



Biochemical constituent, enzyme inhibitory activity, and molecular docking analysis of an endemic plant species, *Thymus migricus*

Abdülmelik Aras¹ · Fikret Türkan² · Umit Yildiko³ · Mehmet Nuri Atalar¹ · Ömer Kılıç⁴ · Mehmet Hakki Alma⁵ · Ercan Bursal⁶ 

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Abstract

Medicinal plants have been used traditionally since ancient times as alternative medications to treat various human diseases. In this work, we examined the chemical constituent, antioxidant activity, and enzyme inhibition of an endemic plant *Thymus migricus*. The plant extracts showed remarkable inhibition effects against the acetylcholinesterase (AChE), butyrylcholinesterase (BChE), glutathione S-transferase (GST), and α -glycosidase (α -Gly) enzymes that are linked with some metabolic disorders. IC₅₀ values (concentration of a sample to inhibit 50% of enzyme activity) for AChE, BChE, GST, and α -Gly were found as 18.23 mg/mL, 17.77 mg/mL, 31.5 mg/mL, and 16.5 mg/mL, respectively. Antioxidant activities of water extract of *T. migricus* (WET) and methanol extract of *T. migricus* (MET) were determined using four in vitro techniques. The extracts showed considerable antioxidant actions on all four techniques. In addition, quinic acid (18.30 mg/g), chlorogenic acid (0.94 mg/g), cynaroside (0.49 mg/g), luteolin (0.21 mg/g), and *p*-coumaric acid (0.19 mg/g) were characterized as major phenolic compounds using advanced LC–MS/MS technique. The interactions of some phenolic compounds with the enzymes have been investigated using molecular docking studies. The docking analysis results revealed that the interactions of the major compounds of the plant with AChE, BChE, GST, and α -Gly enzymes are responsible for the inhibitory activity.

Keywords Antioxidant · Enzyme inhibition · Phenolic compounds · *Thymus migricus* · Docking

Introduction

Reactive oxygen species (ROS) are produced by cellular metabolism or by exogenous reasons such as drugs, chemicals, smoke, and bad environmental conditions. The chemical structure of ROS contains at least one unpaired electron

(Turrens 2003). ROS have been reported to be associated with some of the cancer types, mitochondrial diseases such as diabetes mellitus, insulin resistance, cardiovascular disease, Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease (Bursal et al. 2019a). Antioxidant substances can maintain health by scavenging radicals and reactive oxygen species (Gulcin 2020).

In the last few decades, there was increasing awareness of natural plant-based diet notably important for human health (Nirmala et al. 2018). It is believed that two-thirds of the plant species have medicinal value and have antioxidant potential in certain amounts (Hassan et al. 2017). Plant-based antioxidant substances are widely distributed in plants that are used for many biological functions (Aras et al. 2019). *T. migricus* is a perennial endemic herb that belongs to the Lamiaceae family. *Lamiaceae* family consists of aromatic plants that spread almost in all parts of the world, especially in the Mediterranean region and North-Western Asia. *Thymus* L. is represented in the territory of Turkey with 39 species (60 taxa), 20 of which endemic (Tohidi et al. 2017). Some previous studies reported that various *Thymus*

✉ Ercan Bursal
e.bursal@alparslan.edu.tr; ercanbursal@gmail.com

¹ Department of Biochemistry, Faculty of Science and Arts, Iğdır University, Iğdır, Turkey

² Health Services Vocational School, Iğdır University, Iğdır, Turkey

³ Department of Bioengineering, Architecture and Engineering Faculty, Kafkas University, Kars, Turkey

⁴ Department of Pharmaceutical Sciences, Pharmacy Faculty, Adıyaman University, Adıyaman, Turkey

⁵ Department of Environmental Engineering, Faculty of Engineering, Iğdır University, Iğdır, Turkey

⁶ Department of Nursing, Faculty of Health, Muş Alparslan University, Muş, Turkey

species have strong antioxidant (Köksal et al. 2017), diuretic (Hazzit et al. 2009), antibacterial (Rota et al. 2008), antispasmodic carminative (Dob et al. 2006), antifungal (Gonçalves et al. 2010), and antiviral (Aouam et al. 2019) activities. Phenolic compounds are secondary metabolites of plants with having several benefits for human health. They have correlations with antioxidant properties and preventive effects against oxidation (Koeduka et al. 2014). Natural antioxidants such as flavonoids, phenolic compounds, and other phytochemicals besides preventing the lipid oxidation process can act as free radical scavengers (Baharfar et al. 2015; Silinsin and Bursal 2018).

