Food and Chemical Toxicology 107 (2017) 597–608

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Bioactivities of *Achillea phrygia and Bupleurum croceum* based on the composition of phenolic compounds: *In vitro* and *in silico* approaches

Gokhan Zengin [a, *], Gizem Bulut [b], Adriano Mollica [c], Mehmet Zeki Haznedaroglu [d], Ahmet Dogan [b], Abdurrahman Aktumsek [a]

[a] Selcuk University, Science Faculty, Department of Biology, Campus, 42250, Konya, Turkey
[b] Marmara University, Pharmacy Faculty, Department of Pharmaceutical Botany, Istanbul, Turkey
[c] Department of Pharmacy, University "G. d'Annunzio" of Chieti-Pescara, 66100, Chieti, Italy
[d] Ege University, Pharmacy Faculty, Department of Pharmaceutical Botany, Izmir, Turkey

ARTICLE INFO

Article history:
Received 6 March 2017
Received in revised form
18 March 2017
Accepted 21 March 2017
Available online 22 March 2017

Keywords:
Achiella phrygia
Bupleurum croceum
Bioactive compounds
Natural agents
Turkey

ABSTRACT

This study presents the effects of the *Achillea phrygia* Boiss. et Bal. and *Bupleurum croceum* Fenzl. extracts obtained by different solvents (ethyl acetate, methanol and water) on selected enzyme inhibitory effects and antioxidant ability with screening bioactive compounds. Total and individual bioactive compounds were detected by spectrophotometric and HPLC-DAD techniques. Antioxidant abilities were evaluated by different methods including free radical scavenging (ABTS and DPPH), reducing power (CUPRAC and FRAP), phosphomolybdenum and metal chelating. Enzyme inhibitory effects were tested against cholinesterases, tyrosinase, amylase, glucosidase and lipase. Total phenolic contents were ranged from 20.52 mgGAE/g extract (*B. croceum* methanol extract) to 41.13 mgGAE/g extract (*A. phrygia* methanol extract). Generally, methanol and water extracts showed the strongest antioxidant abilities, while the ethyl acetate extracts had the most promising enzyme inhibitory effects. HPLC analysis revealed the abundance of some phenolics including rutin, quercetin, sinapic acid and chlorogenic acid, respectively. These components were also assessed using molecular modelling with the aim to study their docking properties on a set of six enzymes used in this study. Overall, these species could be suggested as valuable sources of natural-bioactive agents for developing new functional, pharmacological and health-promoting ingredients.

1. Introduction

Natural products have formed the basis of healthcare throughout the world since ancient times and still take attention in both traditional and current approaches. At this point, numerous studies demonstrate the potential of the use of natural products as therapeutic agents (Harvey, 2008). In this context, many phytochemicals are suggested as natural enzyme inhibitors for the treatment of common health problems including Alzheimer's disease, diabetes mellitus and obesity (Doughari, 2012; Lin et al., 2016; Morbidelli, 2016). Phytochemicals especially phenolic compounds are of paramount interest as natural antioxidants, which delay or prevent the negative effects of oxidative stress (Alu'datt et al., 2017). As a result, uninvestigated plants could be considered as a potential pool for discovering new pharmacological and therapeutic agents (Dezsi et al., 2015; Mocan et al., 2015a, 2015b, 2016a, 2016b, 2016c, Savran et al., 2016; Waltenberger et al., 2016).

Turkey flora is very rich with approximately 3000 medicinal and aromatic plants. *Achillea* L. (Asteraceae) is represented in the world by more than 100 species, centered in South West Asia and South East Europe with extensions through Eurasia to North America (Abdel-Rahman et al., 2015). The genus is represented in Turkey by 48 species belonging to 54 taxa, 24 of which endemic in Turkey (Aytaç et al., 2016). *Achillea* species are well known with local names "ayvadana, ayvadanası and civanperçemi" in Turkey (Tuzlaci, 2016). In traditional folk medicine, they are used for especially abdominal pain and stomach diseases (Applequist and Moerman, 2011; Sezik et al., 2001; Tetik et al., 2013). Capitulum of *Achillea phrygia* is used as appetizer, digestive, abdominal pain and nausea in West Anatolia (Deniz et al., 2010). *Bupleurum* L. is a genus of family Umbelliferae (Apiaceae), comprising about 200 species and primarily located in the Northern Hemisphere, Eurasia,

* Corresponding author.
E-mail address: gokhanzengin@selcuk.edu.tr (G. Zengin).

http://dx.doi.org/10.1016/j.fct.2017.03.037
0278-6915/

and North Africa. The genus *Bupleurum* L. comprises 49 taxa in Turkey, of which 21 taxa are endemic (Davis, 1982; Güner et al., 2000). Some *Bupleurum* members are used in the treatment of various ailments, such as influenza, fever, and malaria, in folk medicine (Ashour and Wink, 2011; Pan, 2006; Saraçoğlu et al., 2012). Many biological and chemical studies were performed on both these genera due to the wide variety of well-known uses (Anvari et al., 2016; Ashour et al., 2012, 2014; Hichri et al., 2015; Nan et al., 2014; Radulović et al., 2015; Turkmenoglu et al., 2015; Tušek et al., 2016; Villanueva-Bermejo et al., 2017; Zengin et al., 2017a). However, no information is available on the biological and chemical fingerprints of *Achillea phrygia* Boiss. et Bal. and *Bupleurum croceum* Fenzl. Regarding this, the goals of the current work were (i) to determine total and individual phenolic components of three extracts (ethyl acetate, methanol and water) from these species (ii) to evaluate antioxidant properties by different chemical methods (iii) to examine enzyme inhibitory effects against cholinesterases tyrosinase, amylase, glucosidase and lipase (iv) to determine possible interactions phenolics and enzyme inhibitory effects by *in silico* studies. The obtained results can trigger new insights on the species to assess uses in pharmacological applications.

2. Materials and methods

2.1. Plant materials

Plant material was collected during the flowering period in 2013 and 2014 from Denizli (Acipayam), Turkey. The information's for these species were reported below. Plant materials were identified by Dr. Gizem Bulut and Prof. Dr. Ertan Tuzlaci. Voucher specimens have been deposited at the Herbarium of the Faculty of Pharmacy, Marmara University (MARE), Turkey.

Achillea phrygia Boiss. et Bal. Denizli, Acipayam, between Dedebag and Bedirkoy, 1560 m, 08.06.2014. MARE 17027.

Bupleurum croceum Fenzl. Denizli, Acipayam, Kelekci village, 850 m, 03.07.2013. MARE 16062.

2.2. Total phenolics, flavonoids and phenolic composition

The total phenolics content was determined by Folin-Ciocalteu method (Slinkard and Singleton, 1977; Zengin et al., 2016) with slight modification and expressed as gallic acid equivalents (GAEs/g extract), while total flavonoids content was determined by $AlCl_3$ method (Locatelli et al., 2017; Zengin et al., 2014) with slightly modification and expressed as rutin equivalents (REs/g extract).

Phenolic compounds were evaluated by RP-HPLC (Shimadzu Scientific Instruments, Tokyo, Japan). Detection and quantification were carried out with a LC-10ADvp pump, a Diode Array Detector, a CTO-10Avp column heater, SCL-10Avp system controller, DGU-14A degasser and SIL-10ADvp auto sampler (Shimadzu Scientific Instruments, Columbia, MD). Separations were conducted at 30 °C on Agilent® Eclipse XDB C-18 reversed-phase column (250 mm × 4.6 mm length, 5 μm particle size). Phenolic compositions of the extracts were determined by Zengin et al. (2014). Gallic acid, protocatechuic acid, (+)-catechin, *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid, (−)-epicatechin, syringic acid, vanillin, *p*-coumaric acid, ferulic acid, sinapic acid, benzoic acid, *o*-coumaric acid, rutin, hesperidin, rosmarinic acid, eriodictyol, *trans*-cinnamic acid, quercetin, luteolin, kaempferol and apigenin were used as standards. Identification and quantitative analysis were done by comparison with standards. The amount of each phenolic compound was expressed as microgram per gram of extract using external calibration curves, which were obtained for each phenolic standard.

2.3. Biological activities evaluation

Antioxidant (radical scavenging (ABTS and DPPH), reducing power (CUPRAC and FRAP), phosphomolybdenum, metal chelating (ferrozine method)) and enzyme inhibitory activities (cholinesterase (Elmann's method), tyrosinase (dopachrome method), α-amylase (iodine/potassium iodide method), α-glucosidase (chromogenic PNPG method) and pancreatic lipase (*p*-nitrophenyl butyrate (*p*-NPB) method) determined by the method described by Zengin et al. (2017b).

The antioxidant abilities were expressed as equivalents of trolox (EDTA was used as a standard for evaluating metal chelating activity). The enzyme inhibitory activities of the extracts were obtained as equivalents of standard drugs per gram of the plant sample (galantamine for AChE and BChE, kojic acid for tyrosinase, orlistat for lipase, and acarbose for α-amylase and α-glucosidase inhibition assays).

2.4. Molecular modelling

2.4.1. Receptors preparation

All the crystallographic enzyme structures have been downloaded from the Protein Data Bank RCSB PDB (Berman et al., 2000): acetylcholinesterase (pdb:4 × 3C) (Pesaresi and Lamba) in complex with tacrine-nicotinamide hybrid inhibitor, butyrilcholinesterase (pdb:4BDS) (Nachon et al., 2013) in complex with tacrine, α-amylase (pdb:1VAH) (Zhuo et al., 2004) in complex with *r*-nitrophenyl-α-D-maltoside, α-glucosidase (pdb:3AXI) (Yamamoto et al., 2011) in complex with maltose pancreatic lipase (PDB: 1LPB) (Egloff et al., 1995) in complex with C11-alkyl phosphonate and tyrosinase (pdb:2Y9X) (Ismaya et al., 2011) in complex with tropolone. The enzymes have been prepared for docking by removing the non-catalytic waters, the inhibitors and all the other molecules present in the pdb files using Pymol (DeLano, 2002). The proteins alone were neutralized at pH 7.4 by PROPKA, seleno-cysteines and seleno-methionines, if present, were converted respectively to cysteines and methionines. All the missing fragments and other errors present in the crystal structures were automatically solved by the Wizard Protein Preparation implemented in Maestro 10.2 suite (Maestro, 2011).

