Check for updates

doi.org/10.1002/cbdv.202201181

RESEARCH ARTICLE

CHEMISTRY & BIODIVERSITY

Chemical Characterization and Multidirectional Biological Effects of Different Solvent Extracts of *Arum elongatum*: *in Vitro* and *in Silico* Approaches

Mohamad Fawzi Mahomoodally,[a, b, c] Gokhan Zengin,*[d] Seebaluck-Sandoram Roumita,[a] Giovanni Caprioli,[e] Ahmed M. Mustafa,[e] Diletta Piatti,[e] Evren Yıldıztugay,[f] Gunes Ak,[d] Ayşe Esra Karadağ,[g] Asaad Khalid,[h, i] Ashraf N. Abdalla,[j] Abdullahi Ibrahim Uba,[k] and Fatih Demirci[l]

[a] Department of Health Sciences, Faculty of Medicine and Health Sciences, University of Mauritius, Reduit 80837, Mauritius

[b] Center for Transdisciplinary Research, Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Science, Chennai 600077, India

[c] Center of Excellence for Pharmaceutical Sciences, North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa

[d] Department of Biology, Faculty of Science, Selcuk University, Selcuklu, 42130, Konya, Turkey, e-mail: gokhanzengin@selcuk.edu.tr

[e] School of Pharmacy, University of Camerino, 62032 Camerino, Italy

[f] Department of Biotechnology, Faculty of Science, Selcuk University, Selcuklu, 42130, Konya, Turkey

[g] Department of Pharmacognosy, School of Pharmacy, Istanbul Medipol University, 34810 Istanbul, Türkiye

[h] Substance Abuse and Toxicology Research Center, Jazan University, P.O. Box: 114, Jazan 45142, Saudi Arabia

[i] Medicinal and Aromatic Plants and Traditional Medicine Research Institute, National Center for Research, 2404 Khartoum, Sudan

[j] Department of Pharmacology and Toxicology, College of Pharmacy, Umm Al-Qura University, Makkah 21955, Saudi Arabia

[k] Department of Molecular Biology and Genetics, Istanbul AREL University, 34537 Istanbul, Türkiye

[l] Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Türkiye

Arum elongatum (Araceae) is widely used traditionally for the treatment of abdominal pain, arterial hypertension, diabetes mellitus, rheumatism and hemorrhoids. This study investigated the antioxidant properties, individual phenolic compounds, total phenolic and total flavonoid contents (HPLC/MS analysis), reducing power and metal chelating effects of four extracts obtained from *A. elongatum* (ethyl acetate (EA), methanol (MeOH), methanol/water (MeOH/water) and infusion). The inhibitory activity of the extracts were also determined against acetylcholinesterase, butyrylcholinesterase, tyrosinase, amylase and glucosidase enzymes. The MeOH/water extracts contained the highest amount of phenolic contents (28.85 mg GAE/g) while the highest total flavonoid content was obtained with MeOH extract (36.77 mg RE/g). MeOH/water demonstrated highest antioxidant activity against DPPH$^{\bullet}$ radical at 38.90 mg Trolox equivalent per gram. The infusion extract was the most active against ABTS$^{+\bullet}$ (133.08 mg TE/g). MeOH/water extract showed the highest reducing abilities with the CUPRAC value of 102.22 mg TE/g and the FRAP value of 68.50 mg TE/g. A strong metal chelating effect was observed with MeOH/water extract (35.72 mg EDTAE/g). The PBD values of the extracts ranged from 1.01 to 2.17 mmol TE/g. EA extract displayed the highest inhibitory activity against AChE (2.32 mg GALAE/g), BChE (3.80 mg GALAE/g), α-amylase (0.56 mmol ACAE/g) and α-glucosidase (9.16 mmol ACAE/g) enzymes. Infusion extract was the most active against tyrosinase enzyme with a value of 83.33 mg KAE/g. A total of 28 compounds

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbdv.202201181

 © 2023 Wiley-VHCA AG, Zurich, Switzerland

were identified from the different extracts. The compounds present in the highest concentration were chlorogenic acids, 4-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, ferulic acid, isoquercitrin, delphindin 3,5-diglucoside, kaempferol-3-glucoside and hyperoside. The biological activities of *A. elongatum* extracts could be due to the presence of compounds such as gallic acid, chlorogenic acids, ellagic acid, epicatechin, catechin, kaempferol, 4-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, ferulic acid, quercetin, isoquercitrin, and hyperoside. Extracts of *A. elongatum* showed promising biological activities which warrants further investigations in an endeavor to develop biopharmaceuticals.

Keywords: *Arum*, phenolic composition, bioactive agent, natural enzyme inhibitors.

Introduction

The Araceae plant family is composed of 107 genera and has more than 3700 species that are distributed around the world. *Arum elongatum* Steven belongs to the *Arum* genus. *Arum plants* naturally grow in mountainous, rocky, forested, and red soil areas. The Louf group, which includes *Arum hydrophilum*, *Arum dioscorides*, *Arum elongatum*, and *Arum palaestinum*, are commonly used as spices and cooked as leafy vegetables in Jordan. Additionally, *Arum* species are traditionally used in medicine to treat a variety of ailments including cancer, circulatory issues, obesity, internal bacterial infections, diabetic symptoms, and poisoning.[1] *A. maculatum* is known to treat phlegm from the stomach, chest and lungs. *A. maculatum* tuber is used in Bulgarian traditional and folk medicine to address a range of conditions including kidney stones, colitis, liver disease, hyperacidity, and hemorrhoids. Its extract has also been tested for anti-inflammatory activity in the intestinal and respiratory tract. In Asia, *A. maculatum* is used to treat rheumatism, while in southern Italy, *A. italicum* is traditionally used to treat warts. In Sicily, the leaves and tubers of the plant are macerated in oil and applied to alleviate rheumatic pains. The decoction of *A. elongatum* Steven subsp. detruncatum is consumed on an empty stomach in the morning against abdominal pain, arterial hypertension, diabetes mellitus, and rheumatism.[2] In Bulgaria, the tubers of *A. elongatum* are used against hemorrhoids. In Turkey, the leaves of *A. elongatum* are used in the treatment of abdominal pain, hypertension and diabetes.[3] In Jordan, survey data showed that 35 % of cancer patients consume *A. hydrophilum* and *A. palaestinum* as hot beverages, spice or cooked as a herbal medicine against several types of cancer.[4] *Arum palaestinum* extracts were found effective against leukemia (k562) and colon cancer. The plant extract inhibited the growth of prostatic tumors in mice. *A. maculatum* leaf extracts displayed antibacterial activity. *A. hygrophilum* extract inhibited the gastrointestinal enzymes responsible for digestion and absorption of carbohydrate and lipid. The extract also increased β-cell proliferation in a dose dependent way.[1] In the same study, the ethanolic extract of the plant showed antioxidant activities and a toxicity to brine shrimp. The phytochemical screening of *A. elongatum* aqueous extract showed the presence of saponin, carbohydrates, phenols, tannin and flavonoid while the methanol extract revealed the presence of alkaloids, phenols and flavonoids.[5]

Since there are few published data on the chemical and biological properties of *Arum* species, the aim of this study was to determine the antioxidant properties (free radical scavenging, reducing power, metal chelating effect), enzyme (cholinesterase, tyrosinase, amylase, glucosidase, 5-lipoxygenase and angiotensin II converting) inhibitory activities of different extracts (ethyl acetate, methanol, methanol/water, and water) from *A. elongatum*. Furthermore, the chemical profiles of the different tested extracts were also evaluated using HPLC/MS technique.