Alzheimer's disease (AD) generally affects the memory and behavior of elderly people worldwide. This neurological disease is affected by an acetylcholine (ACh) deficiency and is manifested by increased degeneration of brain tissue (Gülçin et al. 2020). AChE and BChE enzymes are known to be responsible for hydrolyzing the ACh in the human body (Lu et al. 2019). AChE takes charge of break up the acetylcholine in the synaptic of the brain cleft (Işık et al. 2019). Hence by the keeping of the acetylcholine for neurotransmission in the synaptic cleft, AD symptoms being reducing or preventing (Yates et al. 2019). Recent studies demonstrate that ACh as a neurotransmitter decreases because of the decline in acetyltransferase activity and choline (Taslimi et al. 2020a). Therefore, researchers have focused on cholinesterase (ChE) inhibitive treatment as an asymptomatic intervention (Kocuyigit et al. 2018). It is reported that plant secondary metabolites have ChE inhibition which can be utilized for the treatment of the AD (Türkan et al. 2019).

Diabetes mellitus (DM) is a metabolic disease that is characterized by abnormal levels of glucose in the human blood, as well as some disorders including retinopathy, atherosclerosis, cardiovascular diseases, neural damage, and neuropathy (Rendra et al. 2019). DM causes different diseases in different organs such as kidneys, eyes, and veins (Türkan et al. 2019). Since digestive enzymes hydrolyze polysaccharides to monosaccharide units, inhibition of these enzymes is an important treatment method for DM (Gülçin et al. 2018). α -Gly release from intestine cells and hydrolyzes oligosaccharides and polysaccharides to the small monosaccharides. Therefore, α -Gly inhibitors had great importance for controlling DM (Burmaoglu et al. 2019). GST enzyme hydrolyzes the nucleophilic combination of glutathione (GSH) with some electrophilic substrates. This enzyme combination is an important system of detoxification in mammals and some families of herbicides in plants (Gülçin et al. 2016b).

There are numerous studies on the literature about biological properties and enzyme inhibitory effects of various plant species. However, best of our knowledge there is no detailed single study about enzyme inhibitions related to phenolic content and biological activity of *T. migricus*. The

essential oils of *T. migricus* were determined by several former studies (Başer et al. 2002; Küçükbay et al. 2014). In this study, we verified the antioxidant activity of *T. migricus* using in vitro bioanalytical methods and we tested the plant extracts against AChE, BChE, GST, and α -Gly enzymes. Also, the most abundant compounds of *T. migricus* such as chlorogenic acid, cynaroside, luteolin, p-coumaric acid, and quinic acid were observed to be effective with receptor binding scores by molecular docking analysis.

Materials and methods

Plant sample and extraction

Thymus migricus Klokov & Desj.-Shost samples were collected between Adaklı and Bağlarpınarı villages in Bingöl, Turkey on July 2017, by a plant taxonomist Dr. Kılıç (voucher id 5923). The natural habitat of the plant was the steppe areas and the slopes at an altitude 1500–1600 m.

The plant extractions were prepared with both water and methanol solvents. For the water extract of *T. migricus* (WET), 20 g air-dried leaves of the plant was powdered and mixed with 200 mL of pure water. It was stirred at room temperature for 12 h and filtered. The filtrate was lyophilized in a lyophilizer (Labconco, Freezone) at 5 mmHg, $-50\text{ }^{\circ}\text{C}$. For the methanol extract of *T. migricus* (MET), 20 g air-dried leaves of the plant was powdered and mixed with 200 mL of methanol. It was filtered after stirring at room temperature for 12 h. The filtrate was evaporated with a rotary evaporator (Heidolph 94200).

Determination of phenolic content using LC–MS/MS

Ultrahigh performance liquid chromatography (Shimadzu-Nexera) coupled with a tandem mass spectrometer (LCMS-8040 model) was used to determine the phenolic contents of *T. migricus*. The reversed-phase UHPLC was equipped with a SIL-30AC model autosampler, a CTO-10ASvp model column oven, LC-30CE model binary pumps, and a DGU-20A3R model degasser. Different columns such as RP-C18 Inertsil ODS-4 (100 mm $\times$ 2.1 mm, 2 μm) and 120 EC-C18 models (150 mm $\times$ 2.1 mm, 2.7 μm) analytical columns were used and the column temperature was set to 40 $^{\circ}\text{C}$. Methanol and acetonitrile were used as mobile phases, ammonium formate, ammonium acetate, acetic acid, and formic acid were used as mobile phase additives. The gradient elution: 20% B (35–45 min), 100% B (25–35 min), 20–100% B (0–25 min). The flow rate was 0.5 mL/min and the injection volume was 5 μL . Ionization source (ESI) was used for spectrometric detection. ESI was operated in positive ionization mode for vanillin, daidzin, piceid, coumarin, and hesperidin, while for other standards ESI operated in

negative. MS conditions: drying gas (N_2) flow, 15 L/min; nebulizing gas (N_2) flow, 3 L/min; interface temperature, 350 °C, heat block temperature, 400 °C, DL temperature, 250 °C (Yilmaz 2020).

