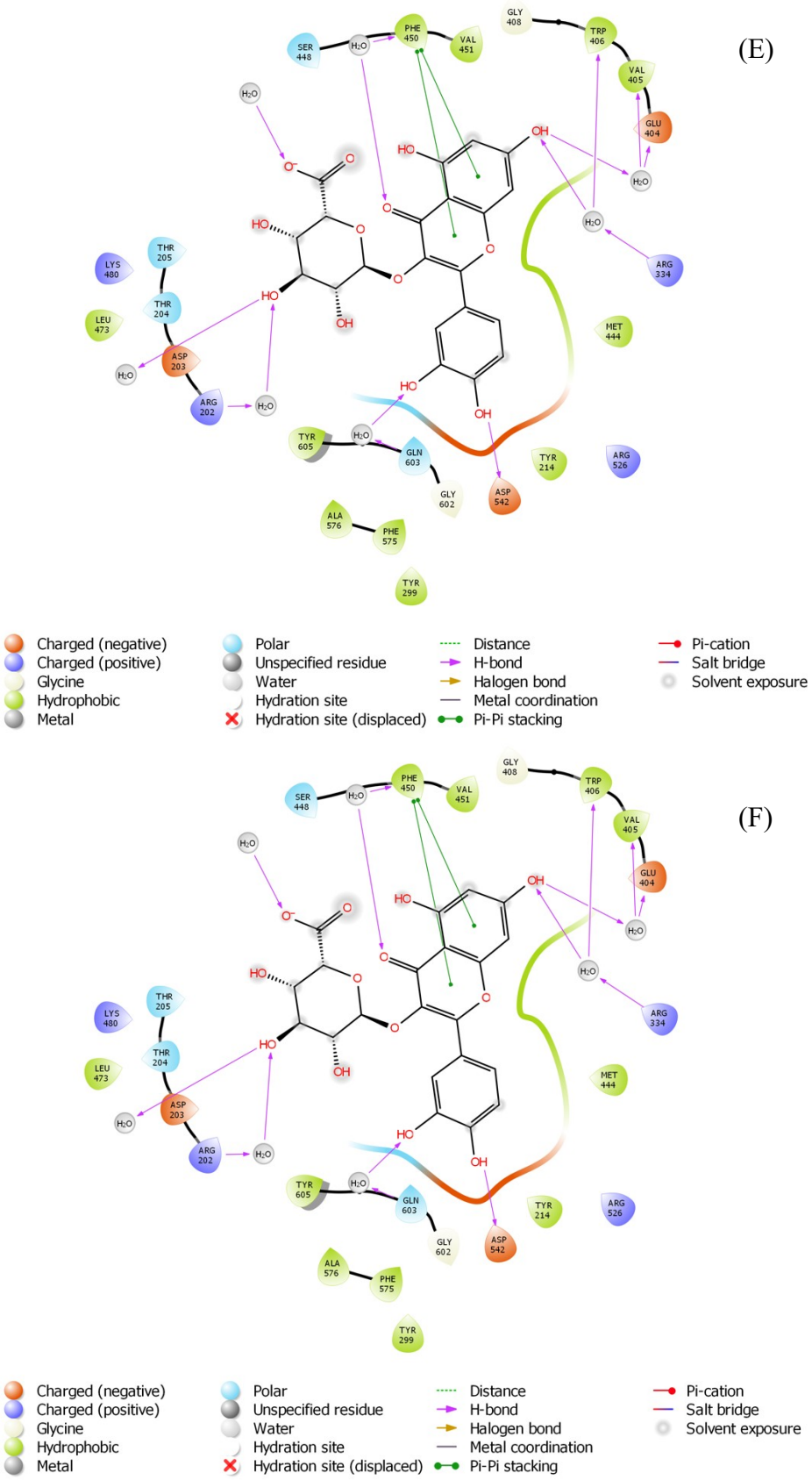



**Figure 6.** Two-dimensional (2D) ligand interactions of miquelianin with *α*-glycosidase enzyme (E), acarbose with *α*-glycosidase (F)

## 4. Conclusion

This study demonstrated that the methanol and water extracts of the aerial part of *A. pseudocartalinica* were shown to be rich in phenolic compounds. In addition, for the first time, to the remarkable biological activities of the extracts by evaluating anti-oxidant, anti-diabetic, anti-Alzheimer's, and anti-glaucoma activities, Through UPLC-MS/MS analysis, the primary phenolic compounds were identified and their potential activity elucidated. The differential binding strengths of these compounds with enzymes provide insights into their potential contribution to the observed effects. A comprehensive evaluation of the antioxidant potential of the two extracts was conducted employing a total of six diverse techniques. Considering the promising biological activity of *A. pseudocartalinica* and its safety our hypothesis will be further strengthened by *in vivo* studies of the plant in our future research. Due to its abundant phenolic and flavonoid contents, *A. pseudocartalinica* extracts have the potential to serve as a natural remedy for various diseases, and can also find applications in the food and pharmaceutical industries. In further studies, the potential mechanisms of biological effects should be explored, and it should be clarified whether the activities are the result of the individual activity of the secondary compounds or the synergistic effect of all phenolic and non-phenolic compounds as a whole.

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## Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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