2.4.2. Ligands preparation

Chlorogenic acid, sinapic acid, rutin, quercetin, luteolin and apigenin, were selected as representative compounds to carry out molecular docking studies, as these compounds were present in abundance in the A. *phrygia* and *B. croceum* extracts. The chemical structures, reported in Fig. 1, have been downloaded from Zinc databases (Irwin et al., 2012) and used for molecular modelling experiments after preparation. The ligands were prepared by the LigPrep tool embedded in Maestro 10.2, neutralized at pH 7.4 by Epik and minimized by force field OPLS3 (Shelley et al., 2007).

2.4.3. Molecular docking

Dockings of the selected substances have been performed for each enzyme employed for the *in vitro* enzymatic inhibition tests in this work. Glide (Friesner et al., 2006) has been employed for the docking calculations by using eXtra Precision (XP) scoring function for all the enzymes, with the exception of the docking to tyrosinase; in this case, Gold 6.0 (Jones et al., 1997) was used with the scoring function GoldScore, which has been previously found to be more suitable to produce reliable poses on metal containing enzymes (Mocan et al., 2016c). In both cases, the binding pocket was determined automatically by centering the grid on the crystallographic inhibitor, extended in a radius of 10 Å from the center. The best pose for each compound docked to the selected enzymes was the best

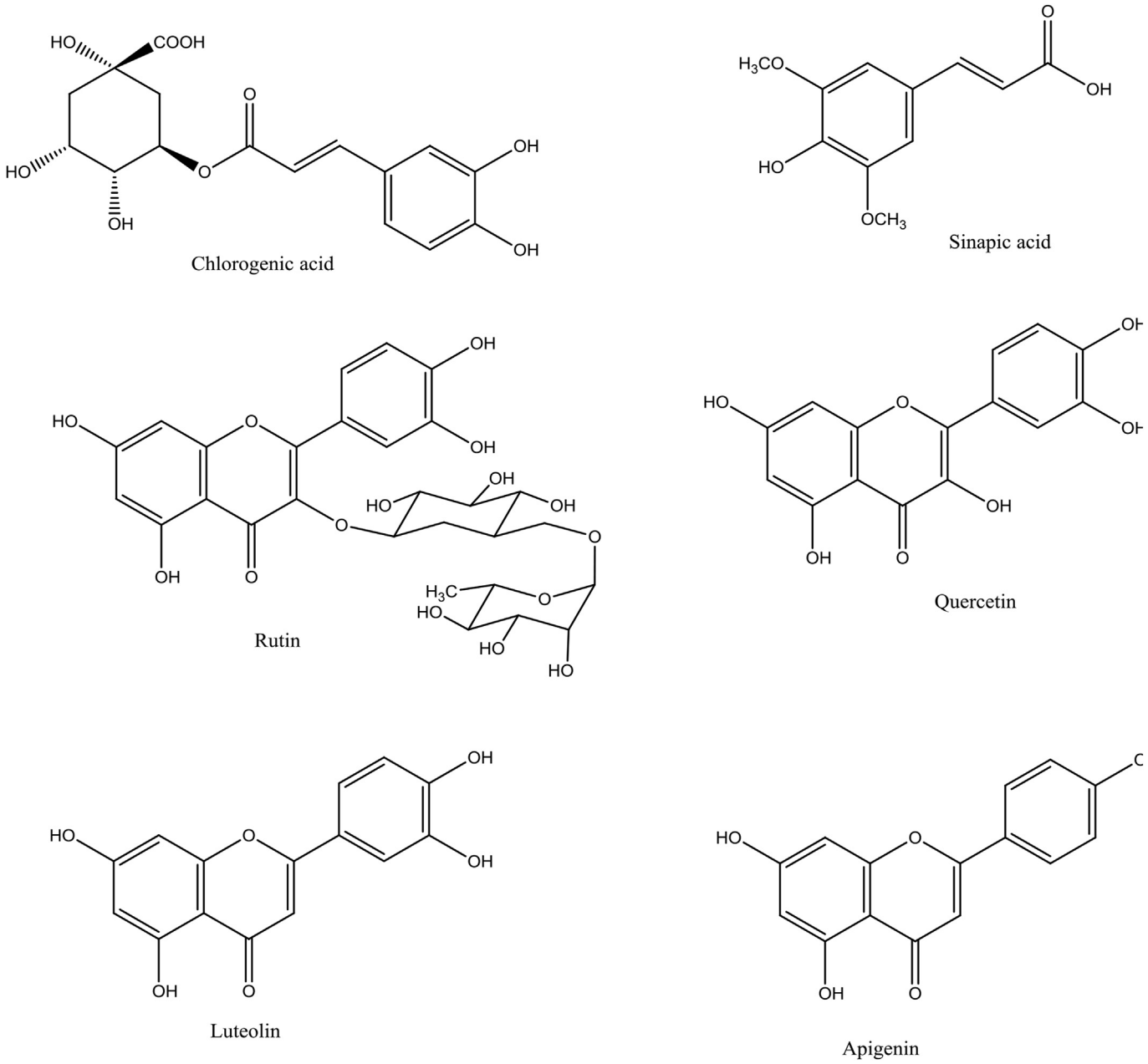

Fig. 1. Chemical structure of Chlorogenic acid, Sinapic acid, Rutin, Quercetin, Luteolin, and Apigenin.

ranked among the 200 generated.

2.5. Statistical analysis

All the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The differences between the different extracts were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference post hoc test with $\alpha = 0.05$. This treatment was carried out using SPSS v. 14.0 program.

3. Results and discussion

3.1. Bioactive composition

Phenolic components have diverse biological activities such as antioxidant, antimicrobial and anticancer. Thus, total and individual phenolic compounds were detected in the present study. Among *A. phrygia* extracts, the methanol extract had the highest total phenolic content with 41.13 mgGAE/g extract, followed by water (35.53 mgGAE/g extract) and ethyl acetate (23.62 mgGAE/g extract) extracts (Table 1). Regarding *B. croceum* extracts, the water extract contains more phenolics as compared to methanol and ethyl acetate extracts. However, the total phenolic content of *B. croceum* methanol extract was very close to that of ethyl acetate extract. Flavonoids have positive impact on human health. As shown in Table 1, the total flavonoid content was highest in the both methanol extracts. In addition, the water extracts contained less flavonoids than ethyl acetate extracts. Taken together, the type of solvent used affects the amount of total bioactive compounds in the extracts studied. Similar findings were reported by some authors (Kallel et al., 2014; Lou et al., 2016).

The results obtained for the polyphenolic components are presented in Table 2. Among the selected phenolics, the *A. phrygia*

Table 1
Total phenolic and flavonoid content and free radical scavenging activity (DPPH and ABTS assays) of the studied extracts.

Species	Extracts	Total phenolic content (mgGAE/g extract)	Total flavonoid content (mgRE/g extract)	DPPH scavenging activity (mgTE/g extract)	ABTS scavenging activity (mgTE/g extract)
A. phrygia	Ethyl acetate	23.62 ± 0.20[a]	14.67 ± 0.32	20.56 ± 0.29	124.64 ± 0.51
	Methanol	41.13 ± 0.74	21.73 ± 1.45	109.34 ± 0.29	329.02 ± 2.07
	Water	35.53 ± 0.81	7.26 ± 0.06	87.27 ± 1.33	360.55 ± 6.46
B. croceum	Ethyl acetate	20.85 ± 0.42	18.56 ± 0.10	33.92 ± 3.03	154.68 ± 2.22
	Methanol	20.52 ± 0.28	22.75 ± 0.30	47.42 ± 0.84	217.91 ± 4.52
	Water	23.50 ± 0.18	12.06 ± 0.13	65.51 ± 1.44	282.51 ± 2.02

[a] Values expressed are means ± S.D. of three parallel measurements. GAE, gallic acid equivalents; RE, rutin equivalents; TE, trolox equivalents.

extracts studied were characterized by the high level of chlorogenic acid, sinapic acid, rutin, quercetin, luteolin and apigenin. Interesting differences in the amounts of each compound were observed. Sinapic acid was the major component in the methanol extract, while chlorogenic acid was the main constituent in the water extract. Our findings were confirmed by several researchers, who reported the higher levels of flavonoids in some *Achillea* species (Jesionek et al., 2015; Tuberoso et al., 2009). Also, Agar et al. (2015) and Vitalini et al. (2011) found chlorogenic acid and its derivatives in the genus *Achillea*.

Concerning *B. croceum* extracts, the predominant components were rutin and quercetin. The level of rutin in the water extract was higher than methanol and ethyl acetate extracts, whereas the highest content of quercetin was observed in the ethyl acetate extract. These extracts also contained important amounts of *p*-hydroxybenzoic acid, gallic acid, epicatechin and kaempferol. However, hesperidin, rosmarinic acid and eridictyol were not identified in both *A. phrygia* and *B. croceum* extracts. Some *Bupleurum* extracts were subjected to different works in order to characterize their phytochemical profile and in accordance with our results, rutin was major flavonoid in these works (Ashour and Wink, 2011; Barrero et al., 1998; Benahmed et al., 2014; Zhang et al., 2007).

3.2. Antioxidant capacity

DPPH and ABTS assays widely used to evaluate free radical scavenging activity of plant extracts or compounds. These assays also reflected electron-donating abilities of antioxidants. The transformation of radical forms to non-radicals in these assays are spectrophotometrically measured (517 nm for DPPH and 734 nm for ABTS). The scavenging effect of each extracts on DPPH and ABTS is given in Table 1. The methanol and water extracts revealed stronger free radical scavenging abilities in these assays as compared to ethyl acetate extracts. Among extracts studied, the methanol extract of *A. phrygia* exhibited the highest DPPH scavenging ability with 109.34 mgTE/g extract. The ABTS radical scavenging activities were also sorted: *A. phrygia* water extract > *A. phrygia* methanol extract > *B. croceum* water extract > *B. croceum* methanol extract. The obtained results may be explained with the higher concentration of phenolic components in these extracts. Similarly, a linear correlation between total phenolic content and free radical scavenging activity was reported by some researchers (Amessis-Ouchemoukh et al., 2017; Samoticha et al., 2017).