Determination of antioxidant activity

Various in vitro techniques such as cupric ion reducing antioxidant capacity (CUPRAC) (Apak et al. 2006), ferric ion reducing antioxidant power (FRAP) (Aras et al. 2017), DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging (Aras et al. 2018), and ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) cation radical scavenging methods (Bursal et al. 2019b) were used to screen the antioxidant activities of the *T. migricus* extracts.

Determination of enzyme inhibition

The inhibition of α -Gly was determined using p nitrophenyl-D-glucopyranoside (p-NPG) substrate (Tao et al. 2013). AChE and BChE inhibitions of *T. migricus* extracts were defined using acetylthiocholine iodide (AChI) and butyrylcholine iodide (BChI) substrates (Bursal et al. 2020). GST enzyme inhibition of *T. migricus* was determined by the method used in a previous study (Gulçin et al. 2018).

Molecular docking studies

Molecular docking analyses of the main compounds of the plant for some metabolic enzymes were performed to investigate the binding mechanisms and the exact binding sites of the ligands on the. The Maestro 11.8 version was applied in the molecular docking studies (Release 2019). The high-resolution (1.55–2.10 Å) crystalline structures of acetylcholinesterase (PDB id: 5HF9), butyrylcholinesterase (PDB id: 5NN0), glutathione s-transferase (PDB id: 1GNW), and α -glycosidase (PDB id: 3A4A) enzymes were downloaded from Protein Data Bank (PDB) (Burley et al. 2019). The structures of the ligands were downloaded in PubChem site in SDF file format. All of the compounds were prepared according to previous studies with Ligprep module. At this stage, all of the water molecules in the crystal structure were deleted. The protein ion balance was optimized by determining the active region of the protein for flexible binding with this module. Using the Receptor grid generation module, grid boxes were created to allow flexible clamping by making grids on the binding regions of the protein according to the previous studies (Karimov et al. 2020; Taslimi et al. 2020b). Ligand–protein docking studies were performed with the Glide docking module. The highest binding energies and binding conformation between ligands and enzymes were calculated. The lowest energy positions

indicate the highest binding affinities. The resulting receptor 3D interactions were visualized with Discovery Studio 4.5 version (Visualizer 2005) (Fig. 1).

Results and discussion

Phenolic content

In the present study, the phenolic compounds of *T. migricus* were identified and quantified using advanced LC–MS/MS techniques. As indicated in Table 1, totally 56 analytes were used as standards.

According to the LC–MS/MS analyses, quinic acid (18.303 mg/g), chlorogenic acid (0.944 mg/g), cynaroside (0.495 mg/g), luteolin (0.21 mg/g), and *p*-coumaric acid (0.194 mg/g) were found to be the major phenolics in *T. migricus* extract. Besides, the lower amounts of acacetin (0.001 mg/g), apigenin (0.003 mg/g), coumarin (0.024 mg/g), hesperidin (0.03 mg/g), isoquercitrin (0.044 mg/g), protocatechuic aldehyde (0.02 mg/g), and tannic acid (0.061 mg/g) were detected as well. The rest of the analytes were not detected in the plant extract by the LC–MS/MS analysis of the present study.

Antioxidant activity

The level of free radicals and antioxidants should be in a balance in the human system (Alisi et al. 2018). Potential antioxidant compounds can transfer electrons to reactive radicals for transforming them into stable and unreactive forms (Bursal et al. 2019b). In the current study, the water and methanol extracts were tested to evaluate the antioxidant potential of the *T. migricus*. DPPH and ABTS methods were used for measuring the radical scavenging capacity. CUPRAC and FRAP methods were used for measuring the reducing power antioxidant activities. In the ABTS method, the IC_{50} values were found as; 25.0 ± 3.3 μ g/mL for WET, 52.2 ± 15.2 μ g/mL for MET, 60.8 ± 16.9 μ g/mL for BHA, 27.9 ± 9.4 μ g/mL for BHT, 41.0 ± 17.1 μ g/mL for ascorbic acid, and 52.9 ± 10.1 μ g/mL for α -tocopherol. The ABTS cation radical inhibition percentages of *T. migricus* sample at same concentration (30 μ g/mL) was calculated as; BHA (16.3%) < MET (18.7%) < α -tocopherol (25.5%) < ascorbic acid (26.8%) < BHT (34.3%) < WET (38.8%). According to the ABTS technique, the water extract of *T. migricus* (WET) had the highest radical scavenging activity among the extracts and standards. Figure 2 demonstrates that radical scavenging potentials of the plant extracts and standards increased by increasing their concentrations.