Results and Discussion

Antioxidant Activities

The amount of total phenolic content varied from 21.65 to 28.85 mg GAE/g. Methanol/water extract contained the highest total phenol value followed by methanol while the extract with the lowest phenol content was ethyl acetate (Table 1). The highest total flavonoid content was obtained with methanol extract (36.77 mg RE/g) followed by ethyl acetate (21.17 mg RE/g) and methanol/water extracts (17.22 mg RE/g). Infusion extract showed lowest total flavonoid content with the value 12.16 mg RE/g. Study from Jaradat and Abualhasan (2016) showed that the total phenols obtained in methanolic extract of *A. elongatum* was 27.49 mg GAE/g. To determine the ability of plant extracts to scavenge free radicals, *in vitro* antioxidant

© 2023 Wiley-VHCA AG, Zurich, Switzerland

16121880, 2023, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.202201181 by Istanbul Arel University, Wiley Online Library on [02/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

CHEMISTRY & BIODIVERSITY

Table 1. Total phenolic, flavonoid contents, and free radical scavenging abilities of the tested extracts.

Solvents	TPC (mg GAE/g)	TFC (mg RE/g)	DPPH (mg TE/g)	ABTS (mg TE/g)
EA	21.65 ± 0.32[b]	21.17 ± 0.40[b]	1.79 ± 0.04[c]	24.36 ± 2.12[d]
MeOH	23.30 ± 2.89[b]	36.77 ± 0.68[a]	37.17 ± 0.89[b]	81.40 ± 4.42[c]
MeOH/Water	28.85 ± 0.47[a]	17.22 ± 0.09[c]	46.65 ± 0.19[a]	122.56 ± 1.35[b]
Infusion	28.84 ± 0.20[a]	12.16 ± 0.24[d]	38.90 ± 1.14[b]	133.08 ± 0.39[a]

Values are reported as mean ± SD of three parallel experiments. GAE: Gallic acid equivalents; RE: Rutin equivalent; TE: Trolox equivalent. Different letters indicate significant differences in the tested extracts ($p < 0.05$).

activity assays were performed using $ABTS^{+\bullet}$ and $DPPH^{\bullet}$, two commonly used radicals. The extracts showed antioxidant activities ranging from 1.79 to 38.90 mg Trolox equivalent per gram for DPPH and from 24.36 to 133.08 mg TE/g for ABTS. Ethyl acetate extract showed the lowest antioxidant activity against DPPH (1.17 mg TE/g) and ABTS (24.36 mg TE/g). Methanol/water extract exhibited the highest antioxidant properties (46.65 mg TE/g) with DPPH assay while the infusion extract was the most potent antioxidant to scavenge $ABTS^{+\bullet}$ cation radical. The endogenous and dietary derived antioxidants constitute the body's antioxidant defense system.[6] In a study by Alan[7] on *A. elongatum* Steven extracts, it was found that ethanol and pure water extracts had low DPPH activity but significant activity in terms of ABTS and total antioxidant. The antioxidant activity was attributed to the presence of identified phytochemicals[8] such as chlorogenic acids, which donate hydrogen atoms to reduce free radicals, leading to the oxidation of phenoxyl radicals.[6] Sukito and Tachibana[9] reported that hyperoside and isoquercitin exhibit antioxidant activities of 67.52 % and 64.33 %, respectively. Caffeic acid functions by interrupting free radical formation and blocking chain reactions. It achieves this by donating electrons or hydrogen, thereby converting free radicals into thermodynamically stable products. The delocalization of electrons in the aromatic ring of caffeic acid (resonance effect) leads to significant stabilization of these product.[10]

Chemical Composition of the Tested Extracts

Ethyl acetate, methanol, methanol/water (70 %) and water extracts of *A. elongatum* plant were analyzed by HPLC–MS/MS searching for 37 marker compounds. Twenty eight bioactive compounds have been identified from the 4 extracts of the investigated plant (*Table 2*). Methanol extract (4505.47 μg/g) showed the highest total content of the identified compounds followed by methanol/water (3934.46 μg/g) and water extract (3207.12 μg/g), while ethyl acetate extract (2618.49 μg/g) showed the lowest content. The most abundant compounds were found to be *p*-coumaric acid, 4-hydroxybenzoic acid, caffeic acid, *trans*-cinnamic acid, chlorogenic acid, ferulic acid, naringin, isoquercitrin, delphindin 3,5-diglucoside, kaempferol-3-glucoside and hyperoside. It was observed that there were variations in the concentrations of the identified compounds in the 4 extracts. For example *p*-coumaric acid was the most abundant compound in methanol extract (1306.39 μg/g), methanol/water (1149.10 μg/g) and ethyl acetate extract (887.03 μg/g), and 4-hydroxybenzoic acid was the major compound in water extract (1623.61 μg/g). The obtained results were in accordance with literature, and previous studies reported the presence of caffeic, ferulic, gallic, rosmarinic acid, quercetin, quercetin-3-O-β-glucoside, quercetin-3-O-rhamnoside, luteolin, isoorientin, and vitexin in extracts of different *Arum* species, e.g., *A. hygrophilum* and *A. dioscoridis*.[11] It is noteworthy that this is the first detailed study on the phenolic profile of *A. elongatum* and this study would enable us to identify the composition of its extracts to integrate with the biological knowledge.

Reducing Power and Metal Chelating Effects of Tested Extracts

Figure 1 presents the reducing ability and metal chelating properties of the studied extracts. The ferric reducing agent assay measures the ability of antioxidants to donate electrons and reduce potassium ferricyanide, indicating their antioxidant activity. Phenolic acids, for instance, can reduce the ferric ion/ferricyanide complex to the ferrous form, forming Perl's Prussian blue complex. Metal chelating properties can be determined spectroscopically by assessing the ability of the extracts to inhibit free radical formation by chelating transition metal ions.[12] MeOH/Water extract showed the highest FRAP value

© 2023 Wiley-VHCA AG, Zurich, Switzerland

16121880, 2023, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.202201181 by Istanbul Arel University, Wiley Online Library on [02/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

16121880, 2023, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.202201181 by Istanbul Arel University, Wiley Online Library on [02/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 2. Chemical composition of the tested extracts (μg g-1 of dried extract).

No.	Compounds	EA	MeOH	MeOH/H_2O	Infusion
1	Gallic acid	11.62	6.26	8.58	11,80
2	Neochlorogenic acid	1.30	2.68	3.30	17,03
3	Catechin	3.01	2.30	0.00	7.26
4	procyanidin B2	25.81	0.00	0.00	80,47
5	Chlorogenic acid	48.60	70.06	44.48	66.93
6	4-Hydroxybenzoic acid	715.49	1183.11	1022.68	1623.61
7	Epicatechin	1.25	1.25	2.18	24.84
8	3-Hydroxybenzoic acid	0.00	0.00	0.00	0,00
9	Caffeic acid	170.42	459.95	425.72	238.13
10	Vanillic acid	14.97	53.45	49.17	21,38
11	Syringic acid	9.84	21.78	27.40	13.35
12	Procyanidin A2	n.d.	n.d.	n.d.	n.d.
13	*p*-Coumaric acid	887.03	1306.39	1149.10	241.24
14	Ferulic acid	53.68	22.09	13.03	125.92
15	3,5-Dicaffeoylquinic acid	1.67	3.95	0.61	1,11
16	Rutin	16.68	54.95	56.05	39,48
17	Isoquercitrin	40.01	119.17	80.26	45,79
18	Delphindin 3,5-diglucoside	38.19	117.18	89.14	53,66
19	Phloridzin	n.d.	n.d.	n.d.	n.d.
20	Quercitrin	0.89	1.03	1.22	3,52
21	Myricetin	n.d.	n.d.	n.d.	n.d.
22	Naringin	110.02	410.79	427.53	244,56
23	Kaempferol-3-glucoside	57.53	234.35	195.17	74,98
24	Ellagic acid	81.93	1.72	0.69	n.d.
25	Quercetin	10.96	29.83	13.81	6,72
26	Phloretin	n.d.	n.d.	n.d.	n.d.
27	Isorhamnetin	2.40	5.33	2.47	0,14
28	Delphindin3-galactoside	n.d.	n.d.	n.d.	n.d.
29	Cyanidin-3-glucoside	n.d.	n.d.	n.d.	n.d.
30	Petunidin-3-glucoside	n.d.	n.d.	n.d.	n.d.
31	Pelargonidin-3-rutinoside	n.d.	n.d.	n.d.	n.d.
32	Pelargonidin-3-glucoside	n.d.	n.d.	n.d.	n.d.
33	Malvidin-3-galactoside	n.d.	n.d.	n.d.	n.d.
34	Hyperoside	38.69	109.40	76.60	32,87
35	Hesperidin	8.39	9.63	6.91	10,86
36	Kaempferol	7.94	12.79	6.60	2,83
37	*trans*-Cinnamic acid	260.17	266.04	231.75	218,64
Total content		2618.49	4505.47	3934.46	3207.12

[a] n.d., not detectable. Relative standard deviation (RSD) for all compounds ranged from 2.26 to 7.87 %.

