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Antioxidant and Enzyme Inhibitory Properties, and HPLC–MS/MS Profiles of Different Extracts of *Arabis carduchorum* Boiss.: An Endemic Plant to Turkey

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Abstract: In this study, six extracts of *Arabis carduchorum* prepared from solvents of varying polarity (water, methanol, ethyl acetate, dichloromethane, and n-hexane) were investigated for their chemical profiles and total phenolic and flavonoid contents (TPC/TFC) using HPLC–MS/MS and spectrophotometric/colorimetric assays, respectively, along with the assessment of their antioxidant and enzyme inhibitory effects. In general, the polar extracts (methanol, water, and infusion) showed higher TPC/TFC than the other extracts (range:13.73–26.62 mg GAE/g; 2.66–52.95 mg RE/g, respectively). The total antioxidant capacity of the polar extracts ranged from 0.90–1.85 mmol TE/g in phosphomolybdenum assay, while the free radical scavenging potential ranged from 4.36–32.13 mg TE/g and 12.58–67.73 mg TE/g in DPPH and ABTS assays, respectively. While the water and infuse extract inhibited only butyrylcholinesterase (2.68 and 2.39 mg GALAE/g, respectively), the other extracts were found to inhibit both acetyl- and butyryl-cholinesterases (0.96–2.73 mg GALAE/g and 1.86–5.11 mg GALAE/g, respectively). On the contrary, all extracts exhibited anti-tyrosinase (40.24–59.22 mg KAE/g) and anti-amylase (0.09–0.63 mmol ACAE/g) activities. Overall, this study highlighted the effect of solvent polarity on the extraction of bioactive compounds and the biological properties of the extracts of *A. carduchorum*; hence, the results obtained could be used to guide optimum extraction in future studies.

Keywords: *Arabis carduchorum*; phenolics; flavonoids; HPLC–MS/MS; solvent polarity; antioxidant; enzyme inhibition

1. Introduction

For many years, extensive research has been conducted to shed light on the significance of botanical families in medicinal ethnobotany [1]. This fact has also made possible the establishment of the medicinal virtues of botanical families and their various species, often associated with their unique phytochemical profiles. Brassicaceae is a broadly spread and diverse plant family comprising many genera, such as *Arabidopsis*, *Camelina*, *Brassica*, *Thellungiella*, *Boechera*, *Raphanus,* and *Arabis*, with significant economic and agricultural importance. The family includes 49 tribes, 325 genera, and 3740 species, which are largely spread throughout the temperate regions of the world, particularly Turkey, which has a great diversity of this family. Species belonging to this family have been scientifically found to have many health benefits, being the source of many health-improving phytochemicals [2]. Due to its rich phytochemical contents, Brassicaceae is one of the most studied families.

Brassicaceae vegetable stands unique because of the high sulfur-containing compounds present in their bioactive metabolites. Additionally, they have been found to help in boosting immunity, maintain bone health, and have anti-inflammatory and anticancer activities, among others. Thus, dietary intake of these plants from the Brassicaceae family could be useful to avert in vivo oxidative damage, associated with various illnesses. Further pharmacological studies on the bioactive constituents from Brassica vegetables have even demonstrated numerous other biological actions, including antimutagenic, antimicrobial, antioxidative, and neuroprotective activity [3].

Within this family, the genus *Arabis* L., is composed of approximately 60 species [4]. Morphologically, the members of the genus are herbs with simple, bifid or branched stellate hairs. Their lower leaves usually form as a rosette and the upper leaves are sessile. The sepals are strongly saccate and their petal colors change from white to violet [5]. The members of this genus are documented to possess multiple benefits, including health benefits. For instance, *A. alpina* is used as an ornamental plant in Turkey's Erzincan province, whereas in the Himalayas region in India, its leaves and shoots are eaten fresh. Moreover, some *Arabis* species are used in traditional medicine, for example, *A. tibetica* is used to heal wounds and *A. glandulosa* can be beneficial in treating abdominal pain. In phytochemical studies, the genus *Arabis* was shown to be rich in glucosides and most of these were glucosinolates. In two earlier studies [6,7], the presence of 9-(methylsulfinyl) nonylisothiocyanate, hirsutin, and 8-methylthio-3-oxooctylglucosinolate in the seeds of *A. alpina* and *A. hirsuta* were reported. In addition, Daxenbichler et al. [8] worked on 14 *Arabis* seeds, and thione derivatives were major components in the tested seeds. In another study conducted by Hasapis et al. [9], the sulfur containing glucosinolates were found in the seeds of *A. purpurea* and *A. kennedyae*. In addition to the presence of glucosinolates, some phenolic components, including rutin, ellagic, and 2,5-dihydroxybenzoic acids, were reported in the extracts of *A. alpina* [4]. In a recent study by Polatoglu et al. [10], the essential oils of two *Arabis* species (*A. purpurea* and *A. cypria*) from Cyprus and some components including nonacosane, heptacosane and phytol were found. However, there is limited scientific evidence on the pharmacological effects of the *Arabis* species. For example, Balpinar [4] reported that the extracts of *A. alpina* exhibited significant antimicrobial effects on *Salmonella typhimurium*. In a recent study by Ozgur et al. [11], a significant antioxidant response was obtained from *A. alpina* during UV-B stress. Some research studies also indicated that plants of the genus *Arabis* have great potential as hyperaccumulators [12–14]. At this point, many *Arabis* species are still underexplored and underused, requiring more investigations on species of this genus. One of them is *Arabis carduchorum,* as its chemical components and biological properties are still unknown.

Thus, the present study is aimed at investigating the phytochemical profile, bioactive contents, and the antioxidant and enzyme inhibition properties of extracts of *Arabis carduchorum* Boiss. prepared using different solvents (methanol, water, ethyl acetate, dichloromethane, and n-hexane). This is the first report demonstrating the effect of solvent polarity

on phytochemical extraction from *A. carduchorum* and its influence in terms of their biological properties.

2. Materials and Methods

2.1. Plant Material

Arabis carduchorum Boiss. samples were collected in Van (Akköprü mountain, Özalap road, forest clearings, Tuşba). The plant was identified by one of the co-authors, who is a plant taxonomist (Dr. Ugur Cakilcioglu) and voucher specimens (Voucher number: UC 19-4) were deposited at the herbarium of Munzur University, Tunceli, Turkey. The aerial parts of the plant samples were dried in shade conditions at room temperature for approximately seven days and then grounded into powder using a mill. The samples were kept in dark conditions.