According to the DPPH method, both of the water and methanol extracts of *T. migricus* demonstrated lower scavenging activities compare to the standards. As a

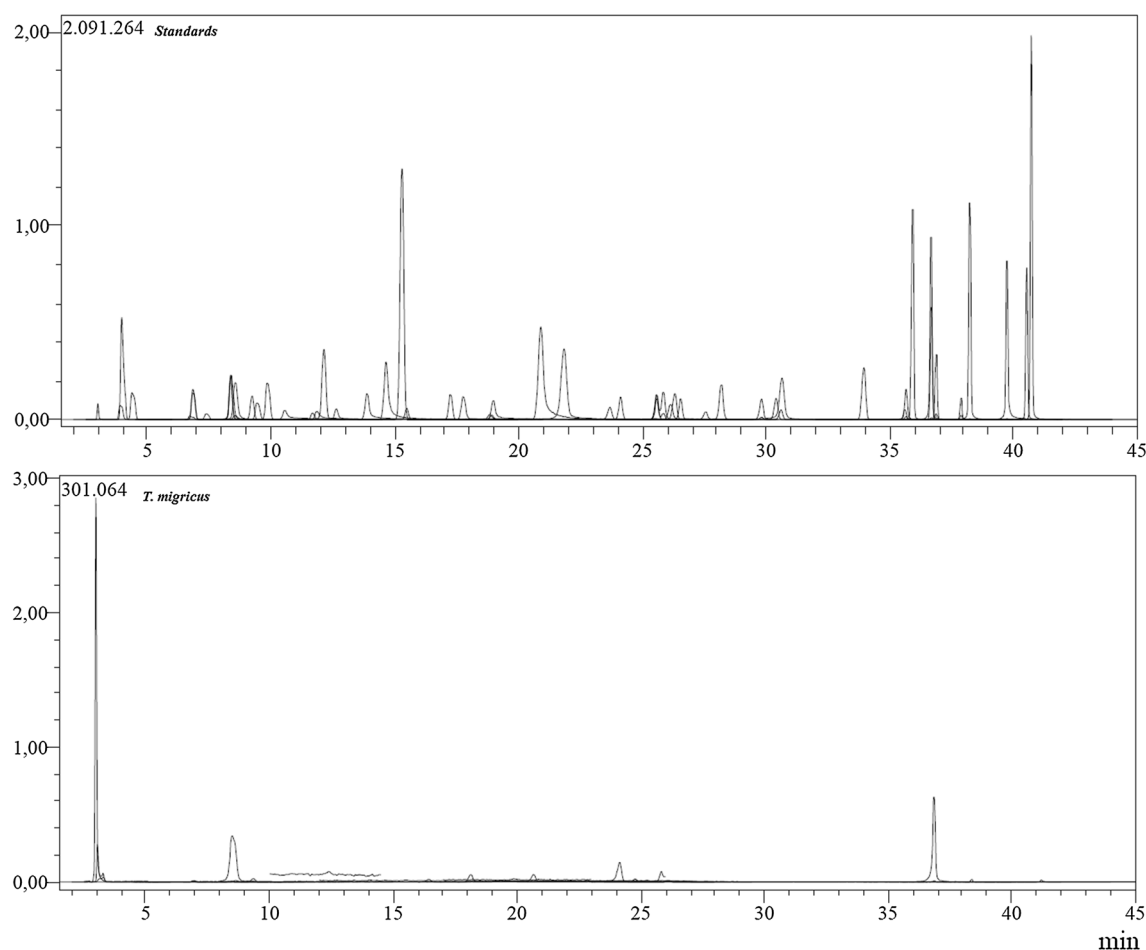


Fig. 1 LC–MS/MS chromatograms of 56 standards and *T. migricus*

consequence, the IC_{50} values of the extracts were higher than the IC_{50} values of the standards. The DPPH scavenging percentages at the same concentration (30 μ M) decreased as, ascorbic acid (94.0%) > BHA (91.2%) > α -tocopherol (90.9%) > BHT (81.8%) > MET (38.8%) > WET (22.1%). Additionally, the IC_{50} values of extracts and standards for both ABTS and DPPH methods were calculated and indicated in Table 2. The lower IC_{50} means higher radical scavenging activity. As understood from the low IC_{50} values, the methanol and water extracts of *T. migricus* had effective ABTS $^{•+}$ cation radical scavenging activities. However, both extracts showed low DPPH free radicals scavenging activity.