(68.50 mg TE/g) followed by infusion (63.63 mg TE/g) and MeOH (51.08 mg TE/g) extracts. EA extract the lowest the lowest reducing potential with a value of 19.23 mg TE/g. The CUPRAC values of the extracts were found to be the highest in Methanol/water extract (102.22 mg TE/g) and lowest in EA extract (50.44 mg TE/g). Results revealed that the extracts have phosphomolybdenum reductive activity in the order of EA > MeOH > infusion ≥ MeOH/water. The phosphomolybdenum assay measures electron transfer based on factors such as redox potential, pH, and the structure of the antioxidant compound. The results of our study showed that the EA and MeOH extracts had high flavonoid content, as indicated by their high TE values. The metal chelating ability of the extracts were found to be in the order of MeOH/Water > infusion > EA > MeOH. Alan[7] showed that the ethanol and pure water extracts of *A. elongatum* exhibited significant antioxidant activity with regard to total reduction and CUPRAC levels.

 © 2023 Wiley-VHCA AG, Zurich, Switzerland

16121880, 2023, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.202201181 by Istanbul Arel University, Wiley Online Library on [02/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

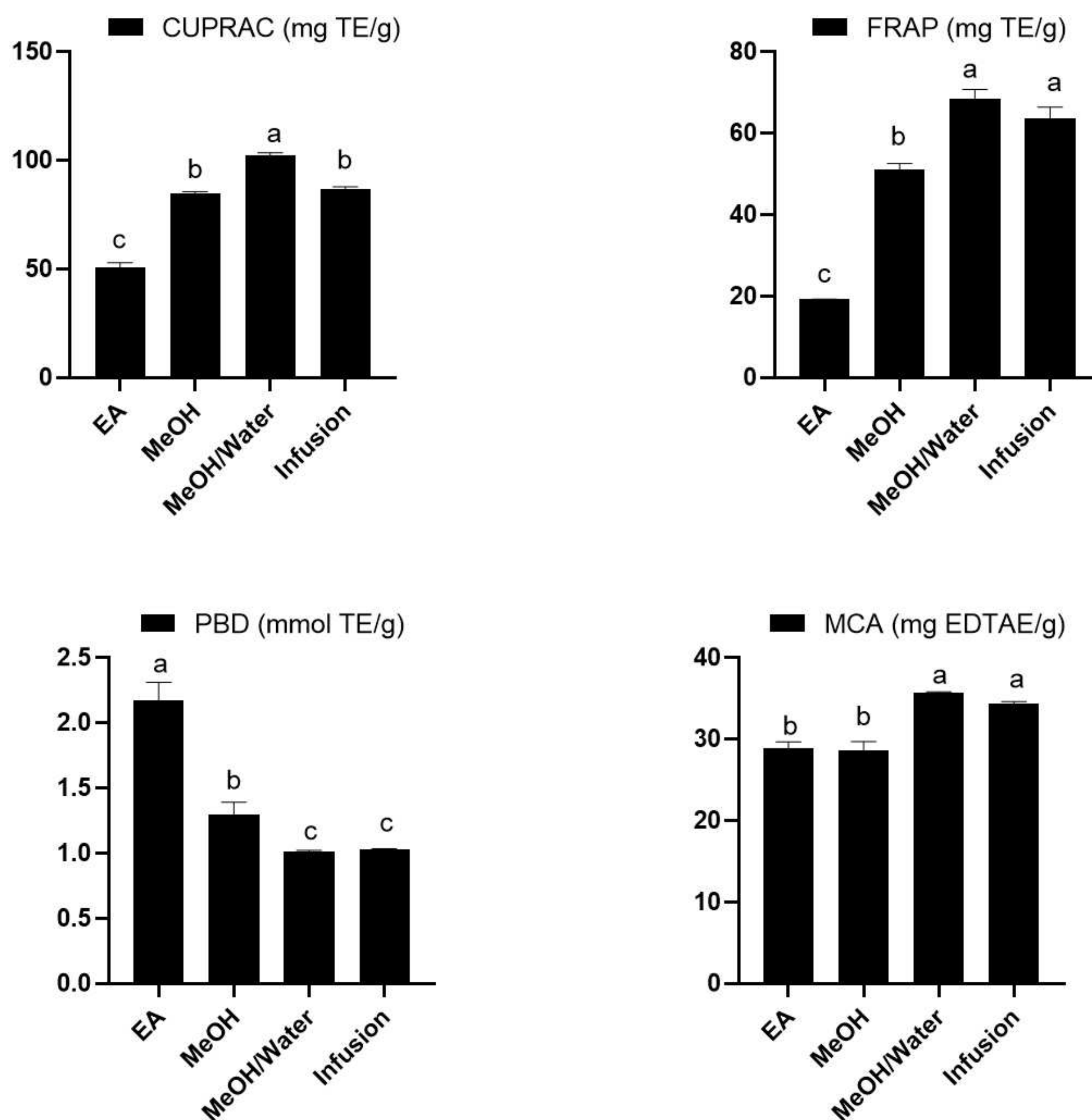

Figure 1. Reducing power and metal chelating abilities of the tested extracts. Values are reported as mean ± SD of three parallel experiments. PBD: Phosphomolybdenum; MCA: Metal chelating ability; TE: Trolox equivalent; EDTAE: EDTA equivalent. Different letters indicate significant differences in the tested extracts ($p < 0.05$).

Enzyme Inhibitory Effects of A. elongatum Extracts

Figure 2 shows the enzyme inhibitory activities of the different extracts tested against acetylcholinesterase, butyrylcholinesterase, tyrosinase, amylase and glucosidase enzymes. EA extract was found to be the most active against AChE with a value of 2.32 mg GALAE/g. MeOH and MeOH/water extracts inhibited AChE enzyme at values 2.29 and 1.72 mg GALAE/g, respectively. Infusion extract showed the lowest inhibitory activity against AChE (0.67 mg GALAE/g). Most of the AChE inhibitors belong to the alkaloid group such as indole, isoquinoline, quinolizidine, piperidine and steroidal alkaloids. However natural sources of terpenoids, flavonoids and other phenolic compounds also act as good AChE inhibitors.[13] Chlorogenic acid has been reported to possess neuroprotective effects by the AChE inhibition.[14] Kaempferol and gallic acid have been reported to show good AChE inhibition with IC50 values of 3.31 and 5.85 μM, respectively. *p*-hydroxybenzoic acid demonstrated non-competitive inhibition against AChE with IC_{50} value of 20.07 μM.[13]