The extracts were prepared using a variety of solvents (hexane, ethyl acetate, dichloromethane, methanol, and water) in this study. A maceration extraction method was employed. In addition, water was also used to make an infusion (as a traditional way). Overnight, the plant material (10 g) was macerated at room temperature with 200 mL of solvents (hexane, ethyl acetate dichloromethane, methanol, and water). Finally, the solvents were evaporated from the mixtures. The plant materials (10 g) were left to soak in 200 mL of boiled water for 15 min before being filtered. The water extracts were lyophilized and stored at 4 °C until further analysis was required.

2.2. Total Phenolic and Flavonoid Content

The total phenolic and flavonoid contents in the tested extracts were determined using spectrophotometric methods (Folin–Ciocalteu and $AlCl_3$ methods, respectively). The experimental details were given in our previous paper [15]. The total phenolic concentration was expressed as mg gallic acid equivalents (GAE)/g dry extract. The total flavonoid content was evaluated as mg rutin equivalents (RE)/g dry extract.

2.3. Quantification of Bioactive Compounds by HPLC-MS/MS System

The dried extracts of *A. carduchorum* were dissolved in methanol (5 mg/mL) and sonicated for 10 min at room temperature. Then, samples were ultracentrifuged at 13,000 rpm for 10 min, and before HPLC–MS/MS analysis it was filtered by a 0.2 µm filter. The quantification of 25 bioactive compounds, including phenolic acids, chlorogenic acids, flavonoids, and secoiridoids, was performed as described previously [16]. The analytical details are given in the supplemental materials.

2.4. Antioxidant Assays

In this investigation, a variety of techniques were used to assess the antioxidant properties of the extracts under investigation [17]. These tests included the radical scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), cupric ion reducing antioxidant capacity (CUPRAC), ferric ion reducing antioxidant power (FRAP), metal chelating ability (MCA), and the phosphomolybdenum (PDA) assay. DPPH, ABTS, CUPRAC, and FRAP test results, for instance, were expressed as mg Trolox equivalents (TE)/g extract, whereas MCA and PDA test results were expressed as mg EDTAE/g and mmol Trolox TE equivalents/g extract, respectively.

2.5. Enzyme Inhibitory Assays

The extracts were tested against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase, amylase, and glucosidase. Data were analyzed in terms of galanthamine equivalents (GALAE) per gram of extract when galanthamine was used as a positive control in AChE and BChE tests. Kojic acid extract (KAE), which was used as a standard inhibitor of tyrosinase, was used to measure the results [17,18]. Acarbose equivalents

(ACAE) per gram of extract were also calculated in the amylase and glucosidase inhibition assays, respectively.

2.6. Statistical Analysis

This study used ANOVA (Tukey's test) to determine whether there were any differences in extract levels between the three samples. The Pearson correlation test was used to examine the relationship between phytoconstituents in tested extracts and biological activities. GraphPad. 9.0 was used for the analysis. Additionally, Principal Component Analysis (PCA) was used to determine whether the tested extracts were similar or different. SIMCA was used to carry out the PCA analysis (version 14.0).

2.7. Molecular Docking

Crystal structures of the target enzymes were downloaded from the protein data bank (https://www.rcsb.org/, accessed on 1 April 2022): AChE (PDB ID: 6O52) [19], BChE (6EQP) [20], tyrosinase (6JU7) [21], and amylase (6TP0) [22]. They were prepared at the physiological pH of 7.4 in which bond orders were corrected and missing atoms were added using Biovia Discovery Studio (DS) (Accelrys Software Inc., San Diego, CA, USA, 2012). Ligand structures were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/, accessed on 1 April 2022) and their geometry was optimized using the ligand preparation toolkit in Biovia DS.

The coordinates of the cocrystal ligand in each PDB structure were used to generate a docking grid file (https://autodock.scripts.edu, accessed on 1 April 2022) [23]. Autodock 4.2, utilizing the Lamarckian genetic algorithm, was used for the ligand conformational search. Torsion angles were assigned to rotatable bonds to generate multiple ligand poses, from which the pose with the lowest binding energy is considered the representative binding mode. Visualization of protein–ligand interactions was conducted in the Biovia DS Visualizer.

3. Results and Discussion

3.1. Chemical Composition

Plant phenolics and flavonoids possess an array of biological potential, which outline the necessity of their determination when investigating the pharmacological properties of plants. For instance, phenolic compounds and flavonoids are widely known as powerful antioxidants and have been found to act as important bioactive agents that are beneficial for human health, having both curative and preventive attributes regarding diseases. The high concentration and variety of these phytochemicals in plants makes them attractive sources for pharmaceutical and medicinal applications, particularly in the field of health promotion. Antioxidant, antibacterial, anti-cancer, and cardioprotective effects, immune system stimulation and anti-inflammatory effects, as well as UV radiation skin protection, are just a few of the many activities they have been found capable of in this context [24]. The current study analyzed the bioactive phytochemical contents using HPLC–MS/MS and determined the total phenolic and flavonoid contents spectrophotometrically using colorimetric assays.

Another focus of the current study was to assess the effect of different solvents on total bioactive contents, phytochemical composition, and biological capacity of the extracts of *A. carduchorum*. A good solvent is characterized by its optimal extraction and its capacity to preserve the stability of the desired chemical compounds, demonstrating the influence of the type of extraction solvent and its polarity on the level of extracted polyphenols [25]. In this study, five solvents ranging from polar to less polar/non-polar, notably water, methanol, ethyl acetate, dichloromethane, and n-hexane, respectively, were used for preparing the different extracts.

Seven compounds, namely shikimic acid (0.48–8.67 μg g^{-1}), 5-caffeoylquinic acid (0.43–35.62 μg g^{-1}), *p*-coumaric acid (1.02–89.98 μg g^{-1}), ferulic acid (0.96–87.20 μg g^{-1}), rutin (0.082–96.11 μg g^{-1}), hyperoside (0.183–23.71 μg g^{-1}), and quercitrin (0.15–46.63 μg g^{-1}), were found in

common in all the tested extracts of *A. carduchorum*, as revealed by HPLC–MS/MS. The methanolic extract yielded the highest bioactive content, followed by water and infusion extracts (326.2, 242.5, and 126.0 μg g^{-1}, whereas the less polar extracts (ethyl acetate, dichloromethane, and hexane) yielded lower bioactive content (5.44–53.1 μg g^{-1}) (Table 1).