FRAP and CUPRAC methods are common techniques to measure reducing antioxidant powers of samples. Antioxidants cause the reduction of ferric (Fe^{3+}) ions to the ferrous (Fe^{2+}) due to their reducing capacities. The FRAP reducing levels of the water and methanol extracts were lower than reducing levels of the standards. At the same concentration (30 μ g/mL), the reducing antioxidant capacities decreased in the order of α -tocopherol > BHT > BHA > ascorbic acid > WET > MET. Similarly, an updated CUPRAC method

was used to measure cupric reducing antioxidant potential of *T. migricus* extracts. The methanol and water extracts of *T. migricus* showed moderate reducing capacities in the CUPRAC method compare to the standards (α -tocopherol, BHA, BHT, and ascorbic acid). The reducing capacities ordered as; BHA > ascorbic acid > BHT > α -tocopherol > WET > MET at the same concentration (30 μ g/mL). The remarkable differences were observed between water and methanol extracts. The results showed that the water extract has higher antioxidant activities than methanol extract using FRAP, CUPRAC, DPPH, and ABTS methods.

Enzyme inhibition

Cholinesterase enzymes catalyze the hydrolysis of the neurotransmitter acetylcholine and butyrylcholine (Aslan et al. 2018). AChE and BChE are two types of cholinesterases in the human nervous system (Cheng et al. 2019). AChE is detected as a predominant enzyme in the brain, while BChE is related to the regulation of acetylcholine level with the secondary role (Chen et al. 2017). GST is a multifunctional

Table 1 LC–MS/MS parameters and phenolic compounds of *T. migricus*

No.	RSD% ^a	RT ^b	MI (m/z) ^c	FI (m/z) ^d	r^2 ^e	Lin- earity range (mg/L)	LOD/LOQ (µg/L) ^f	Recovery (%)	Standards	<i>T. migricus</i>
1	2.42	9.8	515.0	191.0	0.999	0.1–5	5.8/9.4	0.9983	1,5-dicaffeoylquinic acid	ND
2	1.24	10.5	137.2	65.0	0.999	0.2–10	68.4/88.1	1.0032	4-OH Benzoic acid	ND
3	1.67	40.7	283.0	239.0	0.997	0.05–2.5	1.5/2.5	0.9949	Acacetin	0.001
4	2.07	4.0	172.8	129.0	0.971	0.1–5	16.4/31.4	0.9968	Aconitic acid	ND
5	1.35	39.7	537.0	417.0	0.992	0.05–2.5	2.8/5.1	0.9991	Amentoflavone	ND
6	1.17	38.2	268.8	151.0/149.0	0.998	0.05–2.5	1.3/2.0	0.9985	Apigenin	0.003
7	2.08	30.4	447.0	255.0	0.999	0.1–5	6.6/8.2	0.9968	Astragalin	ND
8	1.11	12.1	179.0	134.0	0.999	0.05–2.5	7.7/9.5	1.0015	Caffeic acid	ND
9	2.14	7.4	288.8	203.1	0.999	0.2–10	55.0/78.0	1.0024	Catechin	ND
10	2.15	8.4	353.0	85.0	0.995	0.1–5	13.1/17.6	1.0000	Chlorogenic acid	0.944
11	1.46	40.5	252.8	145.0/119.0	0.999	0.05–2.5	1.5/2.8	0.9922	Chrysin	ND
12	1.65	28.2	431.0	269.0	0.998	0.4–20	6.3/9.2	0.9940	Cosmosiin	ND
13	2.11	20.9	146.9	103.1	0.999	0.05–2.5	214.2/247.3	0.9950	Coumarin	0.024
14	1.56	23.7	447.0	284.0	0.997	0.05–2.5	12.1/16.0	1.0072	Cynaroside	0.495
15	2.18	34.0	253.0	223.0	0.999	0.1–5	9.8/11.6	0.9926	Daidzein	ND
16	2.25	15.2	417.1	199.0	0.996	0.05–2.5	7.0/9.5	0.9955	Daidzin	ND
17	1.57	27.6	301.0	284.0	0.999	0.1–5	56.9/71.0	1.0005	Ellagic acid	ND
18	1.47	11.6	289.0	203.0	0.996	1–50	139.6/161.6	1.0013	Epicatechin	ND
19	1.63	15.5	441.0	289.0	0.997	0.1–5	19.5/28.5	0.9984	Epicatechin gallate	ND
20	1.22	6.7	304.8	219.0	0.998	1–50	237.5/265.9	0.9969	Epigallocatechin	ND
21	1.30	9.4	457.0	305.1	0.999	0.2–10	61.0/86.0	0.9981	Epigallocatechin gallate	ND
22	1.44	18.8	192.8	149.0	0.999	1–50	11.8/15.6	0.9951	Ferulic acid	ND
23	N.A	18.8	196.2	152.1	N.A	N.A	N.A	N.A	Ferulic acid-D3-IS ^h	ND
24	1.75	30.6	285.0	163.0	0.999	0.05–2.5	10.1/12.7	0.9980	Fisetin	ND
25	1.05	3.9	115.2	40.9	0.995	1–50	135.7/167.9	0.9963	Fumaric acid	ND
26	1.60	4.4	168.8	79.0	0.999	0.1–5	13.2/17.0	1.0010	Gallic acid	ND
27	1.48	36.9	269.0	135.0	0.999	0.05–2.5	3.7/5.3	1.0069	Genistein	ND
28	2.01	26.3	431.0	239.0	0.991	0.1–5	14.9/21.7	1.0062	Genistin	ND
29	1.81	8.3	152.8	109.0	0.997	0.1–5	18.5/28.2	0.9963	Gentisic acid	ND
30	2.47	36.7	301.0	136.0/286.0	0.999	0.1–5	7.1/9.1	0.9998	Hesperetin	ND
31	1.84	25.8	611.2	449.0	0.999	0.1–5	19.0/26.0	0.9967	Hesperidin	0.03
32	2.13	25.6	463.0	271.0	0.998	0.1–5	8.7/13.5	1.0057	isoquercitrin	0.044
33	1.49	37.9	285.0	239.0	0.999	0.05–2.5	10.2/15.4	0.9992	Kaempferol	ND
34	1.67	36.7	284.8	151.0/175.0	0.999	0.1–5	2.6/4.1	0.9952	Luteolin	0.21
35	1.31	24.1	477.0	150.9	0.999	0.05–2.5	10.6/14.7	0.9934	Miquelianin	ND
36	2.34	35.9	270.9	119.0	0.999	0.1–5	2.6/3.9	1.0062	Naringenin	ND
37	1.48	30.6	592.9	255.0/284.0	0.999	0.1–5	11.9/16.7	0.9954	Nicotiflorin	ND
38	2.11	26.1	162.8	93.0	0.999	0.1–5	31.8/40.4	1.0044	<i>o</i> -Coumaric acid	ND
39	1.92	17.8	163.0	93.0	0.999	0.1–5	25.9/34.9	1.0049	<i>p</i> -Coumaric acid	0.194
40	1.94	17.2	391.0	135/106.9	0.999	0.05–2.5	13.8/17.8	1.0042	Piceid	ND
41	1.43	6.8	152.8	108.0	0.957	0.1–5	21.9/38.6	0.9972	Protocatechuic acid	ND
42	2.08	8.5	137.2	92.0	0.996	0.1–5	15.4/22.2	1.0002	Protocatechuic aldehyde	0.02
43	1.89	35.7	301.0	272.9	0.999	N.A	15.5/19.0	0.9967	Quercetin	ND
44	N.A	35.6	304.0	275.9	N.A	0.1–5	N.A	N.A	Quercetin-D3-IS ^h	ND
45	2.24	29.8	447.0	301.0	0.999	0.1–5	4.8/6.4	0.9960	Quercitrin	ND
46	0.69	3.0	190.8	93.0	0.996	0.1–5	25.7/33.3	1.0011	Quinic acid	18.303
47	1.24	26.6	359.0	197.0	0.999	0.1–5	16.2/21.2	1.0056	Rosmarinic acid	ND
48	1.38	25.6	608.9	301.0	0.999	N.A	15.7/22.7	0.9977	Rutin	ND