Reducing power is considered as a remarkable marker in the antioxidant mechanism. CUPRAC and FRAP assays were performed to evaluate reducing abilities and the results are depicted in Table 3. Generally, the methanol and water extracts had the strongest reducing abilities in these assays. Regarding CUPRAC and FRAP results, the results are similar to DPPH and ABTS, with the methanol and water extracts of *A. phrygia* showing the best potential. At this point, the observed activity might be attributed to the presence of phenolic compounds and this observation was confirmed that a strongly positive correlation was also found between total phenolic content and reducing abilities. Interestingly, CUPRAC results for *B. croceum* extracts were very close to each other, while the water

Table 2
Phenolic components in the solvent extracts from *Achillea phrygia* and *Bupleurum croceum* (μg/g extract) (mean ± SD)*.

No	Phenolic Components	*A. phrygia-EA*	*A. phrygia -Met*	*A. phrygia -Wat*	*B. croceum - EA*	*B. croceum - Met*	*B. croceum - Wat*
1	Gallic acid	nd	nd	54 ± 1	26 ± 0.4	182 ± 8	134 ± 8
2	Protocatechuic acid	26 ± 0.4	68 ± 0.6	66 ± 1	96 ± 1	50 ± 0.6	72 ± 0.6
3	(+)- Catechin	nd	nd	150 ± 2	42 ± 2	74 ± 2	nd
4	*p*-Hydroxybenzoic acid	16 ± 0.6	54 ± 0.6	nd	146 ± 4	60 ± 1	402 ± 6
5	Chlorogenic acid	222 ± 2	3524 ± 102	2088 ± 16	32 ± 2	98 ± 2	108 ± 2
6	Caffeic acid	20 ± 0.4	84 ± 4	54 ± 0.4	98 ± 4	94 ± 4	28 ± 0.04
7	(−)- Epicatechin	82 ± 6	134 ± 6	nd	136 ± 6	292 ± 6	94 ± 6
8	Syringic acid	42 + 0.2	80 ± 4	80 ± 4	10 ± 0.2	nd	20 ± 0.2
9	Vanilin	nd	nd	nd	6 ± 0.1	12 ± 0.2	nd
10	*p*- Coumaric acid	16 ± 0.1	70 ± 2	74 ± 2	60 ± 2	50 ± 2	40 ± 2
11	Ferulic acid	54 ± 0.4	396 ± 6	nd	88 ± 0.4	60 ± 0.4	60 ± 0.4
12	Sinapic acid	356 ± 2	5072 ± 22	nd	64 ± 2	50 ± 2	72 ± 2
13	Benzoic acid	nd	nd	nd	46 ± 0.6	160 ± 1	nd
14	*o*-Coumaric acid	28 ± 0.1	84 ± 2	nd	nd	nd	nd
15	Rutin	nd	784 ± 18	472 ± 14	500 ± 16	3500 ± 144	3686 ± 146
16	Hesperidin	nd	nd	nd	nd	nd	nd
17	Rosmarinic acid	nd	nd	nd	nd	nd	nd
18	Eriodictyol	nd	nd	nd	nd	nd	nd
19	*trans*-Cinnamic acid	14 ± 1	42 ± 1	24 ± 1	14 ± 1	24 ± 1	88 ± 10
20	Quercetin	386 ± 3	nd	nd	1388 ± 22	1144 ± 42	446 ± 6
21	Luteolin	428 ± 8	422 ± 8	nd	nd	nd	nd
22	Kaempferol	nd	nd	244 ± 4	130 ± 4	120 ± 4	152 ± 4
23	Apigenin	742 ± 18	394 ± 10	nd	nd	nd	nd

nd: not determined. EA: ethyl acetate; Met: methanol; Wat: water.

Table 3
Reducing powers (CUPRAC and FRAP), total antioxidant capacity (by phosphomolybdenum assay) and metal chelating activity of the studied extracts.

Species	Extracts	CUPRAC (mgTE/g extract)	FRAP (mgTE/g extract)	Phosphomolybdenum (mmol TE/g extract)	Metal chelating activity (mgEDTAE/g extract)
A. phrygia	Ethyl acetate	67.47 ± 5.01[a]	52.03 ± 2.00	1.99 ± 0.11	16.97 ± 2.80
	Methanol	175.51 ± 3.66	129.90 ± 2.51	1.61 ± 0.05	16.47 ± 1.23
	Water	140.35 ± 2.74	130.64 ± 1.05	1.17 ± 0.03	16.13 ± 0.07
B. croceum	Ethyl acetate	87.99 ± 1.22	64.67 ± 1.01	1.49 ± 0.09	18.81 ± 2.72
	Methanol	85.80 ± 2.72	72.62 ± 1.16	0.89 ± 0.07	9.52 ± 1.12
	Water	85.15 ± 2.01	87.92 ± 1.74	0.73 ± 0.02	15.88 ± 0.14

[a] Values expressed are means ± S.D. of three parallel measurements. TE, trolox equivalents; EDTAE, EDTA equivalents.

Table 4
Enzyme inhibitory effects of the studied extracts.

Species	Extracts	AChE inhibition (mgGALAE/g extract)	BChE inhibition (mgGALAE/g extract)	Tyrosinase inhibition (mgKAE/g extract)	Amylase inhibition (mmolACAE/g extract)	Glucosidase inhibition (mmolACAE/g extract)	Lipase inhibition (mgOE/g extract)
A. phrygia	Ethyl acetate	1.71 ± 0.07[a]	1.70 ± 0.19	13.05 ± 4.05	0.69 ± 0.03	1.93 ± 0.38	72.73 ± 6.90
	Methanol	1.50 ± 0.05	0.79 ± 0.07	23.06 ± 2.60	0.52 ± 0.02	6.13 ± 0.21	na
	Water	na	na	31.25 ± 0.65	0.13 ± 0.01	4.38 ± 0.23	na
B. croceum	Ethyl acetate	1.73 ± 0.09	1.80 ± 0.14	29.64 ± 1.02	0.72 ± 0.05	2.92 ± 0.16	110.04 ± 6.49
	Methanol	1.43 ± 0.07	1.01 ± 0.08	17.33 ± 0.26	0.52 ± 0.03	2.69 ± 0.04	23.91 ± 3.71
	Water	na	na	47.33 ± 2.72	0.10 ± 0.01	2.33 ± 0.39	na

[a] Values expressed are means ± S.D. of three parallel measurements. GALAE, galanthamine equivalents; ACAE, acarbose equivalents; KAE, kojic acid equivalents; OE, orlistat equivalents; na, not active.

extract of *B. croceum* has the highest FRAP value. The difference observed for *B. croceum* extracts in CUPRAC (both hydrophilic and lipophilic antioxidants) and FRAP assay (hydrophilic antioxidants) may be explained with the physicochemical nature of antioxidants in these tests.

Phosphomolybdenum method is considered a total antioxidant

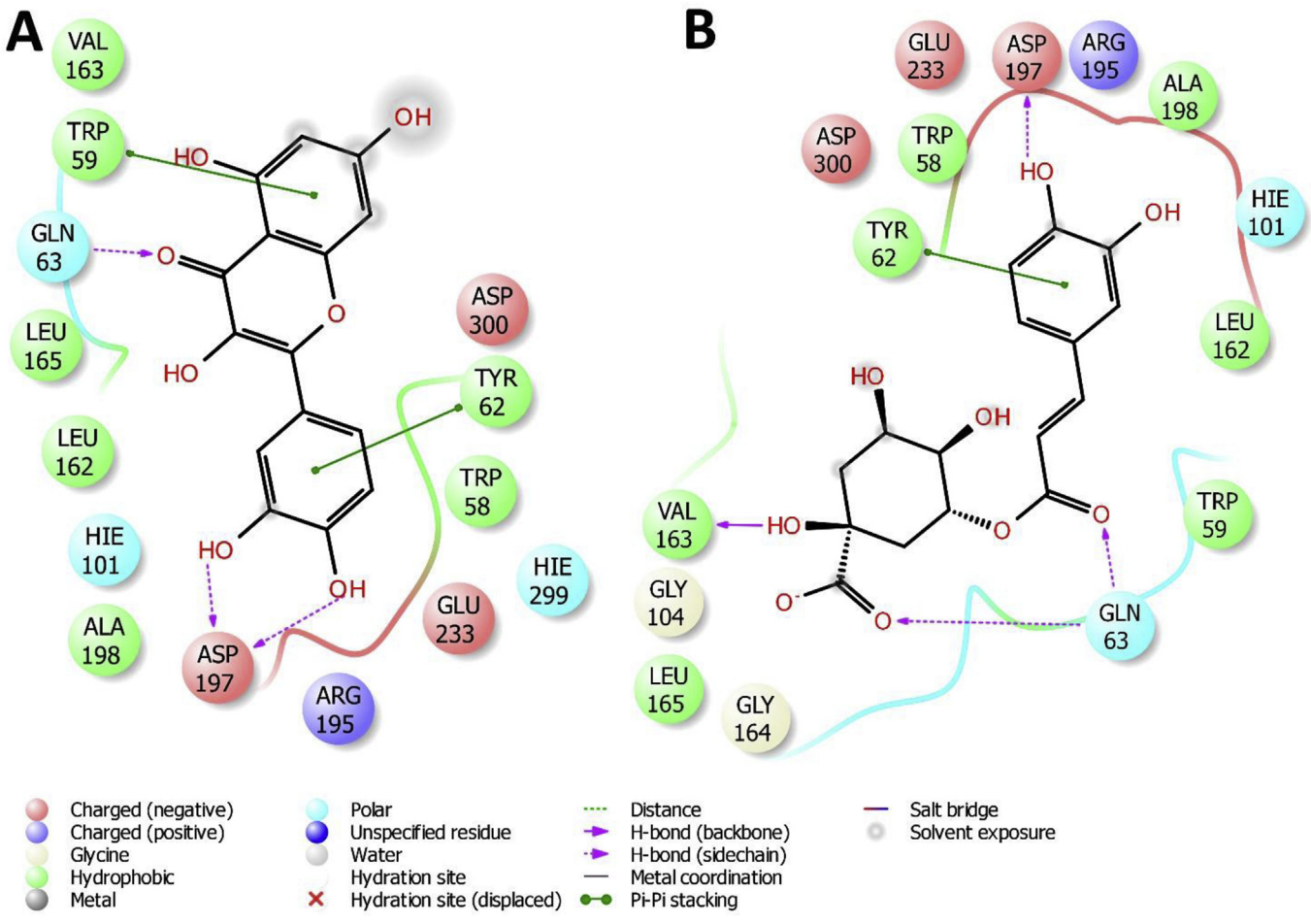

Fig. 2. 2D interactions of the best pose found for (A) Quercetin and (B) Chlorogenic Acid docked to α-amylase.