 © 2023 Wiley-VHCA AG, Zurich, Switzerland

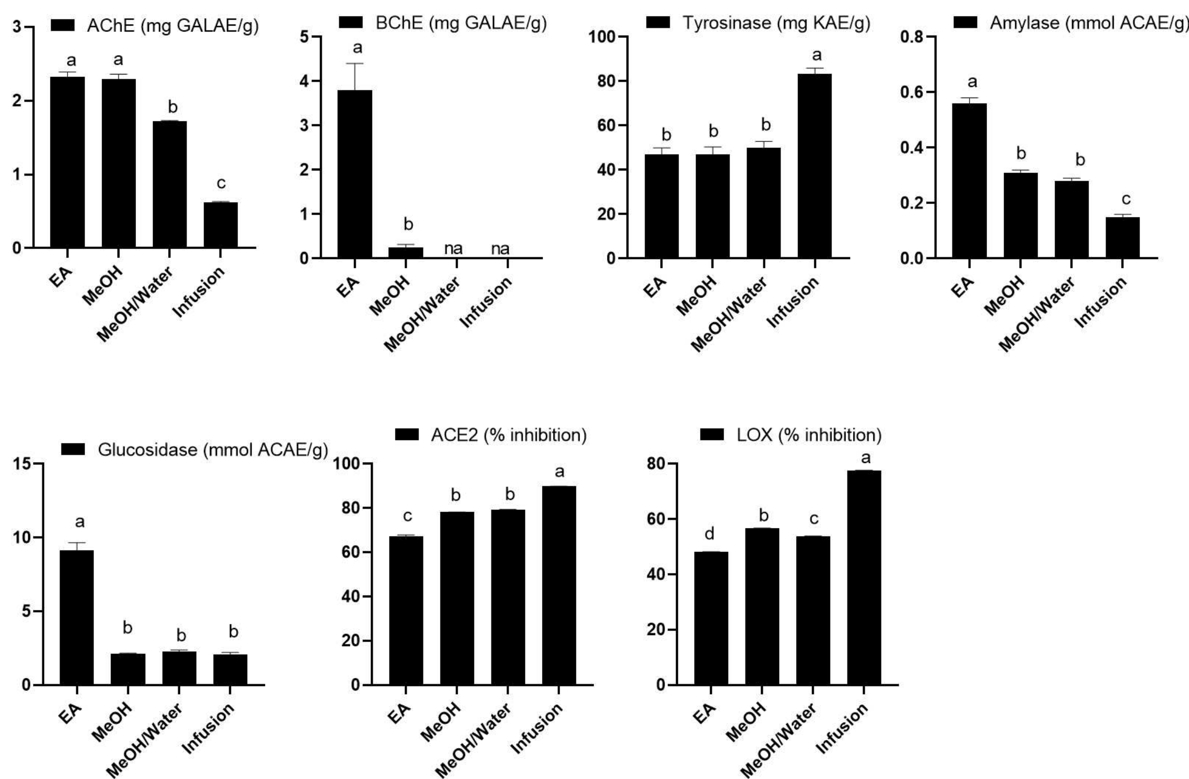

Figure 2. Enzyme inhibitory effects of the tested extracts. Values are reported as mean ± SD of three parallel experiments. GALAE: Galantamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent; na: not active. ACE2 and LOX were tested at concentration of at 20 μg/ml. Different letters indicate significant differences in the tested extracts ($p < 0.05$).

EA and MeOH extracts showed activity against BChE with values 3.80 and 0.25 mg GALAE/g, respectively. Tyrosinase inhibitors have potential clinical applications for the treatment of dermatological issues such as melanin hyperpigmentation, and they can also serve as effective cosmetic ingredients for skin whitening and depigmentation after sunburn.[15] The infusion extract revealed the highest activity against tyrosinase enzyme (83.33 mg KAE/g). Diabetes mellitus (DM) is characterized by hyperglycemia, or high blood glucose levels. Crucial enzymes for carbohydrate digestion and absorption in humans are intestinal α-glucosidase and pancreatic α-amylase. Dietary starch is hydrolyzed into maltose by α-amylase, which is subsequently broken down by α-glucosidase, a membrane-bound enzyme found in the small intestine. Consequently, natural inhibitors of α-glucosidase and α-amylase present promising therapeutic options as supplements or even safe substitutes for existing drugs.[16] The α-amylase inhibitory activity of *A. elongatum* extracts were found to be in the range of 0.15 to 0.56 mmol ACAE/g. Catechin and ellagic acid have shown inhibition against α-amylase.[16] The glucosidase inhibitory capacity of the extracts varied in the range of 2.04 to 9.16 mmol ACAE/g. EA extract showed the highest inhibitory activity against both amylase and glucosidase with the values of 0.56 and 9.16 mmol ACAE/g, respectively. Catechin, ellagic acid, epicatechin, gallic acid, quercetin and kaempferol have been reported to show good alpha glucosidase activity.[17]

The infusion of the plant (89.9 %) at a concentration of 20 μg/mL tested for ACE2 enzyme inhibition showed the highest efficacy (Figure 2). Delphinidin 3,5 glucoside and naringin *in silico* studies gave significant results for the ACE2 enzyme, and the ethyl acetate extract was the poorest in terms of these effective compounds for the tested extracts. Ethyl acetate extract gave the lowest results in terms of ACE2 enzyme inhibition among the tested extracts and the results are consistent in this context. Although these compounds are found at lower levels in infusion compared to methanol extracts, it can be said that the ratios they come together and the extract generally inhibit ACE2 better together with all the compounds it contains. As it is known, synergy does not increase with increasing doses, it can be thought that such a

© 2023 Wiley-VHCA AG, Zurich, Switzerland

16121880, 2023, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.202201181 by Istanbul Arel University, Wiley Online Library on [02/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

CHEMISTRY & BIODIVERSITY

16121880, 2023, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.202201181 by Istanbul Arel University, Wiley Online Library on [02/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

synergy occurs here. The antiviral activity of *Arum* species was investigated in previous studies.[18] The ACE2 enzyme inhibition results obtained in this study also have the potential for antiviral effects against coronaviruses.

LOX enzymes are mostly associated with anti-inflammatory effects.[19] In this study, it was determined that infusion showed the highest LOX inhibition, and close effects were observed in methanol and aqueous methanol extracts (Figure 2). In previous studies, it was revealed that aqueous preparations of *Arum* species are used ethnobotanically as anti-inflammatory.[2] As a matter of fact, the findings obtained from the results of this study also confirm this use.

Molecular Docking

The docking scores calculated in terms of binding energy are shown in *Figure 3*. Although all the selected compounds were able to bind to all 5 enzymes, most of them showed a preference for AChE and BChE. Delphinidin 3,5-diglucoside bound to AChE firmly, forming H-bonds and $\pi-\pi$ stacked interactions with residues in the vicinity of the active site, with van der Waals interactions reinforcing the binding (*Figure 4A*). In the case of BChE-kaempferol-3-*O*-glucoside interaction, the key components were 2 π-anion interactions between the kaempferol moiety and Asp70 deep inside the active site of the enzyme. In addition, the presence of an H-bond, a $\pi-\pi$ stacked, and a few hydrophobic interactions strengthened the binding (*Figure 4B*). Delphinidin 3,5-diglucoside formed strong interaction with tyrosinase via a couple of H-bond and numerous van der Waals interactions. Importantly, the active site Cu^{2+}ion also was involved in van der Waals interaction (*Figure 4C*). Interestingly, naringin occupied the catalytic cavity of amylase, forming H-bonds, a hydrophobic interactions, and a few van der Waals interactions (*Figure 4D*). Likewise, hyperoside occupied the active site of glucosidase via multiple H-bonds and van der Waals interactions along the length of the tunnel (*Figure 4E*).