It is possible to get the best extraction of polyphenols by using a non-polar solvent, but this is not always the case because hydrogen bonds between the polar sites of antioxidative compounds and the solvent make a polar solvent more efficient at solvation [25]. Likewise, in the present study, higher bioactive compounds were extracted from the polar extracts compared to the less polar ones.

Other studies have also attempted to isolate the flavonoids in the flowering herb of *Arabis caucasica*, detected as being isorhamnetin 3-glucoside, quercetin 3-glucoside, isorhamnetin 3-beta-glucoside-7-alpha-rhamnoside, and quercetin 3-beta-glucoside-7-alpha-rhamnoside [26]. The chemical components of other *Arabis* species, such as *A. glabra*, have also been studied. Five compounds were isolated from the whole plant of *A. glabra*, identified by spectroscopic analysis as 2,5-dihydroxybenzoic acid, salicylic acid, astragalin, quercetin−3,7−O−β−D−diglucopyranosidequercetin−3,7−O−β−D−diglucopyranoside, and rutin [27].

An extraction with polar solvents yielded higher TPC (25.17–26.62 mg GAE/g) compared to the less polar solvents (13.73–19.66 mg GAE/g). On the other hand, the methanolic extract yielded the highest TFC (52.95 mg RE/g) (followed by the ethyl acetate extract (19.73 mg RE/g)) compared with the other extracts that possessed relatively low TFC (2.66–9.04 mg RE/g) (Table 2).

Table 1. Content (μg g^{-1} of dried weight extract) of bioactive compounds in *A. carduchorum* extracts.

No.	Compound	*Arabis carduchorum* Extracts					
		Hexane	**EA**	**DCM**	**MeOH**	**H_2O**	**Infusion**
1	Shikimic acid	0.560	0.725	0.482	2.560	0.503	8.699
2	Gallic acid	n.d. [a]	n.d.	n.d.	0.056	0.098	0.175
3	Loganic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4	3-Caffeoylquinic acid	n.d.	n.d.	n.d.	9.107	0.990	0.350
5	Swertiamarin	n.d.	n.d.	n.d.	n.d.	n.d.	2.634
6	(+)-Catechin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7	Delphinidin-3,5-diglucoside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8	Sweroside	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
9	5-Caffeoylquinic acid	0.618	0.493	0.431	35.619	1.398	0.780
10	Vanillic acid	n.d.	3.307	8.189	5.354	35.906	3.307
11	Caffeic acid	n.d.	0.874	0.271	1.043	n.d.	2.971
12	(-)-Epicatechin	n.d.	n.d.	0.188	n.d.	n.d.	n.d.
13	Syringic acid	n.d.	1.465	4.777	4.013	17.771	6.369
14	*p*-Coumaric acid	1.017	25.452	5.065	58.868	89.978	20.011
15	Ferulic acid	0.961	8.576	30.840	33.242	87.204	27.376
16	3,5-Dicaffeoylquinic acid	n.d.	n.d.	n.d.	2.606	0.485	n.d.
17	Naringin	n.d.	0.522	0.043	n.d.	n.d.	n.d.
18	Rutin	1.210	2.017	0.506	96.110	0.082	1.607
19	Hyperoside	0.269	1.636	0.183	23.712	0.733	4.176
20	Resveratrol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
21	Amarogentin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22	Kaempferol-3-glucoside	0.048	0.055	0.021	0.219	n.d.	0.014
23	Quercitrin	0.682	7.233	0.195	49.633	1.310	44.799
24	Quercetin	0.078	0.699	n.d.	4.019	6.039	2.757
25	Isogentisin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total content	**5.441**	**53.054**	**51.190**	**326.162**	**242.496**	**126.024**

[a] n.d.: not detectable. Relative standard deviation (RSD) for all compounds ranged from 3.5 to 12.1%.

Table 2. Total bioactive compounds and total antioxidant capacity (by phosphomolybdenum assays) of the tested extracts.

Extracts	TPC (mg GAE/g)	TFC (mg RE/g)	PBD (mmol TE/g)
n-hexane	13.73 ± 0.41 ^{e}	9.04 ± 0.44 ^{c}	1.45 ± 0.03 ^{b}
Ethyl acetate	18.25 ± 0.22 ^{d}	19.73 ± 0.59 ^{b}	1.85 ± 0.06 ^{a}
DCM	19.66 ± 0.53 ^{c}	3.82 ± 0.65 ^{e}	1.52 ± 0.02 ^{b}
Methanol	25.97 ± 0.48 ^{ab}	52.95 ± 0.34 ^{a}	1.59 ± 0.14 ^{b}
Water	25.17 ± 0.17 ^{b}	2.66 ± 0.04 ^{e}	0.90 ± 0.03 ^{c}
Infusion	26.62 ± 0.11 ^{a}	7.33 ± 0.04 ^{d}	1.06 ± 0.01 ^{c}

Values are reported as mean ± SD. MeOH: Methanol; TPC: Total phenolic content; TFC: Total flavonoid content; PBD: Phosphomolybdenum; GAE: Gallic acid equivalent; RE: Rutin equivalent; TE: Trolox equivalent. Different superscripts indicate significant differences in the tested extracts ($p < 0.05$).

3.2. Antioxidant Potential

As a result of their positive impact on human health, natural antioxidants, particularly polyphenols, are becoming increasingly popular. Thus, plant polyphenols may be able to counteract the harmful effects of oxidative stress, which has been linked to a wide range of diseases, including cancer, inflammation, diabetes, neurodegeneration, and cardiovascular illness [28,29]. In the current investigation, several assays were used to assess the in vitro antioxidant potentials of the different extracts.