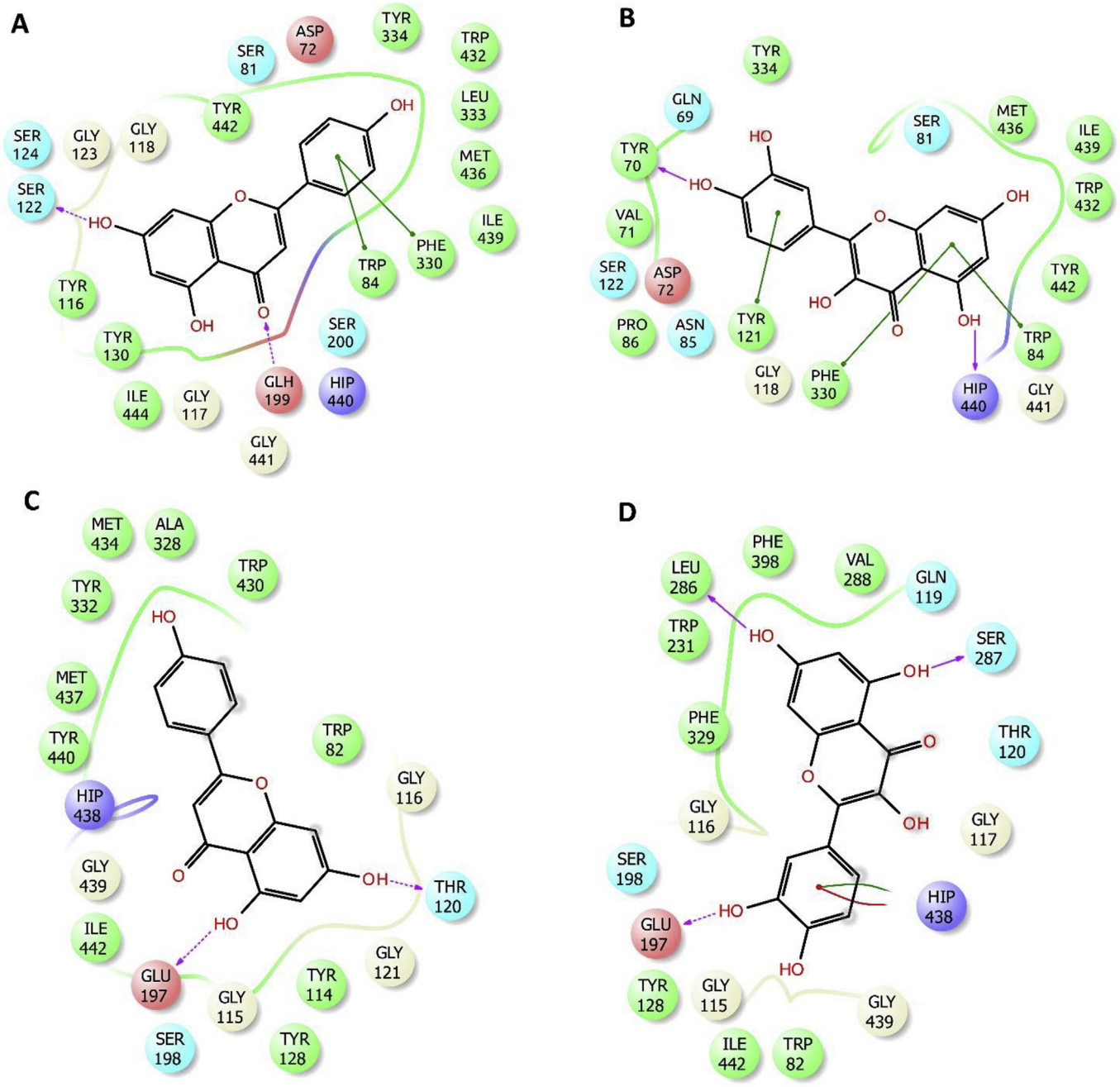

Fig. 3. 2D interactions of the best pose found for (A) Apigenin, (B) Quercetin docked to AChE and of (C) Apigenin and (D) Quercetin docked to BChE.

capacity assay and it is based on the reduction of Mo(VI) to Mo (V) by the antioxidants and then green phosphate/Mo(V) complex is formed. The complex has maximal absorbance at 695 nm. The total antioxidant capacity of the tested extracts was evaluated using the phosphomolybdenum assay, the results being depicted in Table 3. In *A. phrygia* and *B. croceum* extracts, the highest values were noted for the ethyl acetate extracts, which had the lowest total phenolics. On the other hand, the water extracts exhibited the weakest activity. Based on these results, the observed remarkable activities for ethyl acetate extracts could be due to non-phenolic antioxidants such as ascorbic acid or tocopherol. The results are consistent with previous reports that showed a weak correlation between total phenolic content and phosphomolybdenum assay (Ceylan et al., 2016; Chahmi et al., 2015).

Transition metals can produce free radicals (hydroxyl radical) and thus promote lipid peroxidation by Fenton reaction. In this perspective, chelation of transition metals could be envisaged due to its importance was in the antioxidant mechanisms. For this reason, the metal chelating abilities of extracts studied were detected by ferrozine method and the results are summarized in Table 3. Similar to phosphomolybdenum assay, the ethyl acetate extracts displayed the strongest metal chelating abilities. However, the activities for *A. phrygia* extracts were very close to each other. Our findings can be explained with the presence of non-phenolic chelators such as citric acid and polypeptides in the ethyl acetate extracts. From this point, some authors have claimed that metal chelating ability has minor impact on the overall antioxidant mechanism of phenolic compounds (Rice-Evans et al., 1996). In

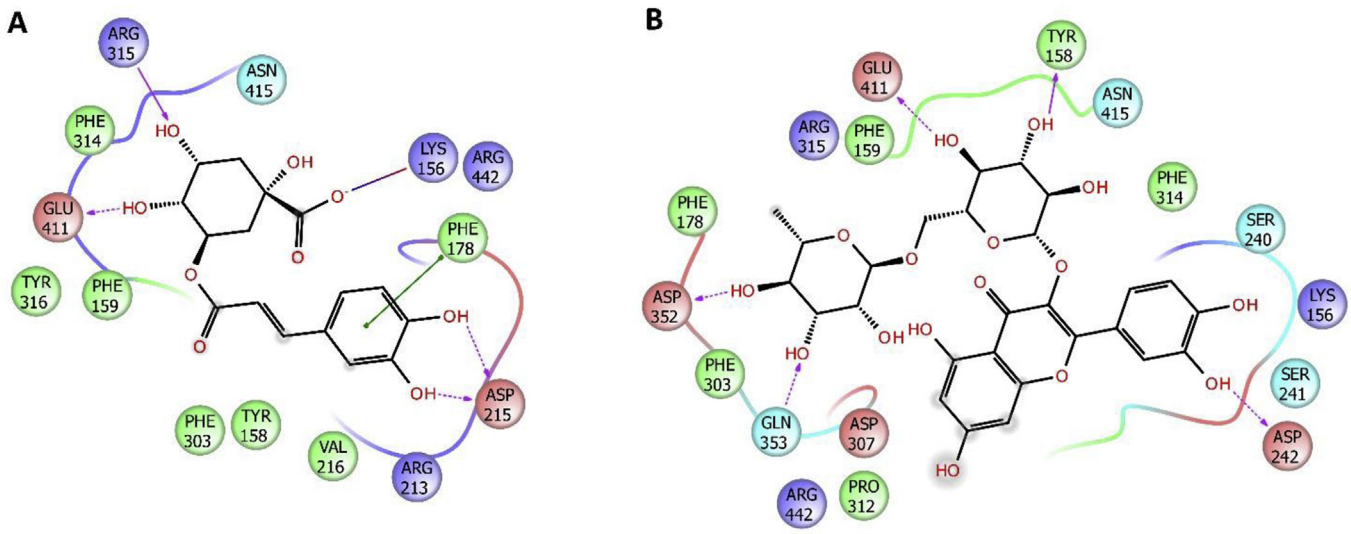

Fig. 4. 2D interactions of the best pose found for (A) Chlorogenic acid and (B) Rutin docked to α-glucosidase.

summary, the antioxidant picture reflected that antioxidant effects significantly depend on the type of solvent used.

3.3. Enzyme inhibitory potentials

The prevalence of global health problems including Alzheimer's disease (AD), diabetes mellitus (DM) and obesity has dramatically increased worldwide. For example, 46 million people affected with AD and this expected to multiply 3-fold (about 131 million) by 2050 (ADI, 2015). Also, about 1.9 billion adults have obesity in 2014 and the worldwide prevalence is more than doubled since 1980 (Patel, 2015). Thus, more attention is needed on the development of novel therapeutic strategies and the inhibition of key enzymes involved in metabolic process is one of the most appealing approach. According to this theory, inhibition of key enzymes (cholinesterases for AD; carbohydrate-hydrolyzing enzymes (amylase and glucosidase) for DM, tyrosinase for skin disorders and lipase for obesity) could help to alleviate the symptoms of above-mentioned diseases. From this point of view, several synthetics (galatamine for cholinesterase; acarbose for amylase and glucosidase; kojic acid for tyrosinase; and orlistat for lipase) are chemically produced as inhibitors but most of them have unpleasant side effects such as gastrointestinal disturbances and toxicities (Giacobini, 2004; Sridhar et al., 2017; Xie et al., 2016). Thus, the discovery of safe and effective inhibitors from natural sources is to great importance all over the world.