Furthermore, the interactions of some of the bioactive compounds with ACE2 and lipoxygenase were analyzed. Delphinidin 3,5-diglucoside, naringin, kaempferol-3-*O*-glucoside, and hyperoside were found to demonstrate high binding potential to both ACE2 (*Figures 5A*–C) and lipoxygenase (*Figures 5D*–*F*) with binding energy ranging from −8.07 to −12.82 kcal/mol (*Figure 3*). These compounds were completely buried in the active sites of the two enzymes, forming several interactions, including multiple H-bonds, with the active site residues. Taken together, these bioactive compounds from the extracts of *Arum elongatum* likely inhibited the biological activity of these

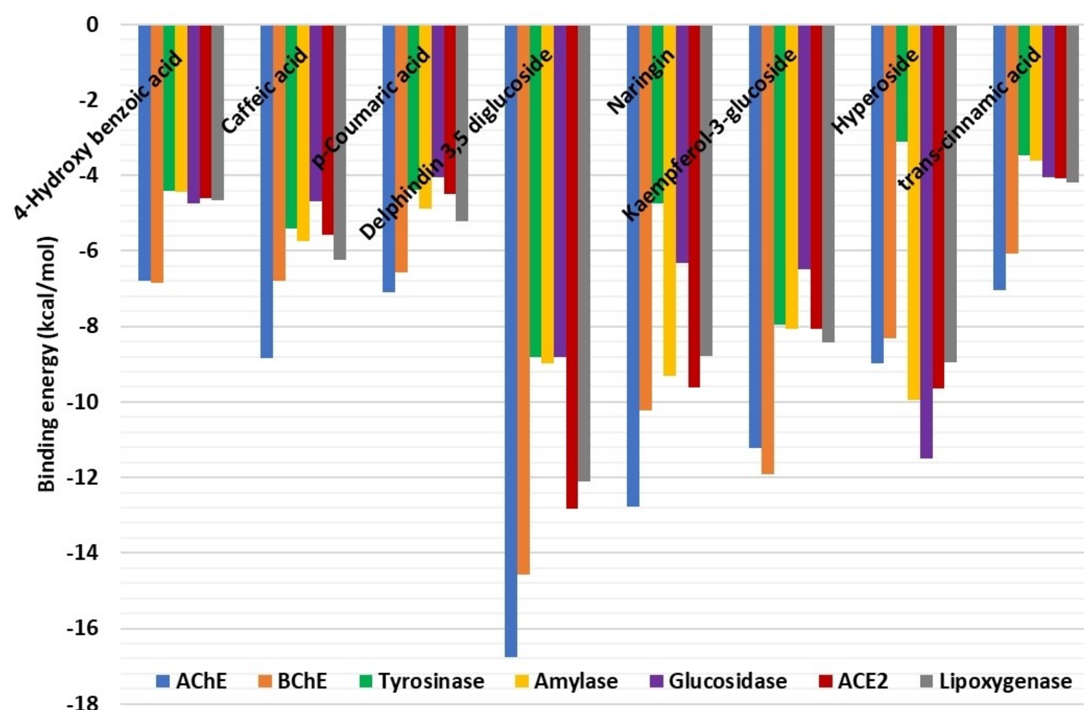

Figure 3. Calculated molecular docking scores of the dominant phytochemicals from *Arum elongatum* extracts.

 © 2023 Wiley-VHCA AG, Zurich, Switzerland

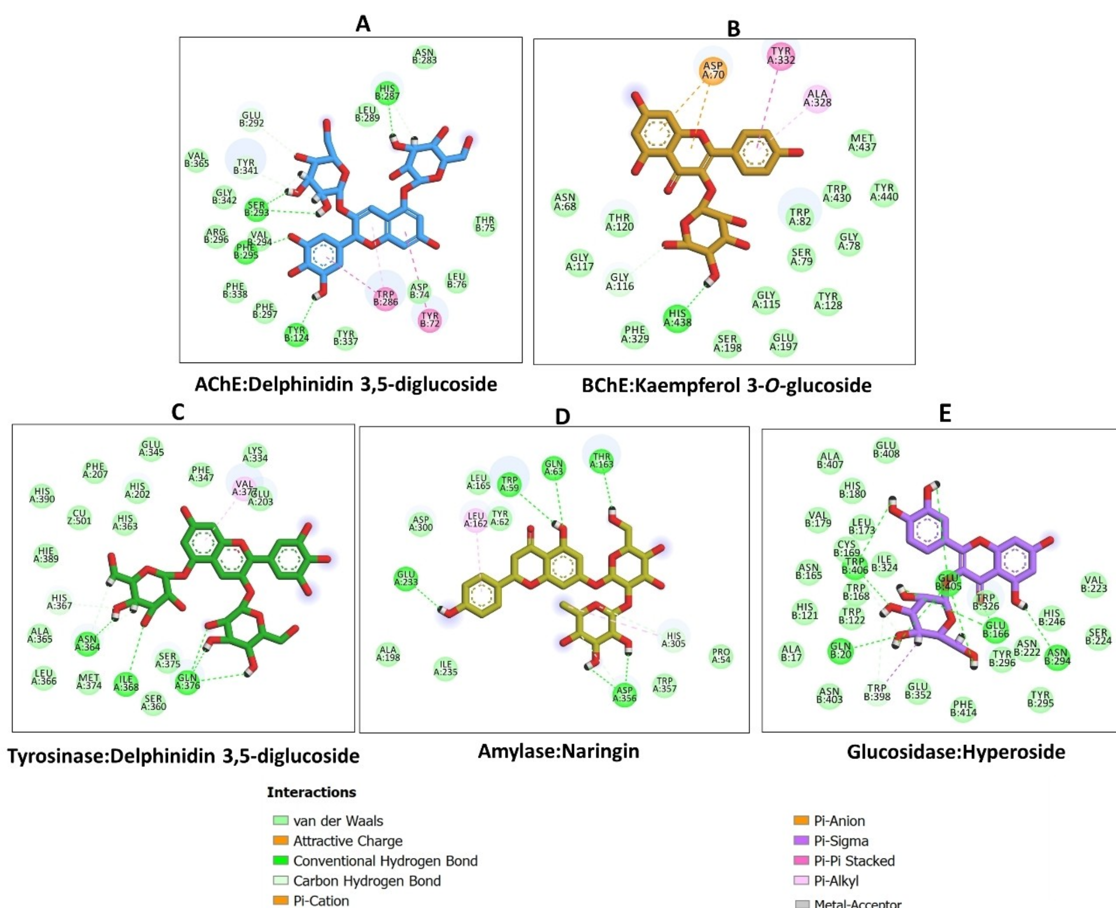

Figure 4. (A) AChE and delphinidin 3,5-diglucoside, (B) BChE and kaempferol 3-*O*-glucoside, (C) tyrosinase and delphinidin 3,5-diglucoside, (D) amylase and Naringin, and (E) glucosidase and hyperoside.

target enzymes by binding to their active site via different types of interactions.

Conclusion

In this study, the different extracts showed good antioxidant activities against free radicals such as $DPPH^{\bullet}$ and $ABTS^{+\bullet}$. MeOH/water contained the highest total phenolic contents and was more active against $DPPH^{\bullet}$ while the highest flavonoid contents were observed in MeOH extract. The infusion extract demonstrated highest activity against $ABTS^{+\bullet}$. Among the 28 identified compounds present in the different extracts, chlorogenic acids, 4-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, ferulic acid, isoquercitrin, delphindin 3,5-diglucoside, kaempferol-3-glucoside and hyperoside were found in the highest concentration. MeOH/water extract showed the highest reducing potential with respect to CUPRAC, FRAP and displayed a strong metal chelating effect. EA extract indicated the highest inhibitory activity against AChE, BChE, α-amylase and α-glucosidase enzymes while the infusion extract was more active against tyrosinase, ACE2 and lipoxygenase enzymes. Thus, the *A. elongatum* extracts can be considered as promising candidates for the use as a natural antioxidant and for the treatment or management of diseases such as Alzheimer, diabetes, coronavirus diseases, inflammatory illnesses and hyperpigmentation of the skin. However, further studies are needed to evaluate the toxicity of the different extracts.