For instance, the total antioxidant capacity of the extracts revealed by the phosphomolybdenum assay was in the range from 0.90–1.85 mmol TE/g (Table 3). However, no significant correlation was observed between the total antioxidant capacity and the TPC and TFC. Furthermore, all the studied extracts showed radical scavenging potential in DPPH and ABTS assays (4.36–32.13 mg TE/g and 12.58–67.73 mg TE/g, respectively) (Table 3). While methanolic extract showed the strongest scavenging activity in the DPPH assay, infusion extract was the most potent in the ABTS assay. However, in both scavenging assays, the less polar solvent extracts showed the lowest scavenging activity compared to the polar extracts. Similarly, all extracts were found to possess reducing activity, revealed in CUPRAC and FRAP assays (40.80–73.45 mg TE/g and 17.56–39.40 mg TE/g, respectively) (Table 3). Interestingly, the same pattern could be observed for the extracts' reducing activities, whereby the less polar extracts demonstrated lower reducing potential than the polar ones. Unlike the other antioxidant assays, the less polar extracts, particularly the ethyl acetate and DCM extracts exhibited higher activity in the metal chelating assay (22.46–28.52 mg EDTAE/g) than the less polar extracts. For instance, while the methanolic extract did not show metal chelating activity, water and infusion extract displayed the metal chelating activity of 12.21 and 27.58 mg EDTAE/g, respectively (Table 3).

Table 3. Antioxidant properties of the tested extracts.

Extracts	DPPH (mg TE/g)	ABTS (mg TE/g)	CUPRAC (mg TE/g)	FRAP (mg TE/g)	MCA (mg EDTAE/g)
n-hexane	5.09 ± 0.15 ^{e}	12.58 ± 0.61 ^{d}	40.80 ± 1.07 ^{e}	17.56 ± 0.28 ^{d}	22.46 ± 0.54 ^{a}
Ethyl acetate	6.58 ± 0.34 ^{d}	12.79 ± 0.80 ^{d}	45.33 ± 1.34 ^{d}	18.01 ± 0.71 ^{cd}	28.52 ± 3.42 ^{a}
DCM	4.36 ± 0.43 ^{e}	12.62 ± 0.11 ^{d}	54.51 ± 0.43 ^{c}	18.73 ± 0.25 ^{c}	27.02 ± 0.94 ^{a}
Methanol	32.13 ± 0.67 ^{a}	49.41 ± 3.42 ^{c}	73.45 ± 1.52 ^{a}	39.40 ± 0.50 ^{a}	na
Water	20.05 ± 0.82 ^{c}	53.90 ± 1.39 ^{b}	52.81 ± 0.74 ^{c}	30.50 ± 0.15 ^{b}	12.21 ± 0.54 ^{b}
Infusion	26.49 ± 0.58 ^{b}	67.73 ± 0.95 ^{a}	61.13 ± 0.36 ^{b}	38.58 ± 0.24 ^{a}	27.58 ± 5.36 ^{a}

Values are reported as mean ± SD. MeOH: Methanol; TE: Trolox equivalent; EDTAE: EDTA equivalents; na: not active. Different superscript indicates significant differences in the tested extracts ($p < 0.05$).

The relatively higher antioxidant activity of the polar extracts (infusion, water, and methanol) observed in scavenging and reducing assays could be attributed to the richness in bioactive compounds of the extracts compared to the other studied extracts. This fact was also confirmed by correlation analysis. The correlation heatmap is given in Figure 1. This is in line with previous reports suggesting a correlation relationship between phytochemical content and antioxidant activity of plant extracts and the presence of specific antioxidant compounds [30]. The polar extracts contained higher concentrations of phenolic acids such as ferulic and *p*-coumaric acids, also present in a wide variety of plants that are known to be good antioxidants. The antioxidant effect of phenolic compounds is due to the reactivity of phenol moiety. Although there are numerous mechanisms for the antioxidant activity owing to this reactivity of the phenol moiety of phenolic acids, the radical scavenging via hydrogen atom donation is considered to be the principal mechanism [31]. Moreover, rutin was found to be present in a much-elevated quantity in the methanolic extract compared to the other polar extracts. Rutin is well-known to be a potent antioxidant and studies are reporting its strong radical scavenging ability and effective inhibition of lipid peroxidation [32]. Rutin has been found to contribute to the antioxidant capacity of some plants [33]. In our correlation analysis, rutin was strongly correlated with the antioxidant properties (Figure 1). In addition, methanolic extract was seen to contain 5-caffeoylquinic acid in greater quantity unlike the other extracts also known to possess antioxidant properties [34]. Hence, the presence of these antioxidant compounds in higher amounts could have accounted for the higher antioxidant potential of the methanolic extract in most of the antioxidant assays (Figure 1).