Table 1 (continued)

No.	RSD% ^a	RT ^b	MI (m/z) ^c	FI (m/z) ^d	r ^{2e}	Lin-earity range (mg/L)	LOD/LOQ (μg/L) ^f	Recovery (%)	Standards	<i>T. migricus</i>
49	N.A	25.5	612.2	304.1	N.A	0.1–5	N.A	N.A	Rutin-D3-IS ^h	ND
50	1.48	21.8	137.2	65.0	0.999		6.0/8.3	0.9950	Salicylic acid	ND
51	1.45	18.9	222.8	193.0	0.999	0.2–10	65.2/82.3	1.0031	Sinapic acid	ND
52	1.18	12.6	196.8	166.9	0.998	1–50	82.3/104.5	1.0006	Syringic acid	ND
53	2.51	14.6	181.0	151.1	0.999	0.4–20	19.7/28.0	1.0001	Syringic aldehyde	ND
54	2.40	9.2	182.8	78.0	0.999	0.05–2.5	15.3/22.7	0.9970	Tannic acid	0.061
55	1.92	11.8	166.8	108.0	0.999	1–50	141.9/164.9	1.0022	Vanillic acid	ND
56	1.10	13.9	153.1	125.0	0.996	0.1–5	24.5/30.4	1.0009	Vanillin	ND