The enzyme inhibitory effects of extracts studied were tested against cholinesterase (AChE and BChE), tyrosinase, amylase, glucosidase and pancreatic lipase. The results are disposed in Table 4. The ethyl acetate extract of *B. croceum* was the most active and the ethyl acetate extracts exhibited stronger AChE and BChE inhibitory effects as compared to the methanol extracts. However, the water extracts were not active on cholinesterases. This finding may be linked to non-phenolic inhibitors such as alkaloids in the ethyl acetate extracts. Our findings were supported by some researchers who reported no correlation between total phenolic and anti-cholinesterase effects (Russo et al., 2015; Samaradivakara et al., 2016). Regarding tyrosinase inhibitory effects, the best activities were obtained by water extracts (47.33 mgKAE/g extract for *B. croceum* and 31.25 mgKAE/g extract for *A. phrygia*). This activity might be associated with the higher concentrations of phenolic compounds in these extracts. Some phenolics (kaempferol, cholorogenic acid, etc.) could chelate copper in the active site of tyrosinase (Kim and Uyama, 2005) and thus the enzyme inhibited. The ethyl acetate extract of *A. phrygia* had also the least activity with 13.05 mgKAE/g extract. In contrast to tyrosinase inhibition, the ethyl acetate extracts exhibited very attractive α-amylase inhibitory abilities (0.72 mmolACAE/g extract for *B. croceum* and 0.69 mmolACAE/g extract for *A. phrygia*). The extracts had remarkable levels of apigenin (*A. phrygia* ethyl acetate) and quercetin (*B. croceum* ethyl acetate), which are known as amylase inhibitors. In addition, the water extracts were found to be the lowest α-amylase inhibition abilities. The methanol extract of *A. phrygia* exhibited the superior α-glucosidase inhibitory effects, while *B. croceum* extracts were very close to each other. Three extracts were active on lipase and it can be ranked as *B. croceum* ethyl acetate > *A. phrygia* ethyl acetate > *B. croceum* methanol. Similar to amylase inhibition, the observed lipase activity may be explained with the presence of apigenin and quercetin. These results corroborate earlier reports that the phenolics can inhibit lipase (Gatto et al., 2002; Guo et al., 2016). To the best our knowledge, the enzyme inhibitory activities of *A. phrygia* and *B. croceum* are reported for the first time in this study. In this regard, the current work can provide new insights on these species.

3.4. Molecular modelling

Enzymatic assays performed on the extracts of *A. phrygia* evidenced overall, a low inhibition activity to cholinesterases and α-amylase, a good inhibition of tyrosinase of α-glucosidase and very good on Lipase, whereas the *B. croceum* extracts have shown an analogous pattern, with low inhibition activity to cholinesterases and α-amylase, medium on α-glucosidase and relevant toward tyrosinase and lipase. The best and most representative enzyme-ligand complexes interactions are reported in Figs. 2–6. Among the identified bioactive compounds, the most abundant compounds found in the extracts of *A. phrygia* were chlorogenic acid, sinapic acid, quercetin, rutin, apigenin and in lower amount ferulic acid, quercetin and luteolin. In the extracts of *B. croceum* the most abundant compounds found were rutin and quercetin, whereas chlorogenic acid, sinapic acid, apigein, ferulic acid and luteolin have been found in considerably lower amount.

As reported in literature, several flavonoids and polyphenols are capable to inhibit the cholinesterase enzymes (AChE and BChE), in

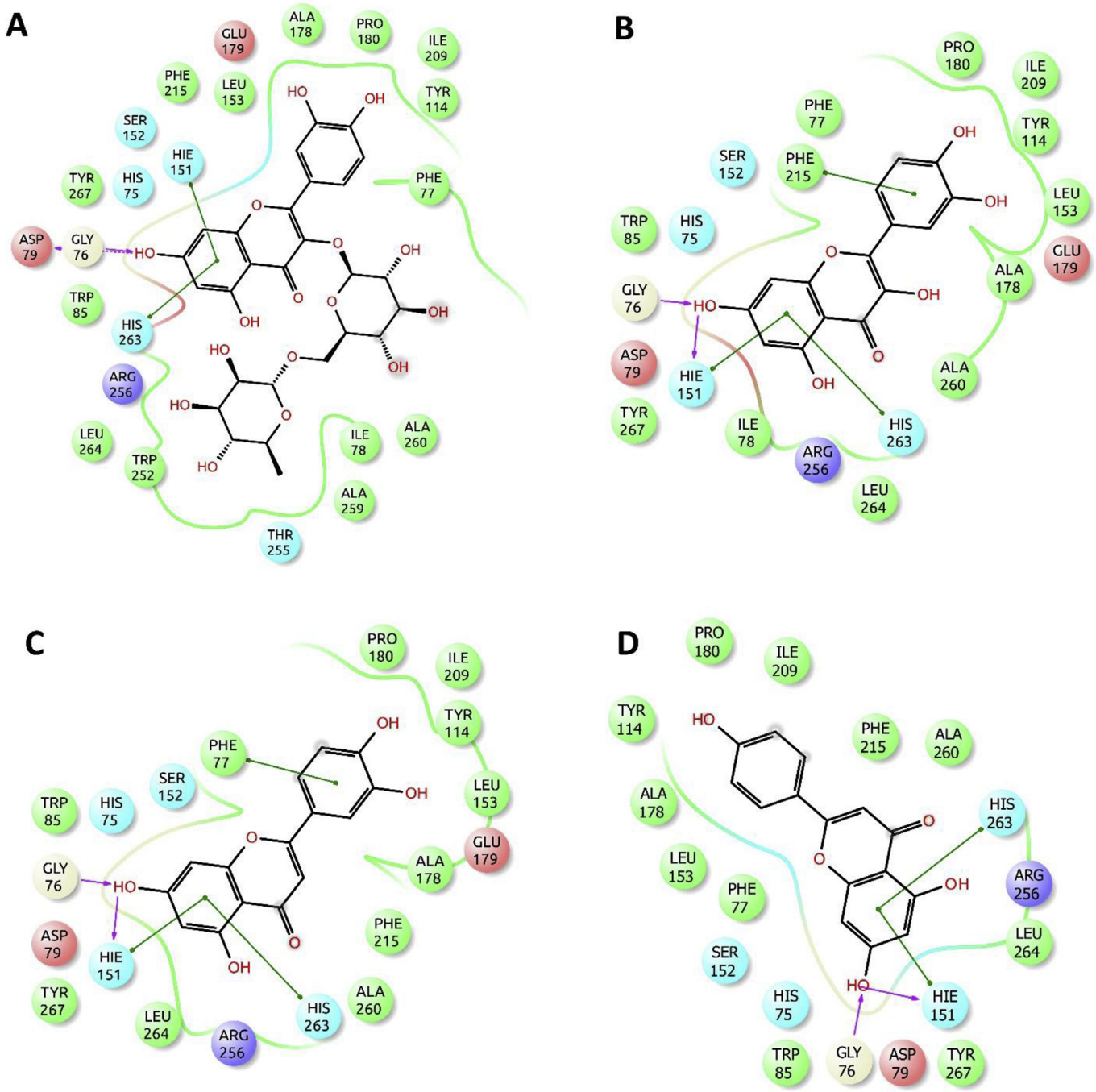

Fig. 5. 2D interactions of the best pose found for (A) Rutin, (B) Quercetin, (C) Luteolin and (D) Apigenin docked to pancreatic lipase.

this case the activity found for both herbs is modest and the activity can be attributed most of all to the presence of apigenin and quercetin, which have been previously reported to be moderate inhibitor of cholinesterases (Abdalla et al., 2013; Jung and Park, 2007; Katalinić et al., 2010, 2014; Xie et al., 2014). Also, the same consideration can be done for the α-amylases, indeed the activity found on this enzyme is overall weak, thus, it has been previously reported in literature that quercetin and chlorogenic acid are good inhibitors of this enzyme (Meng et al., 2016; Oboh et al., 2015).

On the other hand, we have found that the good inhibition activity toward α-glucosidase of the extracts of *A. phrygia* could be in part explained by the relevant presence of chlorogenic acid and rutin as previously reported (Li et al., 2009; Meng et al., 2016; Oboh et al., 2015) and the elevated activity on tyrosinase can be also attributed to the presence of chlorogenic acid and rutin. The remarkable activity on the inhibition of the pancreatic lipase could be due to the presence of apigenin, and quercetin (Lunagariya et al., 2014; Ong et al., 2016; Sudeep and Shyam Prasad, 2014). Also considering the relative abundance in the different extracts examined. With regards to the extracts of *B. croceum*, the major activity has been found on tyrosinase and lipase, both are inhibited by apigenin and sinapic acid (de Freitas et al., 2016; Ha et al., 2003; Li et al., 2014; Zhang et al., 2008).