Experimental Section

Plant Materials and Extraction

The aerial parts of the plants (*Arum elongatum* Steven) H.Riedl were collected in Alanya Turkey (GPS coordinate: 36°.53′,4330″E; 32°.18.1940″N, Tosmur Valley,

© 2023 Wiley-VHCA AG, Zurich, Switzerland

16121880, 2023, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.202201181 by Istanbul Arel University, Wiley Online Library on [02/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

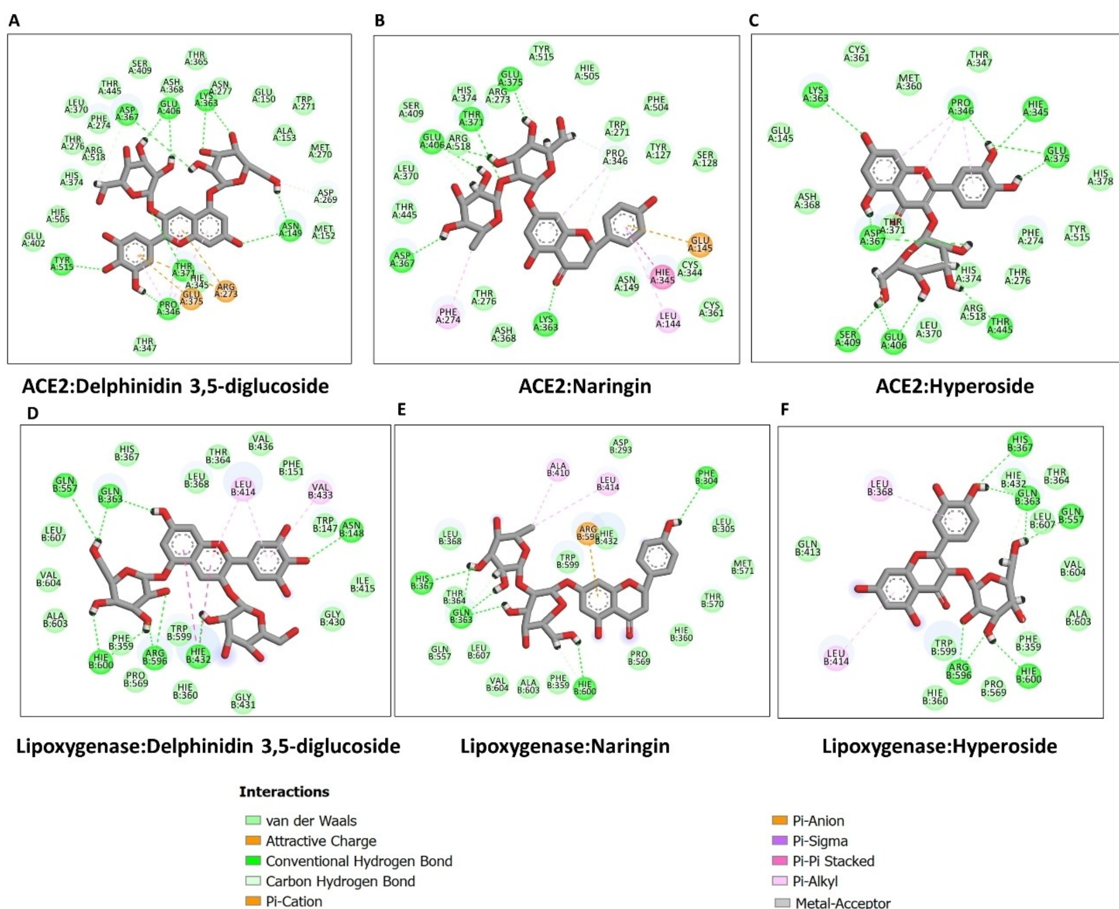

Figure 5. Interaction of ACE2 with (A) delphinidin 3,5-diglucoside, (B) naringin, and (C) hyperoside, and lipoxygenase with (D) delphinidin 3,5-diglucoside, (E) naringin, and (F) hyperoside.

2025 m, Collection date: 10.06.2020). The plant was identified by Dr. Evren Yildiztugay of Selcuk University, who is a botanist and a co-author of this manuscript. Voucher specimens were stored at the herbarium of Selcuk University (Voucher number: EY-3034).

Four solvents (ethyl acetate, methanol, methanol/water (70%) and water) were used for extraction of the plant extracts. The Aerial parts (as mix) of the plant were used to prepare extracts. The ethyl acetate, methanol, and methanol/water extracts were prepared using the maceration technique. To prepare the extracts, 10 g of plant material were stirred with 200 mL of methanol at room temperature for 24 h. The mixtures were filtered using Whatman filter paper and the solvents were removed using a rotary evaporator. The water extract was prepared using a traditional infusion method, whereby 10 g of plant material were steeped in boiled water (200 mL) for 15 min, filtered, and lyophilized for 48 h. All extracts were stored at 4 °C until further analysis.

Profile of Bioactive Compounds

The Folin-Ciocalteu method was employed to determine the total phenolic content while the $AlCl_3$ assay was used to estimate the total flavonoid content.[20] The obtained results were expressed as gallic acid equivalents (mg GAEs/g extract) and rutin equivalents (mg REs/g extract), respectively.

HPLC/MS Analysis

The contents of 37 phenolic compounds belonging to the phenolic acids, flavonols, flavan-3-ols, flavones, proanthocyanidins, anthocyanins, and non-phenolic acids in the different plant extracts were analysed using a modified version of the method proposed by.[21] After dissolving the dried extracts in methanol

© 2023 Wiley-VHCA AG, Zurich, Switzerland

16121880, 2023, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.202201181 by Istanbul Arel University, Wiley Online Library on [02/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

(5 mg/mL), sonication was done for 2 min at room temperature before filtering the solution using a 0.2 m syringeless filter. The extract was then injected into the HPLC–MS/MS system. HPLC analysis was performed using Agilent 1290 Infinity series coupled with Triple Quadrupole 6420 purchased from Agilent Technology (Santa Clara, CA). Other details are given in supplemental materials and the mass spectrometer parameters for the analysed compounds are given in *Table S1*.

Determination of Antioxidant and Enzyme Inhibitory Effects

The antioxidant and enzyme inhibitory activities of the tested extracts were assessed as previously described.[22,23] The tested parameters, including DPPH and ABTS radical scavenging activity, cupric ion reducing antioxidant capacity (CUPRAC), ferric ion reducing antioxidant power (FRAP), metal chelating ability (MCA), total antioxidant activity (phosphomolybdenum assay, PBD), AChE and BChE inhibitory activities, tyrosinase inhibitory activity, and amylase and glucosidase inhibitory activities were expressed as follows: mg Trolox equivalents (TE)/g extract for DPPH, ABTS, CUPRAC, and FRAP; mg EDTA equivalents (EDTAE)/g extract for MCA; mmol TE/g extract for PBD; mg galantamine equivalents (GALAE)/g extract for AChE and BChE inhibitory activities; mg kojic acid equivalents (KAE)/g extract for tyrosinase inhibitory activity; and mmol acarbose equivalents (ACAE)/g extract for amylase and glucosidase inhibitory activities.