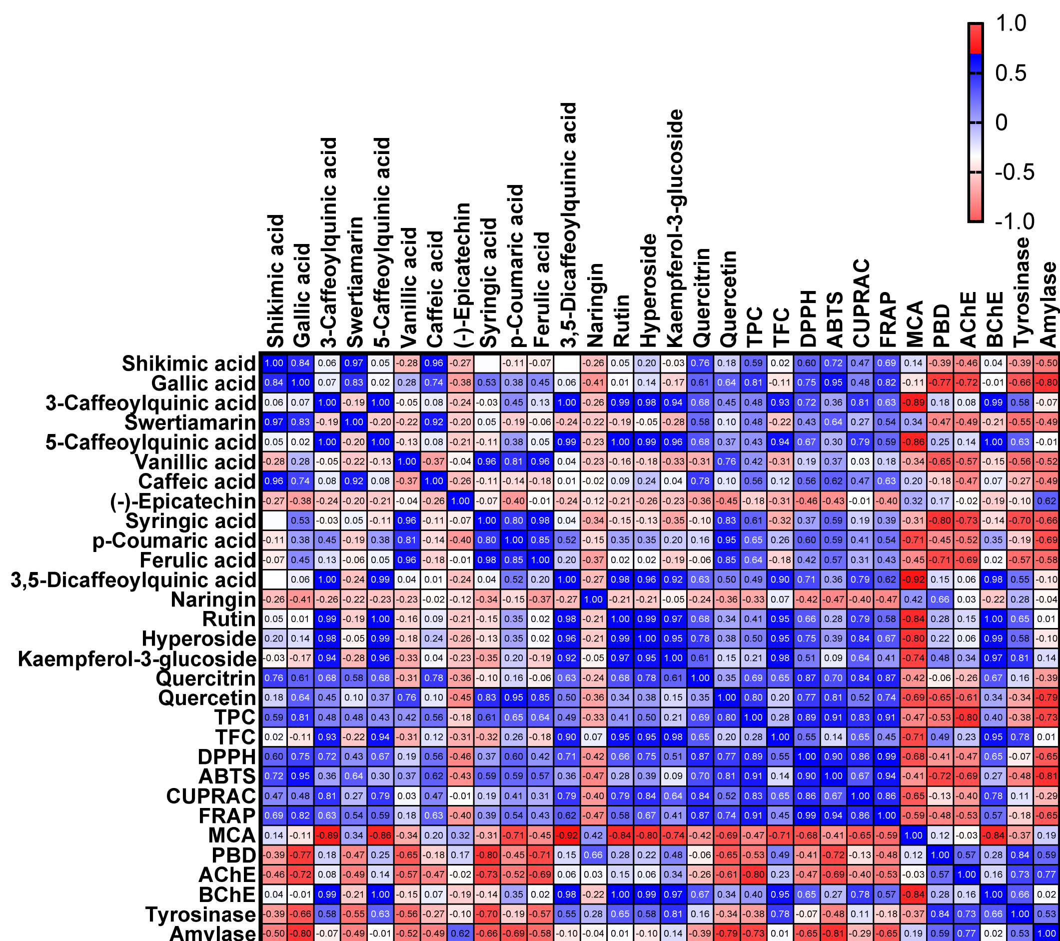

Figure 1. Correlation heat map between chemical components and biological activities of the tested extracts ($p < 0.05$).

In addition, the TFC in the methanolic extract was found to be higher than that in the other extracts in this study. Flavonoids have recently received much attention because of their ability to reduce free radical formation and scavenge free radicals. There have been numerous studies on the potential of flavonoids to act as antioxidants in vitro, thereby establishing structure–activity relationships of flavonoids' antioxidant activity [35–37].

3.3. Enzyme Inhibitory Effects

The use of enzyme inhibitors has valuable implications in disease management and treatment, and, accordingly, comprehensive studies have and are still being conducted in the search of novel enzyme inhibitors to improve therapies given to patients and as a result, remain an important research area in biomedicine. Here, four key enzymes (acetyl-/butyryl-cholinesterase, tyrosinase, and amylase) were considered to assess the efficacy of the extracts as enzyme inhibitors.

The enzyme acetylcholinesterase terminates neurotransmission at the cholinergic synapses by rapidly causing the hydrolysis of the neurotransmitter acetylcholine; inhibitors of this enzyme are employed in the treatment of several neurological disorders and are the main drugs permitted so far by the FDA for managing Alzheimer's disease (AD) [38]. Another enzyme, butyrylcholinesterase, is also believed to play a minor part in the regulation of brain acetylcholine levels. Hence, both enzymes are important therapeutic targets for amending the cholinergic deficit responsible for the deteriorations in cognitive, behavioral, and overall functioning typical of AD [39]. In this context, naturally-occurring cholinesterase inhibitors, particularly botanicals, have received considerable attention due to their efficacy in AD therapy [40,41].

In the present study, except for water and infusion extracts which did not possess anti-AChE activity, all the other extracts showed AChE inhibitory activity in the range from 0.96–2.73 mg GALAE/g. On the other hand, all the extracts exhibited anti-BChE activity (1.86–5.11 mg GALAE/g). Interestingly, the n-hexane extract was found to display the highest activity against both cholinesterase enzymes (Table 4).

The downregulation of tyrosinase, a key enzyme that catalyzes a rate-limiting step in melanin synthesis, is the most appropriate strategy for developing inhibitors of melanogenesis. A number of tyrosinase inhibitors have been developed in recent years [42]. However, because of their poor safety profiles, side effects, or low effectiveness, only a minority of them are still in use. Hence, there is a pressing need to find and develop more effective and safer tyrosinase inhibitors [43]. Above all, tyrosinase inhibitors from natural sources have received significant consideration for their latent use as hypopigmenting agents [44].

All the extracts were found to act as inhibitors of tyrosinase (inhibitory activity: 40.24–59.22 mg KAE/g). The methanolic extract demonstrated the highest tyrosinase inhibition (59.22 mg KAE/g), while the water and infusion extracts showed relatively lower anti-tyrosinase activity compared to the other extracts (Table 4).

Key indicators of anti-melanogenesis include antioxidant and tyrosinase inhibitory activities. Antioxidant activity and direct inhibitory effects on tyrosinase activity or the regulation of tyrosinase expression are two ways that many skin-whitening agents exhibit anti-melanogenic effects [45]. Thus, the antioxidant activities of the extracts could have contributed to some extent to their anti-tyrosinase effects.

Herein, the inhibition of amylase was demonstrated by all extracts (0.09–0.63 mmol ACAE/g), however, the least activity was observed by the water and infusion extracts, while the less polar extracts showed higher anti-amylase potential (Table 4). This was unlike other studies that stated high polarity extracts, such as methanol and aqueous-methanol extracts, to display higher potency than the non-polar and low polarity extracts (hexane- and dichloromethane-extracts) against diabetes-related enzymes, amylase, and glucosidase [46].

Table 4. Enzyme inhibitory effects of the tested extracts.

Extracts	AChE (mg GALAE/g)	BChE (mg GALAE/g)	Tyrosinase (mg KAE/g)	Amylase (mmol ACAE/g)
n-hexane	2.73 ± 0.01 [a]	5.11 ± 0.08 [a]	53.28 ± 0.47 [c]	0.55 ± 0.01 [c]
Ethyl acetate	1.07 ± 0.12 [c]	4.28 ± 0.48 [a]	53.57 ± 0.87 [c]	0.63 ± 0.01 [a]
DCM	0.96 ± 0.11 [c]	4.70 ± 0.56 [a]	57.49 ± 0.62 [b]	0.60 ± 0.01 [b]
Methanol	1.32 ± 0.15 [b]	1.86 ± 0.47 [b]	59.22 ± 0.28 [a]	0.34 ± 0.01 [d]
Water	na	2.68 ± 0.22 [b]	40.24 ± 0.55 [d]	0.09 ± 0.01 [f]
Infusion	na	2.39 ± 0.24 [b]	40.34 ± 0.66 [d]	0.13 ± 0.01 [e]

Values are reported as mean ± SD. MeOH: Methanol; GALAE: Galantamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent; na: not active. Different superscript indicates significant differences in the tested extracts ($p < 0.05$).