In order to better understand the activity of the above

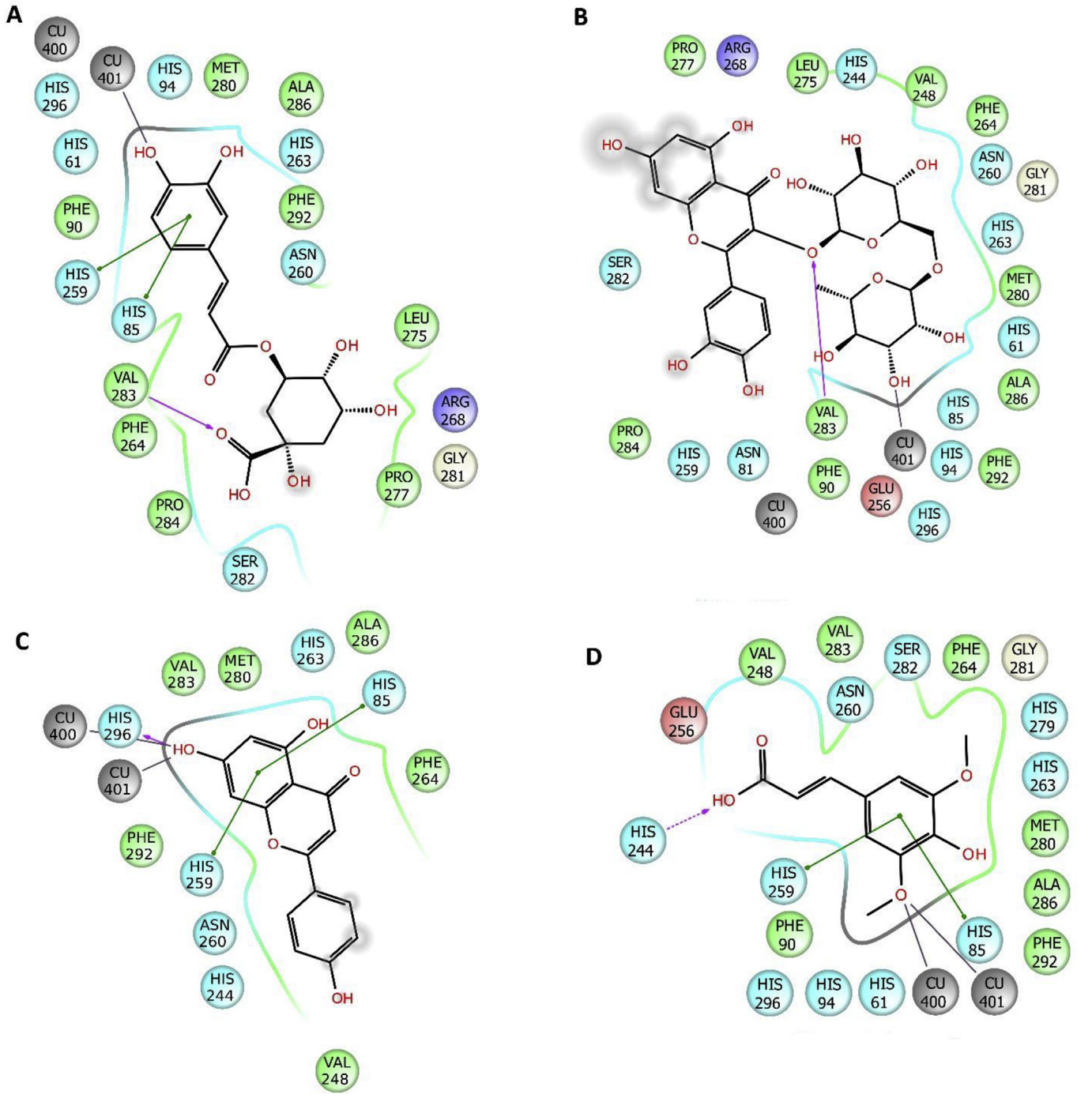

Fig. 6. 2D interactions of the best pose found for (A) Chlorogenic acid, (B) Rutin, (C) Apigenin and (D) Sinapic acid, docked to tyrosinase.

mentioned compounds on the target enzyme pool, molecular docking experiments have been conducted. The best docking pose obtained for quercetin on α-amylase is stabilized by three hydrogen bonds to Gln63, Asp197 and by two Pi-Pi interactions to Trp59 and Tyr62, whereas chlorogenic acid interacts to the cavity of α-amylase by forming four hydrogen bonds to Val163, Asp197, Gln63 and one Pi-Pi interaction with Tyr62, see Fig. 2.

The best docking pose for apigenin and quercetin on cholinesterases are reported on Fig. 3. Apigenin interacts with the residues present in the enzymatic cavity of AChE by forming two H bonds to Ser122 and Gln199 and two Pi-Pi interactions to the aromatic ring of Trp84 and Phe330. Apigenin interacts to the enzymatic cavity of BChE by forming two hydrogen bonds to Glu197 and Thr120. Quercetin interacts efficaciously to the enzymatic cavity of AChE by forming two hydrogen bonds with Tyr70 and His440 and three Pi-Pi interactions with Trp84, Phe330 and Tyr120, analogously to apigenin. Quercetin interacts to the cavity of BChE by forming three hydrogen bonds to Leu286, Ser287, Glu197 and two Pi-Pi interactions to His438.

Interesting interactions of chlorogenic acid and rutin have been also found in the docking with α-glucosidase. Chlorogenic acid interacts to the enzymatic cavity by forming four hydrogen bonds to Arg315, Glu411, Asp215 one Pi-Pi interaction to Phe178 and one saline bridge to Lys156. Rutin, is capable to establish several

hydrogen bonds to Glu411, Tyr158, Asp242, Gln353, Asp352. (Fig. 4).

The elevated activity toward pancreatic lipase could be explained by the presence of rutin, quercetin, luteolin and apigenin, their docking interactions to lipase are reported in Fig. 5. Rutin interacts to the enzymatic pocket of lipase by forming two hydrogen bonds to Asp79 and Gly76, and two Pi-Pi interactions to His151 and His263, quercetin interacts to the enzyme by forming two hydrogen bonds to Gly76 and His151 and three Pi-Pi interactions to Phe215, His151 and His263. Luteolin interacts to lipase by forming two hydrogen bonds to Gly76 and His151 and three Pi-Pi interactions to Phe77, His151, His263. Apigenin interacts in the same binding mode of luteolin (Fig. 6).

Tyrosinase, is strongly inhibited by chlorogenic acid, rutin, apigenin, and sinapic acid. These bioactive compounds are all capable to penetrate into the enzymatic cavity of tyrosinase and bind efficaciously to one or both copper atoms present in the cavity and also to the histidines that are coordinated to the metal atoms. Chlorogenic acid interacts into the cavity of tyrosinase with one hydrogen bond to Val283 and by two Pi-Pi stacks to the His259 and His85 and by binding to the Cu401. An analogue binding mode have been found for apigenin, whereas rutin binds to tyrosinase at Val283 and Cu401. Sinapic acid best poses possess, among the selected substances, the most efficacious interactions with tyrosinase, with one hydrogen bond to His244, two Pi-Pi interactions to His259 and His85 and by binding to both Cu atoms present in the cavity (Cu400 and Cu401), Fig. 6.

4. Conclusion

Natural bioactive agents play a significant role in the management of major health problems, including Alzheimer's disease, diabetes mellitus and cancer; the modern scientific studies on these agents have gained great interest in recent years. From this point of view, this study was designed to explore biological and chemical properties of *A. phrygia* and *B. croceum*. Generally, the methanol and water extracts showed a better antioxidant potentials with the higher levels of phenolics. However, the ethyl acetate extracts had remarkable enzyme inhibitory effects. Also, these extracts were very rich phenolic compounds and major components were docked to understand possible phenolic-enzyme interactions. This is the first detailed study on these species. From the obtained results, *A. phrygia* and *B. croceum* have a high potential for developing new functional and nutraceutical products as sources of natural-bioactive agents. Thus, the present study could be considered as a starting point for these species. However, additional investigations are necessary to evaluate their toxic effects of these species.

Acknowledgements

We thank Prof. Dr. Ertan Tuzlacı for identification of plant (*Bupleurum croceum*) and his valuable comments.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2017.03.037.

References

Abdalla, F.H., Cardoso, A.M., Pereira, L.B., Schmatz, R., Gonçalves, J.F., Stefanello, N., Fiorenza, A.M., Gutierres, J.M., da Silva Serres, J.D., Zanini, D., 2013. Neuroprotective effect of quercetin in ectoenzymes and acetylcholinesterase activities in cerebral cortex synaptosomes of cadmium-exposed rats. Mol. Cell. Biochem. 381, 1–8.

Abdel-Rahman, R., Alqasoumi, S., El-Desoky, A., Soliman, G., Paré, P., Hegazy, M.-E., 2015. Evaluation of the anti-inflammatory, analgesic and anti-ulcerogenic potentials of *Achillea fragrantissima* (Forssk.). South Afr. J. Bot. 98, 122–127.

ADI, 2015. The Global Impact of Dementia: an Analysis of Prevalence, Incidence, Cost and Trends. World Alzheimer Report. Alzheimer's Disease International (ADI), London, UK.

Agar, O.T., Dikmen, M., Ozturk, N., Yilmaz, M.A., Temel, H., Turkmenoglu, F.P., 2015. Comparative studies on phenolic composition, antioxidant, wound healing and cytotoxic activities of selected *Achillea* L. species growing in Turkey. Molecules 20, 17976–18000.

Alu'datt, M.H., Rababah, T., Alhamad, M.N., Al-Mahasneh, M.A., Almajwal, A., Gammoh, S., Ereifej, K., Johargy, A., Alli, I., 2017. A review of phenolic compounds in oil-bearing plants: distribution, identification and occurrence of phenolic compounds. Food Chem. 218, 99–106.

Amessis-Ouchemoukh, N., Ouchemoukh, S., Meziant, N., Idiri, Y., Hernanz, D., Stinco, C.M., Rodríguez-Pulido, F.J., Heredia, F.J., Madani, K., Luis, J., 2017. Bioactive metabolites involved in the antioxidant, anticancer and anticalpain activities of *Ficus carica* L., *Ceratonia siliqua* L. and *Quercus ilex* L. extracts. Industrial Crops Prod. 95, 6–17.

Anvari, S., Bahaoddini, A., Moein, M., Khosravi, A.R., 2016. The effect of hydroalcoholic extract of Achillea eriophora dc. on blood pressure of anaesthetized male rat. Excli J. 15, 797–806.

Applequist, W.L., Moerman, D.E., 2011. Yarrow (*Achillea millefolium* L.): a neglected Panacea? A review of ethnobotany, bioactivity, and biomedical Research1. Econ. Bot. 65, 209–225.

Ashour, M.L., El-Readi, M.Z., Hamoud, R., Eid, S.Y., El Ahmady, S.H., Nibret, E., Herrmann, F., Youns, M., Tahrani, A., Kaufmann, D., 2014. Anti-infective and cytotoxic properties of *Bupleurum marginatum*. Chin. Med. 9, 4.

Ashour, M.L., El-Readi, M.Z., Tahrani, A., Eid, S.Y., Wink, M., 2012. A novel cytotoxic aryltetraline lactone from *Bupleurum marginatum* (Apiaceae). Phytochem. Lett. 5, 387–392.