For ACE2 enzyme inhibition 'Angiotensin II Converting Enzyme (ACE2) Inhibitor Screening Kit' (BioVision, catalog number: K310) were used. Prepared stock solutions using DMSO and transferred 20 μg/mL of test samples into each well. Added 40 μL of the prepared ACE2 enzyme solution and substrate solution to each well. Incubated the reaction mixture and measured the fluorescence at Ex/Em = 320/420 nm using a microplate reader (SpectraMax i3) in fluorescence mode. Expressed the results as % inhibition values.[24]

5-Lipoxygenase (LOX) enzyme inhibition was studied by Baylac's method.[25] The % inhibition was calculated by comparing the absorbance change per minute of enzyme activity to that of the test samples. Nordihydroguaiaretic acid was used as a positive control. The experiments were carried out in triplicate, and the results are presented as the mean ± standard deviation (SD).[26]

Molecular Modeling

The crystal structures of human AChE (PDB ID: 6O52),[27] BChE (PDB ID: 6EQP),[28] pancreatic α-amylase (PDB ID: 1B2Y),[29] angiotensin-converting enzyme 2 (ACE2) (PDB ID: 14RL),[30] and Lipoxygenase (PDB ID: 6 N2 W)[31] were obtained from the RCSB protein data bank (PDB) (https://www.rcsb.org/). Also, the crystal structures of *Priestia megaterium* tyrosinase (PDB ID: 6QXD)[32] and *Mus musculus* α-glucosidase (PDB ID: 7KBJ)[33] were retrieved. These structures were then used as templates for homology modeling of human α-amylase (UniProt ID: P14679), and α-glucosidase (Uniprot ID: P0DUB6), respectively. The details of the modeling procedure was described in our previous work.[34] All proteins were prepared using Playmolecule ProteinPrepare module,[35] where predicted pKa of titratable residues were utilized to prepare the proteins at pH of 7.4. The chemical structure of each ligand was obtained in 'sdf' format from the PubChem (https://pubchem.ncbi.nlm.nih.gov/) and geometrically optimized using Frog2.[36] The cocrystal ligand was used to define the docking grid box x, y, z coordinates and sizes with 0.375 Å spacing using AutoDockTools 1.5.6 input file preparation program and docking was conducted using AutoDock 4.2.6 software (https:// https://autodock.scripps.edu/).[37] The docking protocol used was adopted from Refs. [38–41]. The docking scores were estimated, and 2D protein-ligand interaction diagrams were generated using Biovia DS Visualizer (Dassault Systèmes Biovia Software Inc, 2012).

Statistical Analysis

We conducted a one-way analysis of variance with Tukey's post-hoc test, with statistical significance defined as $p < 0.05$. We performed the statistical analysis using GraphPad version 9.0, and report the results as mean ± standard deviation ($n = 3$).

List of abbreviations

ABTS	2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
AChE	Acetylcholinesterase
BChE	Butyrylcholinesterase
DPPH	2,2-diphenyl-1-picrylhydrazyl

© 2023 Wiley-VHCA AG, Zurich, Switzerland

16121880, 2023, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.202201181 by Istanbul Arel University, Wiley Online Library on [02/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

CHEMISTRY & BIODIVERSITY

FRAP	Ferric reduction activity power
HPLC/MS	High-performance liquid chromatography-mass spectrometry
GAE	Gallic acid equivalent
RE	Rutin equivalent
TE	Trolox equivalent
EDTAE	EDTA equivalent
CUPRAC	Cupric reducing antioxidant capacity
GALAE	Galantamine equivalent
KAE	Kojic acid equivalent
ACAE	Acarbose equivalent
LOX	5-Lipoxgenase
ACE2	Angiotensin II Converting Enzyme

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

[1] H. M. Jaber, K. D. Al-Hamaideh, H. I. Al-Daghistani, N. H. Amer, M. N. Nassar, A. Al-Latif, M. Saleh, A. H. Al-Nuaimi, 'Antibacterial Activity and Chemical Composition of *Arum hygrophilum* Boiss Crude Extracts', *Jordan Journal of Biological Sciences* **2020**, *13*.

[2] V. Kochmarov, E. Kozuharova, Z. Naychov, G. Momekov, I. Mincheva, 'Ethnobotany and ethnopharmacology of *Arum maculatum* L.(Araceae) in Bulgaria with an emphasis on its effect against haemorrhoids', *Int J Pharm Chem Biol Sci* **2015**, *5*, 394–402.

[3] A. Azab, 'Arum: a plant genus with great medicinal potential', *European Chemical Bulletin* **2017**, *6*, 59–68.

[4] H. Al-Daghistani, L. Abu-Niaaj, Y. Bustanji, K. Al-Hamaideh, H. Al-Salamat, M. Nassar, H. Jaber, N. Amer, B. Abu-Irmaileh, A. Al-Nuaimi, 'Antibacterial and cytotoxicity evaluation of *Arum hygrophilum* Bioss', *European Review for Medical and Pharmacological Sciences* **2021**, *25*, 7306–7316.

[5] N. Jaradat, M. Abualhasan, 'Comparison of phytoconstituents, total phenol contents and free radical scavenging capacities between four *Arum* species from Jerusalem and Bethlehem', *Pharm. Sci.* **2016**, *22*, 120–125.

[6] N. Liang, D. D. Kitts, 'Role of chlorogenic acids in controlling oxidative and inflammatory stress conditions', *Nutrients* **2015**, *8*, 16.

[7] Y. Alan, 'Evaluation of phenolic substance content and biological activities of *Arum elongatum* steven extracts', *BEU J Sci* **2018**, *7*, 370–379.

[8] R. Manuja, S. Sachdeva, A. Jain, J. Chaudhary, 'A comprehensive review on biological activities of *p*-hydroxybenzoic acid and its derivatives', *Int. J. Pharm. Sci. Rev. Res* **2013**, *22*, 109–115.

[9] A. Sukito, S. Tachibana, 'Isolation of hyperoside and isoquercitrin from *Camellia sasanqua* as antioxidant agents', *Pakistan Journal of Biological Sciences: PJBS* **2014**, *17*, 999–1006.

[10] K. M. M. Espíndola, R. G. Ferreira, L. E. M. Narvaez, A. C. R. Silva Rosario, A. H. M. Da Silva, A. G. B. Silva, A. P. O. Vieira, M. C. Monteiro, 'Chemical and pharmacological aspects of caffeic acid and its activity in hepatocarcinoma', *Frontiers in oncology* **2019**, 541.

[11] F. U. Afifi, V. Kasabri, S. Litescu, I. F. Abaza, K. Tawaha, 'Phytochemical and biological evaluations of *Arum hygrophilum* boiss.(Araceae)', *Pharmacogn. Mag.* **2017**, *13*, 275.

[12] H. Uguzlar, E. Maltas, S. Yildiz, 'Antioxidant Activity and Fatty Acid Compositions of *Arum dioscoridis* Extracts', *Biosciences Biotechnology Research Asia* **2016**, *8*, 75–82.

[13] A. P. Murray, M. B. Faraoni, M. J. Castro, N. P. Alza, V. Cavallaro, 'Natural AChE Inhibitors from Plants and their Contribution to Alzheimer's Disease Therapy', *Curr. Neuropharmacol.* **2013**, *11*, 388–413.

[14] N. Ouattara, R. N.-T. Meda, A. Hilou, S. Guenné, K. Konaté, A. Y. Coulibaly, M. Kiendrébeogo, J. F. Millogo, O. G. Nacoulma, 'Anti-acetylcholinesterase and antioxidant activities and HPLC/MS analysis of polyphenol from extracts of *Nelsonia canescens* (Lam.) Spreng', *Asian Pacific Journal of Tropical Disease* **2013**, *3*, 382–388.

[15] P. M. Souza, S. T. Elias, L. A. Simeoni, J. E. de Paula, S. M. Gomes, E. N. S. Guerra, Y. M. Fonseca, E. C. Silva, D. M. Silveira, P. O. Magalhaes, 'Plants from Brazilian Cerrado with potent tyrosinase inhibitory activity', *PLoS One* **2012**, *7*, e48589.

[16] N. Laaraj, M. Bouhrim, L. Kharchoufa, S. Tiji, H. Bendaha, M. Addi, S. Drouet, C. Hano, J. M. Lorenzo, M. Bnouham, 'Phytochemical Analysis, α-Glucosidase and α-Amylase Inhibitory Activities and Acute Toxicity Studies of Extracts from Pomegranate (*Punica granatum*) Bark, a Valuable Agro-Industrial By-Product', *Food* **2022**, *11*, 1353.

[17] S. Kumar, S. Narwal, V. Kumar, O. Prakash, 'α-glucosidase inhibitors from plants: A natural approach to treat diabetes', *Pharmacognosy reviews* **2011**, *5*, 19.

[18] M. Farid, S. Hussein, A. Trendafilova, M. Marzouk, A. El Oqlah, M. Saker, 'Phytochemical constituents of the butanol fraction of *Arum palaestinum* Boiss.: cytotoxic and antiviral screening', *Mater. and Envir. Sci* **2017**, *8*, 2585–2591.