ND Not detected

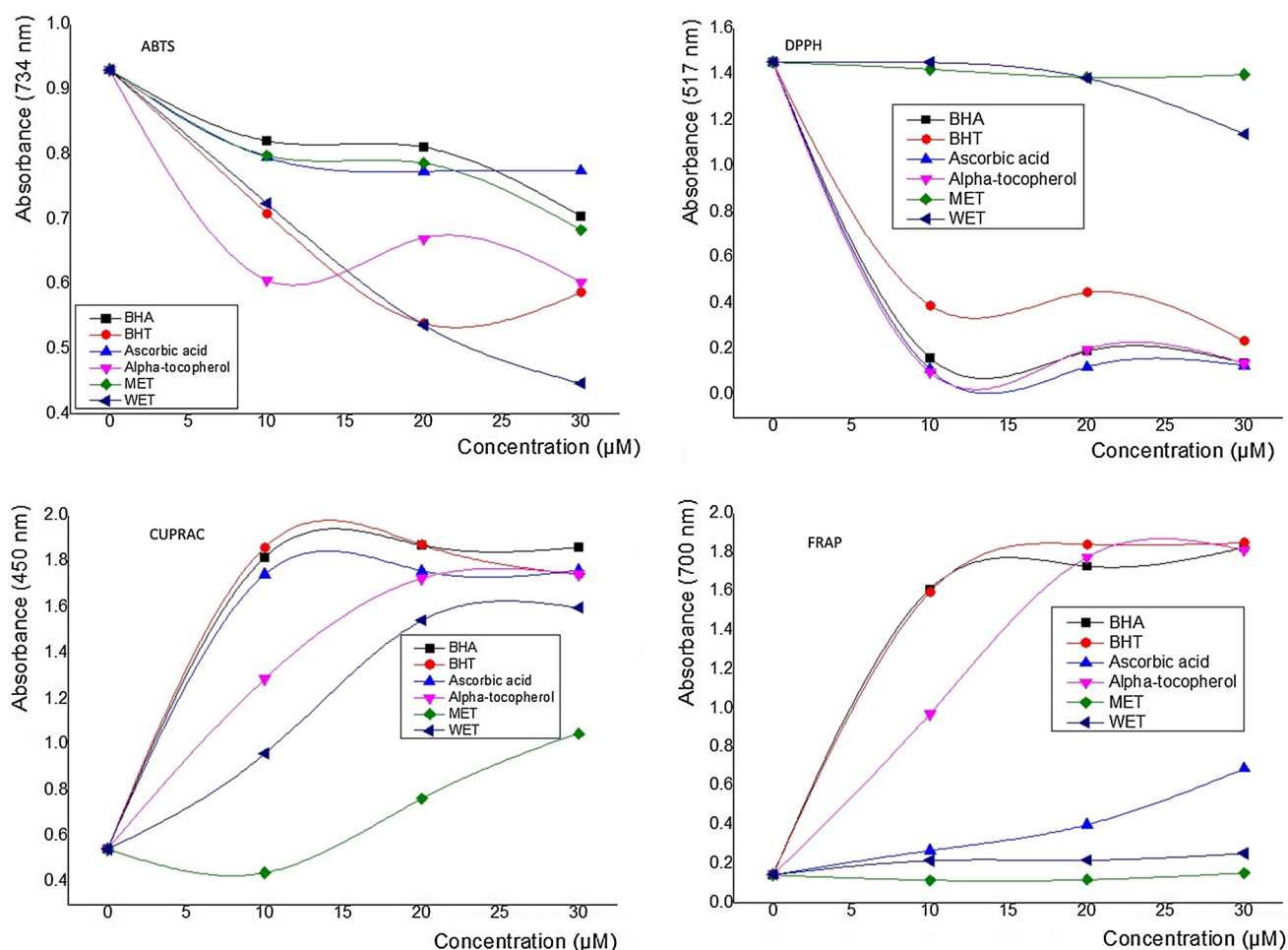
^aRelative standard deviation^bRetention time^cMolecular ions of the standard analytes (*m/z* ratio)^dFragment ions^eCoefficient of determination^fLimit of detection/quantification**Fig. 2** Antioxidant activities of *T. migricus* extracts and standards using ABTS, DPPH, CUPRAC, and FRAP methods

Table 2 The IC₅₀ values of *T. migricus* extracts for antioxidant and enzyme inhibitory methods