Ashour, M.L., Wink, M., 2011. Genus *Bupleurum*: a review of its phytochemistry, pharmacology and modes of action. J. Pharm. Pharmacol. 63, 305–321.

Aytaç, Z., Duman, H., Ekici, M., 2016. Two new *Achillea* L. (Asteraceae) species from Turkey. Turkish J. Bot. 40, 373–379.

Barrero, A.F., Haídour, A., Munoz-Dorado, M., Akssira, M., Sedqui, A., Mansour, I., 1998. Polyacetylenes, terpenoids and flavonoids from *Bupleurum spinosum*. Phytochemistry 48, 1237–1240.

Benahmed, M., Akkal, S., Elomri, A., Laouer, H., Vérité, P., Seguin, E., 2014. Constituents from *Bupleurum montanum* (coss. & dur.)(apiaceae). Arabian J. Chem. 7, 1065–1069.

Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N., Bourne, P.E., 2000. The protein data bank. Nucleic acids Res. 28, 235–242.

Ceylan, R., Katanić, J., Zengin, G., Matić, S., Aktumsek, A., Boroja, T., Stanić, S., Mihailović, V., Guler, G.O., Boga, M., 2016. Chemical and biological fingerprints of two Fabaceae species (*Cytisopsis dorycniifolia* and *Ebenus hirsuta*): are they novel sources of natural agents for pharmaceutical and food formulations? Industrial Crops Prod. 84, 254–262.

Chahmi, N., Anissi, J., Jennan, S., Farah, A., Sendide, K., El Hassouni, M., 2015. Antioxidant activities and total phenol content of Inula viscosa extracts selected from three regions of Morocco. Asian Pac. J. Trop. Biomed. 5, 228–233.

Davis, P., 1982. Flora of Turkey and the East Aegean Islands. Edinburgh Univ. Press, Edinburgh, pp. 393–418.

de Freitas, M.M., Fontes, P.R., Souza, P.M., Fagg, C.W., Guerra, E.N.S., de Medeiros Nóbrega, Y.K., Silveira, D., Fonseca-Bazzo, Y., Simeoni, L.A., Homem-de-Mello, M., 2016. Extracts of morus nigra L. Leaves standardized in chlorogenic acid, rutin and isoquercitrin: tyrosinase inhibition and cytotoxicity. PLoS one 11, e0163130.

DeLano, W.L., 2002. The PyMOL Molecular Graphics System.

Deniz, L., Serteser, A., Kargıoğlu, M., 2010. Uşak Üniversitesi Ve Yakın Çevresindeki Bazı Bitkilerin Mahalli Adları Ve Etnobotanik Özellikleri, pp. 57–72.

Dezsi, Ş., Bădărău, A.S., Bischin, C., Vodnar, D.C., Silaghi-Dumitrescu, R., Gheldiu, A.-M., Mocan, A., Vlase, L., 2015. Antimicrobial and antioxidant activities and phenolic profile of *Eucalyptus globulus* Labill. and *Corymbia ficifolia* (F. Muell.) KD Hill & LAS Johnson leaves. Molecules 20, 4720–4734.

Doughari, J.H., 2012. Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents. INTECH Open Access Publisher.

Egloff, M.-P., Marguet, F., Buono, G., Verger, R., Cambillau, C., van Tilbeurgh, H., 1995. The 2.46. ANG. Resolution structure of the pancreatic lipase-colipase complex inhibited by a C11 alkyl phosphonate. Biochemistry 34, 2751–2762.

Friesner, R.A., Murphy, R.B., Repasky, M.P., Frye, L.L., Greenwood, J.R., Halgren, T.A., Sanschagrin, P.C., Mainz, D.T., 2006. Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. J. Med. Chem. 49, 6177–6196.

Gatto, M.T., Falcocchio, S., Grippa, E., Mazzanti, G., Battinelli, L., Nicolosi, G., Lambusta, D., Saso, L., 2002. Antimicrobial and anti-lipase activity of quercetin and its C2-C16 3-O-acyl-esters. Bioorg. Med. Chem. 10, 269–272.

Giacobini, E., 2004. Cholinesterase inhibitors: new roles and therapeutic alternatives. Pharmacol. Res. 50, 433–440.

Guo, X., Liu, J., Cai, S., Wang, O., Ji, B., 2016. Synergistic interactions of apigenin, naringin, quercetin and emodin on inhibition of 3T3-L1 preadipocyte differentiation and pancreas lipase activity. Obes. Res. Clin. Pract. 10, 327–339.

Güner, A., Özhatay, N., Ekim, T., Başer, K., 2000. Second Supplement, Edinburgh 11.