[19] R. Mashima, T. Okuyama, 'The role of lipoxygenases in pathophysiology; new insights and future perspectives', *Redox Biol* **2015**, *6*, 297–310.

[20] G. Zengin, A. Aktumsek, 'Investigation of antioxidant potentials of solvent extracts from different anatomical parts of *Asphodeline anatolica* E. Tuzlaci: An endemic plant to Turkey', *African Journal of Traditional, Complementary and Alternative Medicines* **2014**, *11*, 481–488.

[21] A. M. Mustafa, S. Angeloni, D. Abouelenein, L. Acquaticci, J. Xiao, G. Sagratini, F. Maggi, S. Vittori, G. Caprioli, 'A new

 © 2023 Wiley-VHCA AG, Zurich, Switzerland

16121880, 2023, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.202201181 by Istanbul Arel University, Wiley Online Library on [02/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

CHEMISTRY & BIODIVERSITY

HPLC/MS/MS method for the simultaneous determination of 36 polyphenols in blueberry, strawberry and their commercial products and determination of antioxidant activity', *Food Chem.* **2022**, *367*, 130743.

[22] S. Uysal, G. Zengin, M. Locatelli, M. B. Bahadori, A. Mocan, G. Bellagamba, E. De Luca, A. Mollica, A. Aktumsek, 'Cytotoxic and enzyme inhibitory potential of two *Potentilla* species (P. speciosa L. and P. reptans Willd.) and their chemical composition', *Front. Pharmacol.* **2017**, *8*, 290.

[23] D. M. Grochowski, S. Uysal, A. Aktumsek, S. Granica, G. Zengin, R. Ceylan, M. Locatelli, M. Tomczyk, 'In vitro enzyme inhibitory properties, antioxidant activities, and phytochemical profile of *Potentilla thuringiaca*', *Phytochem. Lett.* **2017**, *20*, 365–372.

[24] S. N. Biltekin, A. E. E. Karadagˇ, B. L. Demirci, F. Demirci, 'ACE2 and LOX Enzyme Inhibitions of Different Lavender Essential Oils and Major Components Linalool and Camphor', *ACS Omega* **2022**, *7*, 36561–36566.

[25] S. Baylac, P. Racine, 'Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts', *International Journal of Aromatherapy* **2003**, *13*, 138–142.

[26] F. Demirci, A. E. Karadağ, S. N. Biltekin, B. Demirci, 'In vitro ACE2 and 5-LOX inhibition of Rosmarinus officinalis L. Essential oil and its major component 1, 8-cineole', *Rec. Nat. Prod.* **2022**.

[27] O. Gerlits, K.-Y. Ho, X. Cheng, D. Blumenthal, P. Taylor, A. Kovalevsky, Z. Radić, 'A new crystal form of human acetylcholinesterase for exploratory room-temperature crystallography studies', *Chem.-Biol. Interact.* **2019**, *309*.

[28] T. Rosenberry, X. Brazzolotto, I. Macdonald, M. Wandhammer, M. Trovaslet-Leroy, S. Darvesh, F. Nachon, 'Comparison of the Binding of Reversible Inhibitors to Human Butyrylcholinesterase and Acetylcholinesterase: A Crystallographic, Kinetic and Calorimetric Study', *Molecules* **2017**, *22*.

[29] R. Maurus, A. Begum, L. K. Williams, J. R. Fredriksen, R. Zhang, S. G. Withers, G. D. Brayer, 'Alternative Catalytic Anions Differentially Modulate Human α-Amylase Activity and Specificity', *Biochemistry* **2008**, *47*, 3332–3344.

[30] P. Towler, B. Staker, S. G. Prasad, S. Menon, J. Tang, T. Parsons, D. Ryan, M. Fisher, D. Williams, N. A. Dales, M. A. Patane, M. W. Pantoliano, 'ACE2 X-Ray Structures Reveal a Large Hinge-bending Motion Important for Inhibitor Binding and Catalysis', *J. Biol. Chem.* **2004**, *279*, 17996–18007.

[31] N. C. Gilbert, J. Gerstmeier, E. E. Schexnaydre, F. Börner, U. Garscha, D. B. Neau, O. Werz, M. E. Newcomer, 'Structural and mechanistic insights into 5-lipoxygenase inhibition by natural products', *Nat. Chem. Biol.* **2020**, *16*, 783–790.

[32] L. Ielo, B. Deri, M. P. Germanò, S. Vittorio, S. Mirabile, R. Gitto, A. Rapisarda, S. Ronsisvalle, S. Floris, Y. Pazy, A. Fais, A. Fishman, L. De Luca, 'Exploiting the 1-(4-fluorobenzyl)piperazine fragment for the development of novel tyrosinase inhibitors as anti-melanogenic agents: Design, synthesis, structural insights and biological profile', *Eur. J. Med. Chem.* **2019**, *178*, 380–389.

[33] S. S. Karade, M. L. Hill, J. L. Kiappes, R. Manne, B. Aakula, N. Zitzmann, K. L. Warfield, A. M. Treston, R. A. Mariuzza, 'N-Substituted Valiolamine Derivatives as Potent Inhibitors of Endoplasmic Reticulum α-Glucosidases I and II with Antiviral Activity', *J. Med. Chem.* **2021**, *64*, 18010–18024.

[34] H. A. A. Omer, G. Caprioli, D. Abouelenein, A. M. Mustafa, A. I. Uba, G. Ak, R. B. Ozturk, G. Zengin, S. Yagi, 'Phenolic Profile, Antioxidant and Enzyme Inhibitory Activities of Leaves from Two *Cassia and* Two *Senna* Species', *Molecules* **2022**, *27*.

[35] G. Martínez-Rosell, T. Giorgino, G. De Fabritiis, 'PlayMolecule ProteinPrepare: A Web Application for Protein Preparation for Molecular Dynamics Simulations', *J. Chem. Inf. Model.* **2017**, *57*, 1511–1516.

[36] M. A. Miteva, F. Guyon, P. Tuffery, 'Frog2: Efficient 3D conformation ensemble generator for small compounds', *Nucleic Acids Res.* **2010**, *38*, W622-W627.

[37] G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, A. J. Olson, 'AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility', *J. Comput. Chem.* **2009**, *30*, 2785–2791.

[38] A. I. Uba, K. Yelekçi, 'Pharmacophore-based virtual screening for identification of potential selective inhibitors of human histone deacetylase 6', *Comput. Biol. Chem.* **2018**, *77*, 318–330.

[39] A. İ Uba, K. YelekÇİ, 'Exploration of the binding pocket of histone deacetylases: the design of potent and isoform-selective inhibitors', *Turk. J. Biol.* **2017**, *41*, 901–918.

[40] G. Zengin, S. Dall'Acqua, K. I. Sinan, A. I. Uba, S. Sut, G. Peron, O. K. Etienne, M. Kumar, C. L. Cespedes-Acuña, J. Alarcon-Enos, A. Mollica, M. F. Mahomoodally, 'Gathering scientific evidence for a new bioactive natural ingredient: The combination between chemical profiles and biological activities of *Flueggea virosa* extracts', *Food Bioscience* **2022**, *49*.

[41] G. Zengin, N. M. Fahmy, K. I. Sinan, A. I. Uba, A. Bouyahya, J. M. Lorenzo, E. Yildiztugay, O. A. Eldahshan, S. Fayez, 'Differential Metabolomic Fingerprinting of the Crude Extracts of Three Asteraceae Species with Assessment of Their In Vitro Antioxidant and Enzyme-Inhibitory Activities Supported by In Silico Investigations', *Processes* **2022**, *10*.

Received December 14, 2022
Accepted March 9, 2023

© 2023 Wiley-VHCA AG, Zurich, Switzerland

16121880, 2023, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/cbdv.202201181 by Istanbul Arel University, Wiley Online Library on [02/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License