Based on the chemical composition and biological activities of the tested extracts, three groups were obtained by PLS-DA analysis (Figure 2). Apolar and less polar extracts, namely n-hexane, ethyl acetate, and dichloromethane, were in the same group. Methanol was very far from the other extracts, and it was characterized by the high amount of phenolics as well as significant antioxidant properties. The last group contained water extracts (macerated and infused water).

3.4. Molecular Docking

The binding energy values of each ligand against each enzyme target are given in Table 5. The representative bioactive compounds demonstrated binding potential to AChE, BChE, and amylase, with no/modest binding to tyrosinase. Rutin (quercetin 3-O-glucoside), kaempferol 3-O-rutinoside, and hyperoside bound well to AChE, BChE, and amylase. H-bonds are the major contributor, and several van der Waals interactions along with a couple of hydrophobic contacts added to the overall binding affinity. Rutin and kaempferol 3-O-rutinoside filled up the cavity of and formed H-bonds with polar amino acid residues of AChE and BChE via multiple hydroxyl groups (Figure 3A–C). Hyperoside bound strongly to amylase, mainly via H-bonds as well (Figure 3D). Shikimic acid fitted the narrow pocket of tyrosinase, forming 3 H-bonds and a couple of van der Waals interactions deep inside (Figure 3E), while the catalytic channel of amylase was bound by the quercetin via several H-bonds and a few van der Waals interactions.

Table 5. Calculated binding energy values of bioactive compounds *Arabis carduchorum.*

Compound	AChE	BChE	Tyrosinase	Amylase
			Kcal/mol	
Shikimic acid	−8.25	−8.56	−4.26	−4.44
5-Caffeoylquinic acid	−9.76	−9.68	−4.99	−7.74
3-Caffeoylquinic acid	−11.30	−11.32	−4.08	−5.78
Vanillic acid	−7.44	−6.99	−5.20	−5.77
Caffeic acid	−8.85	−6.79	−3.37	−4.87
Syringic acid	−6.74	−6.52	−3.67	−5.78
p-Coumaric acid	−7.10	−6.58	−1.56	−4.70
Ferulic acid	−6.99	−6.99	−2.49	−4.33
Rutin	−13.89	−11.63	−6.23	−9.88
Hyperoside	−8.97	−8.32	−3.10	−9.95
Kaempferol-3-O-glucoside	−11.21	−11.92	−6.17	−9.95
Quercitrin	−8.68	−8.78	−3.34	−9.19
Quercetin	−6.89	−6.78	−4.67	−6.22

ADMET properties of the dominant bioactive compounds in *A. carduchorum* extracts were predicted using the SwissADME web server (http://www.swissadme.ch/, accessed on 1 April 2022). The predicted ADMET properties of each compound are shown in Table 6 and Figure 4. Vanillic acid, caffeic acid, syringic acid, p-Coumaric acid, and ferulic acid are predicted to have high gastrointestinal (GI) absorption and can cross the blood-brain barrier. While shikimic acid has high GI absorption, it cannot penetrate the blood–brain barrier. The rest of the compounds have low GI absorption and cannot pass the blood–brain barrier due to high polarity and violate some of Lipinski's "rule of 5" parameters because of their large size. Importantly, however, except for quercetin, all the compounds show no potential inhibition of liver metabolic CYP450 enzymes and are, therefore, considered non-toxic to the organ.

Table 6. Drug-like and ADMET properties of the dominant bioactive *Arabis carduchorum* extracts predicted using the SwissADME server.

Compound	GI Absorption	BBB Permeant	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	Lipinksi Rule	PAINS
Shikimic acid	High	No	No	No	No	No	No	Yes; 0 violation	0 alert
5-Caffeoylquinic acid	Low	No	No	No	No	No	No	Yes; 1 violation: NHorOH > 5	1 alert: catechol Amine
3-Caffeoylquinic acid	Low	No	No	No	No	No	No	Yes; 1 violation: NHorOH > 5	1 alert: catechol Amine
Vanillic acid	High	No	No	No	No	No	No	Yes; 0 violation	0 alert
Caffeic acid	High	No	No	No	No	No	No	Yes; 0 violation	1 alert: catechol Amine
Syringic acid	High	No	No	No	No	No	No	Yes; 0 violation	0 alert
p-Coumaric acid	High	Yes	No	No	No	No	No	Yes; 0 violation	0 alert:
Ferulic acid	High	Yes	No	No	No	No	No	Yes; 0 violation	0 alert:
Rutin	Low	No	No	No	No	No	No	No; 3 violations: MW > 500; HBA > 10; HBD > 5	1 alert: catechol Amine
Hyperoside	Low	No	No	No	No	No	No	No; 2 violations: NorO > 10, NHorOH > 5	1 alert: catechol Amine
Kaempferol-3-O-glucoside	Low	No	No	No	No	No	No	No; 3 violations: MW > 500; HBA > 10; HBD > 5	0 alert
Quercitrin	Low	No	No	No	No	No	No	No; 2 violations: NorO > 10, NHorOH > 5	1 alert: catechol Amine
Quercetin	High	No	Yes	No	No	Yes	Yes	Yes; 0 violation	1 alert: catechol Amine

GI: gastrointestinal absorption; BBB: blood–brain barrier; CYP: cytochrome P450; MW: molecular weight; NorO: Nitrogen or Oxygen; NHorOH: NH or OH groups.