Flora of Turkey and the East Aegean Islands, vol. 11, pp. 143–144.
Ha, T.J., Jang, D.S., Lee, J.R., Lee, K.D., Lee, J., Hwang, S.W., Jung, H.J., Nam, S.H., Park, K.H., Yang, M.S., 2003. Cytotoxic effects of Sesquiterpene lactones from the flowers of *Hemisteptia lyrata* B. Archives pharmacal Res. 26, 925.
Harvey, A.L., 2008. Natural products in drug discovery. Drug Discov. today 13, 894–901.
Hichri, F., Znati, M., Jannet, H.B., Bouajila, J., 2015. A new sesquiterpene lactone and secoguaianolides from *Achillea cretica* L. growing in Tunisia. Industrial Crops Prod. 77, 735–740.
Irwin, J.J., Sterling, T., Mysinger, M.M., Bolstad, E.S., Coleman, R.G., 2012. ZINC: a free tool to discover chemistry for biology. J. Chem. Inf. Model. 52, 1757–1768.
Ismaya, W.T., Rozeboom, H.J., Weijn, A., Mes, J.J., Fusetti, F., Wichers, H.J., Dijkstra, B.W., 2011. Crystal structure of Agaricus bisporus mushroom tyrosinase: identity of the tetramer subunits and interaction with tropolone. Biochemistry 50, 5477–5486.
Jesionek, W., Majer-Dziedzic, B., Choma, I.M., 2015. Separation, identification, and investigation of antioxidant ability of plant extract components using TLC, LC–MS, and TLC–DPPH•. J. Liq. Chromatogr. Relat. Technol. 38, 1147–1153.
Jones, G., Willett, P., Glen, R.C., Leach, A.R., Taylor, R., 1997. Development and validation of a genetic algorithm for flexible docking. J. Mol. Biol. 267, 727–748.
Jung, M., Park, M., 2007. Acetylcholinesterase inhibition by flavonoids from *Agrimonia pilosa*. Molecules 12, 2130–2139.
Kallel, F., Driss, D., Chaari, F., Belghith, L., Bouaziz, F., Ghorbel, R., Chaabouni, S.E., 2014. Garlic (*Allium sativum* L.) husk waste as a potential source of phenolic compounds: influence of extracting solvents on its antimicrobial and antioxidant properties. Industrial Crops Prod. 62, 34–41.
Katalinić, M., Bosak, A., Kovarik, Z., 2014. Flavonoids as inhibitors of human butyrylcholinesterase variants. Food Technol. Biotechnol. 52, 64–67.
Katalinić, M., Rusak, G., Barović, J.D., Šinko, G., Jelić, D., Antolović, R., Kovarik, Z., 2010. Structural aspects of flavonoids as inhibitors of human butyrylcholinesterase. Eur. J. Med. Chem. 45, 186–192.
Kim, Y.-J., Uyama, H., 2005. Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective for the future. Cell. Mol. Life Sci. 62, 1707–1723.
Li, H.-R., Habasi, M., Xie, L.-Z., Aisa, H.A., 2014. Effect of chlorogenic acid on melanogenesis of B16 melanoma cells. Molecules 19, 12940–12948.
Li, Y.Q., Zhou, F.C., Gao, F., Bian, J.S., Shan, F., 2009. Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of α-glucosidase. J. Agric. Food Chem. 57, 11463–11468.
Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., 2016. An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules 21, 1374.
Locatelli, M., Zengin, G., Uysal, A., Carradori, S., De Luca, E., Bellagamba, G., Aktumsek, A., Lazarova, I., 2017. Multicomponent pattern and biological activities of seven *Asphodeline* taxa: potential sources of natural-functional ingredients for bioactive formulations. J. Enzyme Inhibition Med. Chem. 32, 60–67.
Lou, S.-N., Lai, Y.-C., Hsu, Y.-S., Ho, C.-T., 2016. Phenolic content, antioxidant activity and effective compounds of kumquat extracted by different solvents. Food Chem. 197, 1–6.
Lunagariya, N.A., Patel, N.K., Jagtap, S.C., Bhutani, K.K., 2014. Inhibitors of pancreatic lipase: state of the art and clinical perspectives. EXCLI J. 13, 897–921.
Maestro, S., 2011. Version 9.2. LLC, New York.
Meng, Y., Su, A., Yuan, S., Zhao, H., Tan, S., Hu, C., Deng, H., Guo, Y., 2016. Evaluation of total flavonoids, myricetin, and quercetin from hovenia dulcis thunb. As inhibitors of α-amylase and α-glucosidase. Plant Foods Hum. Nutr. 71, 444–449.
Mocan, A., Schafberg, M., Crişan, G., Rohn, S., 2016a. Determination of lignans and phenolic components of Schisandra chinensis (Turcz.) Baill. using HPLC-ESI-ToF-MS and HPLC-online TEAC: contribution of individual components to overall antioxidant activity and comparison with traditional antioxidant assays. J. Funct. Foods 24, 579–594.
Mocan, A., Vlase, L., Arsene, A.L., Vodnar, D., Bischin, C., Silaghi-Dumitrescu, R., Crisan, G., 2015a. Hplc/ms analysis of caffeic and chlorogenic acids from three Romanian *veronica* species and their antioxidant and antimicrobial properties. Farmacia 63, 890–896.
Mocan, A., Vlase, L., Raita, O., Hanganu, D., Păltinean, R., Dezsi, Ş., Gheldiu, A.-M., Oprean, R., Crişan, G., 2015b. Comparative studies on antioxidant activity and polyphenolic content of *Lycium barbarum* L. and *Lycium chinense* Mill. leaves. Pak. J. Pharm. Sci. 28, 1511–1515.
Mocan, A., Zengin, G., Crişan, G., Mollica, A., 2016b. Enzymatic assays and molecular modeling studies of *Schisandra chinensis* lignans and phenolics from fruit and leaf extracts. J. Enzyme Inhibition Med. Chem. 31, 200–210.
Mocan, A., Zengin, G., Uysal, A., Gunes, E., Mollica, A., Degirmenci, N.S., Alpsoy, L., Aktumsek, A., 2016c. Biological and chemical insights of *Morina persica* L.: a source of bioactive compounds with multifunctional properties. J. Funct. Foods 25, 94–109.
Morbidelli, L., 2016. Polyphenol-based nutraceuticals for the control of angiogenesis: analysis of the critical issues for human use. Pharmacol. Res. 111, 384–393.
Nachon, F., Carletti, E., Ronco, C., Trovaslet, M., Nicolet, Y., Jean, L., Renard, P.-Y., 2013. Crystal structures of human cholinesterases in complex with huprine W and tacrine: elements of specificity for anti-Alzheimer's drugs targeting acetyl-and butyryl-cholinesterase. Biochem. J. 453, 393–399.
Nan, X., Ya-Nan, S., Zhong, X., Yue, C., Li, W., Tian-Zhu, J., 2014. A new saikogenin from the roots of Bupleurum bicaule. Chin. J. Nat. Med. 12, 305–308.
Oboh, G., Agunloye, O.M., Adefegha, S.A., Akinyemi, A.J., Ademiluyi, A.O., 2015. Caffeic and chlorogenic acids inhibit key enzymes linked to type 2 diabetes (in vitro): a comparative study. J. basic Clin. physiology Pharmacol. 26, 165–170.
Ong, S.L., Mah, S.H., Lai, H.Y., 2016. Porcine pancreatic lipase inhibitory agent isolated from medicinal herb and inhibition kinetics of extracts from eleusine indica (L.) gaertner. J. Pharm. 2016.
Pan, S.-L., 2006. Bupleurum Species: Scientific Evaluation and Clinical Applications. CRC Press.
Patel, D., 2015. Pharmacotherapy for the management of obesity. Metabolism 64, 1376–1385.
Pesaresi, A., Lamba, D., Torpedo Californica Acetylcholinesterase in Complex with a Tacrine-nicotinamide Hybrid Inhibitor.
Radulović, N.S., Mladenović, M.Z., Randjelovic, P.J., Stojanović, N.M., Dekić, M.S., Blagojević, P.D., 2015. Toxic essential oils. Part IV: the essential oil of *Achillea falcata* L. as a source of biologically/pharmacologically active trans-sabinyl esters. Food Chem. Toxicol. 80, 114–129.
Rice-Evans, C.A., Miller, N.J., Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. 20, 933–956.
Russo, D., Valentão, P., Andrade, P.B., Fernandez, E.C., Milella, L., 2015. Evaluation of antioxidant, antidiabetic and anticholinesterase activities of Smallanthus sonchifolius landraces and correlation with their phytochemical profiles. Int. J. Mol. Sci. 16, 17696–17718.
Samaradivakara, S.P., Samarasekera, R., Handunnetti, S.M., Weerasena, O.J., 2016. Cholinesterase, protease inhibitory and antioxidant capacities of Sri Lankan medicinal plants. Industrial Crops Prod. 83, 227–234.
Samoticha, J., Wojdyło, A., Golis, T., 2017. Phenolic composition, physicochemical properties and antioxidant activity of interspecific hybrids of grapes growing in Poland. Food Chem. 215, 263–273.
Saraçoğlu, H.T., Zengin, G., Akin, M., Aktümsek, A., 2012. A comparative study on the fatty acid composition of the oils from five *Bupleurum* species collected from Turkey. Turkish J. Biol. 36, 527–532.
Savran, A., Zengin, G., Aktumsek, A., Mocan, A., Glamoćlija, J., Ćirić, A., Soković, M., 2016. Phenolic compounds and biological effects of edible *Rumex scutatus* and *Pseudosempervivum sempervivum*: potential sources of natural agents with health benefits. Food & Funct. 7, 3252–3262.
Sezik, E., Yeşilada, E., Honda, G., Takaishi, Y., Takeda, Y., Tanaka, T., 2001. Traditional medicine in Turkey X. Folk medicine in central Anatolia. J. Ethnopharmacol. 75, 95–115.
Shelley, J.C., Cholleti, A., Frye, L.L., Greenwood, J.R., Timlin, M.R., Uchimaya, M., 2007. Epik: a software program for pK a prediction and protonation state generation for drug-like molecules. J. computer-aided Mol. Des. 21, 681–691.
Slinkard, K., Singleton, V.L., 1977. Total phenol analysis: automation and comparison with manual methods. Am. J. Enology Vitic. 28, 49–55.
Sridhar, S., Ginson, G., Reddy, P.V., Tantak, M.P., Kumar, D., Paul, A.T., 2017. Synthesis, evaluation and molecular modelling studies of 2-(carbazol-3-yl)-2-oxoacetamide analogues as a new class of potential pancreatic lipase inhibitors. Bioorg. Med. Chem. 25, 609–620.
Sudeep, H., Shyam Prasad, K., 2014. Computational studies of the antiobesity effect of polyphenols from pomegranate leaf. J. Chem. Pharm. Res. 6, 278–281.
Tetik, F., Civelek, S., Cakilcioglu, U., 2013. Traditional uses of some medicinal plants in Malatya (Turkey). J. Ethnopharmacol. 146, 331–346.
Tuberoso, C.I.G., Montoro, P., Piacente, S., Corona, G., Deiana, M., Dessì, M.A., Pizza, C., Cabras, P., 2009. Flavonoid characterization and antioxidant activity of hydroalcoholic extracts from *Achillea ligustica* All. J. Pharm. Biomed. analysis 50, 440–448.
Turkmenoglu, F.P., Agar, O.T., Akaydin, G., Hayran, M., Demirci, B., 2015. Characterization of volatile compounds of eleven *Achillea* species from Turkey and biological activities of essential oil and methanol extract of A. hamzaoglui arabacı & budak. Molecules 20, 11432–11458.
Tušek, A.J., Benković, M., Cvitanović, A.B., Valinger, D., Jurina, T., Kljusurić, J.G., 2016. Kinetics and thermodynamics of the solid-liquid extraction process of total polyphenols, antioxidants and extraction yield from Asteraceae plants. Industrial Crops Prod. 91, 205–214.
Tuzlaci, E., 2016. Türkiye Bitkileri Sözlüğü. Alfa publisher, Istanbul, Turkey.
Villanueva-Bermejo, D., Zahran, F., Troconis, D., Villalva, M., Reglero, G., Fornari, T., 2017. Selective precipitation of phenolic compounds from *Achillea millefolium* L. extracts by supercritical anti-solvent technique. J. Supercrit. Fluids 120, 52–58.
Vitalini, S., Beretta, G., Iriti, M., Orsenigo, S., Basilico, N., Dall'Acqua, S., Iorizzi, M., Fico, G., 2011. Phenolic compounds from Achillea millefolium L. and their bioactivity. Acta Biochim. Pol. 58, 203–209.
Waltenberger, B., Mocan, A., Šmejkal, K., Heiss, E.H., Atanasov, A.G., 2016. Natural products to counteract the epidemic of cardiovascular and metabolic disorders. Molecules 21, 807.
Xie, W., Zhang, H., He, J., Zhang, J., Yu, Q., Luo, C., Li, S., 2016. Synthesis and Biological Evaluation of novel hydroxybenzaldehyde-based Kojic Acid analogues as inhibitors of mushroom tyrosinase. Bioorg. Med. Chem. Lett. 5, 530–532.
Xie, Y., Yang, W., Chen, X., Xiao, J., 2014. Inhibition of flavonoids on acetylcholine esterase: binding and structure–activity relationship. Food & Funct. 5, 2582–2589.
Yamamoto, K., Miyake, H., Kusunoki, M., Osaki, S., 2011. Steric hindrance by 2 amino acid residues determines the substrate specificity of isomaltase from Saccharomyces cerevisiae. J. Biosci. Bioeng. 112, 545–550.
Zengin, G., Aktumsek, A., Ceylan, R., Uysal, S., Mocan, A., Guler, G.O., Mahomoodally, M.F., Glamoćlija, J., Ćirić, A., Soković, M., 2017a. Shedding light on the biological and chemical fingerprints of three Achillea species (A. biebersteinii, A. millefolium and A. teretifolia). Food & Funct. http://dx.doi.org/

10.1039/C6FO01847E.
Zengin, G., Locatelli, M., Ceylan, R., Aktumsek, A., 2016. Anthraquinone profile, antioxidant and enzyme inhibitory effect of root extracts of eight *Asphodeline* taxa from Turkey: can Asphodeline roots be considered as a new source of natural compounds? J. Enzyme Inhibition Med. Chem. 31, 754–759.
Zengin, G., Sarikurkcu, C., Aktumsek, A., Ceylan, R., 2014. *Sideritis galatica* Bornm.: a source of multifunctional agents for the management of oxidative damage, Alzheimer's's and diabetes mellitus. J. Funct. Foods 11, 538–547.
Zengin, G., Uysal, A., Aktumsek, A., Mocan, A., Mollica, A., Locatelli, M., Custodio, L., Neng, N.R., Nogueira, J.M., Aumeeruddy-Elalfi, Z., 2017b. *Euphorbia denticulata* Lam.: a promising source of phyto-pharmaceuticals for the development of novel functional formulations. Biomed. Pharmacother. 87, 27–36.
Zhang, D.-M., Zhou, N.-D., Zhou, H., Chen, T., Li, G.-X., 2008. Electrochemical study of the inhibitory effect of sinapic acid and its derivatives on tyrosinase reactivity [j]. Chem. J. Chin. Univ. 2, 012.
Zhang, T., Zhou, J., Wang, Q., 2007. Flavonoids from aerial part of *Bupleurum chinense* DC. Biochem. Syst. Ecol. 35, 801–804.
Zhuo, H., Payan, F., Qian, M., 2004. Crystal structure of the pig pancreatic α-amylase complexed with ρ-nitrophenyl-α-d-maltoside-flexibility in the active site. protein J. 23, 379–387.