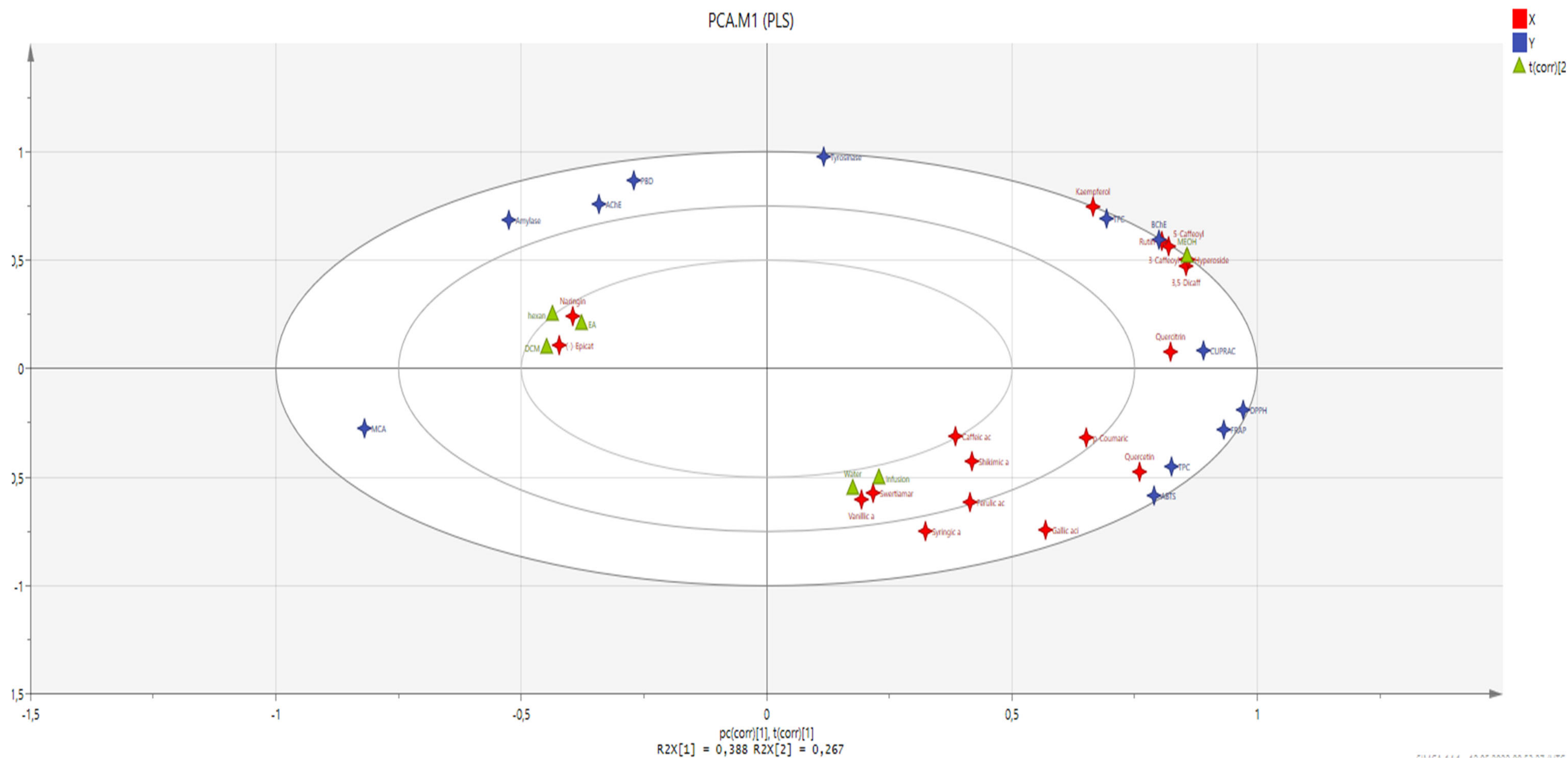

Figure 2. PLS-DA scatter plot of *Arabis carduchorum* extracts based on chemical components and biological activities (antioxidant and enzyme inhibitory abilities).

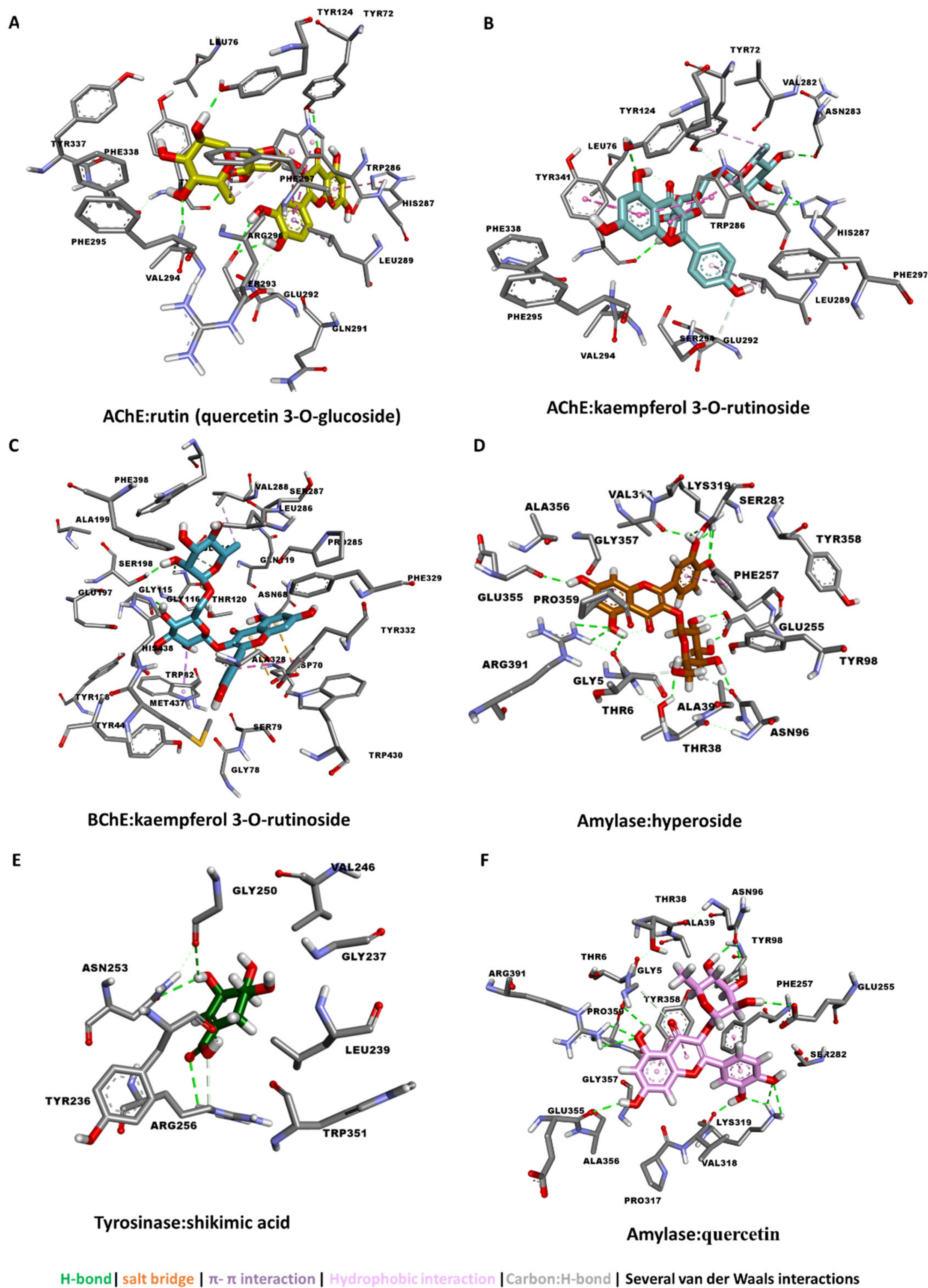

Figure 3. Protein–ligand interaction: (**A**) AChE and rutin (quercetin 3-O-glucoside), (**B**) AChE and kaempferol 3-O-glucoside, (**C**) BChE and kaempferol-3-O-glucoside, (**D**) amylase and hyperoside, (**E**) tyrosinase and shikimic acid, and (**F**) amylase and quercetin.

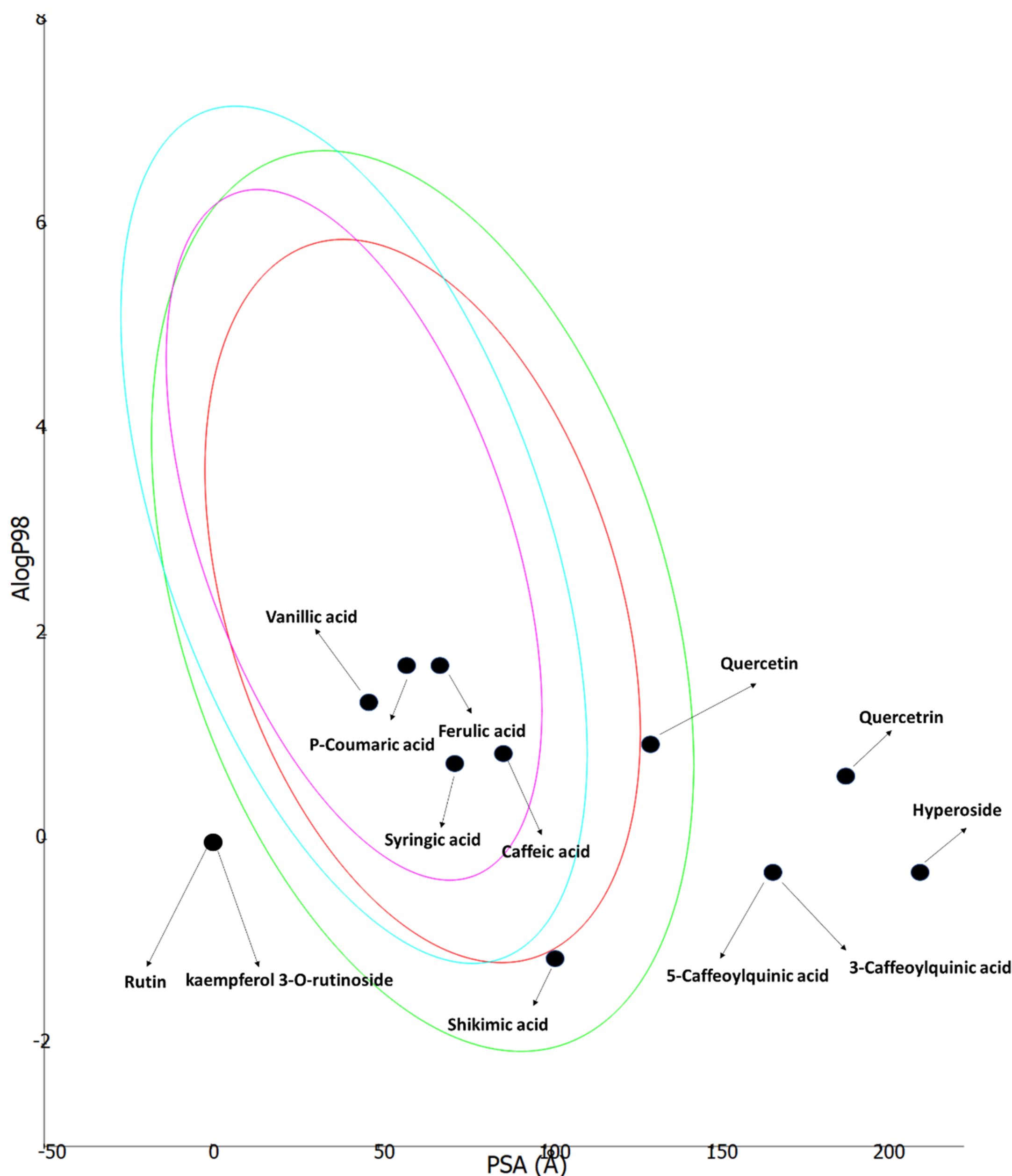

Figure 4. ADMET plot: logarithm of octanol–water partition coefficient (LogP) versus topological polar surface area (PA), The 4 ellipses define areas of well-absorbed molecules: 95 and 99% of a molecule are expected to be in ellipses colored in red and green, respectively, and 95 and 99% of a molecule are within the ellipses colored in magenta and aqua, respectively, for blood–brain barrier permeability.

4. Conclusions

In this study, the extracts of *A. carduchorum*, prepared using five solvents with different degrees of polarity, were investigated for their phytochemical profile and tested for antioxidant and enzyme inhibition properties. While the extracts showed varying antioxidant potential, the polar extracts and, in particular, the methanolic extract showed better antioxidant abilities, which could be related to the richness in bioactive contents as revealed by HPLC–MS/MS. Moreover, while the polar extracts were better antioxidants in terms of the radical scavenging and reducing activities, the less polar extracts were better enzyme inhibitors. The findings from this study, therefore, demonstrated that the solvent is an important factor to consider in the extraction of *A. carduchorum*, as it clearly influenced the total bioactive contents and the biological activities. This study could be used

to guide optimum extraction of phytochemicals from *A. carduchorum* and direct further studies (animal, toxicity, bioavailability, etc.) to maximize the extraction of bioactive compounds and investigate the effects of groups of compounds on the biological potencies of extracts.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/article/10.3390/app12136561/s1, Table S1. HPLC–MS/MS acquisition parameters, working as "dynamic Multiple Reaction Monitoring" mode, including retention time (Rt) and delta retention time (Δ Rt) for each transition.

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