Methods	BHA	BHT	Ascorbic acid	α-Tocopherol	Methanol extract (MET)	Water extract (WET)	Acarbose
DPPH	11.0 ± 5.4	12.0 ± 5.4	10.6 ± 5.3	10.9 ± 5.2	24.9 ± 4.0	45.2 ± 22.5	–
ABTS	18.7 ± 6.8	38.8 ± 15.3	16.3 ± 6.9	34.3 ± 9.5	26.8 ± 17.3	25.5 ± 10.8	–
GST	–	–	–	–	31.50 ($R^2=0.91$)	–	–
AChE	–	–	–	–	18.23 ($R^2=0.90$)	–	–
BChE	–	–	–	–	17.77 ($R^2=0.95$)	–	–
α-Gly	–	–	–	–	16.50 ($R^2=0.99$)	–	22.80 ($R^2=0.99$)

enzyme and used for catalyzing the metabolic pathway for detoxification. It is used as a catalyst in the first step in forming mercapturic acid, which is the water-soluble end product (Gülçin et al. 2016b). Catalyzed by GST in this first step, glutathione (GSH) binds the endogenous and exogenous hydrophobic electrophiles (Gülçin et al. 2016a). In this study, AChE, BChE, GST, and α-glycosidase enzymes were efficiently inhibited by *T. migricus* extracts as shown in Fig. 3. IC₅₀ values for *T. migricus* methanol extract on metabolic enzymes were obtained 31.50 mg/mL for GST, 18.23 mg/mL for AChE, 17.77 mg/mL for BChE and 16.50 mg/mL for α-Gly. Overall, AChE, BChE, GST, and α-Gly were inhibited by *T. migricus* methanol extracts.

Molecular modelling and docking studies

ADME analysis is important for drug molecule design as a preliminary study in performance analysis related to

the absorption, distribution, metabolism, and excretion (Taslimi et al. 2020b). Traditionally, after the determination of these features as a preliminary study with the progression of computer-aided studies clinical trials of the drug are carried out. The Lipinski Five Rule (Lipinski et al. 1997) was designed to assist the development of bioavailable drugs as a guide for all small molecule drugs when evaluating ADME analysis. Under this rule, molecular weight < 500 g/mol, more than five hydrogen bond donors, more than ten hydrogen bond acceptors, and a division coefficient (log *P*) value < 5 are expected. These properties correspond to the poor absorption of the drug in the membrane and the ability to cross the intestinal barriers. In this study, SwissADME software was used to determine ADME features (Table 3). Among the compounds used in the study, it contains < 500 g/mol by weight, Lipophilicity consensus log *P* < 5 and other properties close values.

Fig. 3 The enzyme inhibition of *T. migricus* extract against AChE, BChE, GST, and α-glycosidase enzymes

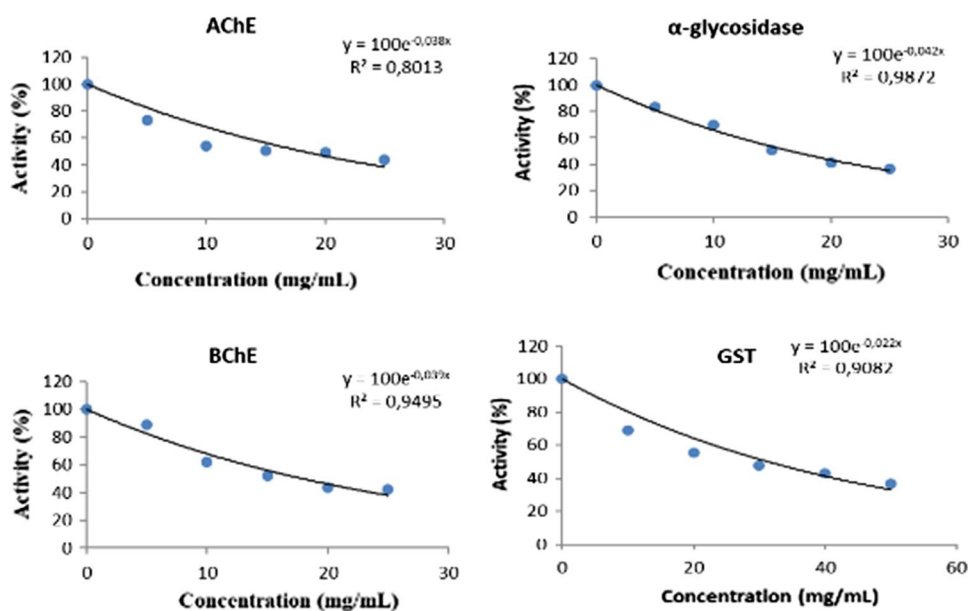


Table 3 Lipophilicity and physicochemical properties of the major compounds of *T. migratus* using SwissADME software

Compounds	Lipophilicity con-sensus log P	MW ^a (g/mol)	Heavy atoms	Aromatic heavy atoms	Rot. bond	H-bond acc	H-bond don	MR ^b	TPSA ^c (Å ²)	% ABS ^d
Chlorogenic acid	− 0.39	354.31	25	6	5	9	6	83.50	164.75	52.16
Cynaroside	0.15	448.38	32	16	4	11	7	108.13	190.28	43.45
Luteolin	1.73	286.24	21	16	1	6	4	76.01	111.13	70.71
p-Coumaric acid	1.26	164.16	12	6	2	3	2	45.13	57.53	88.99
Quinic acid	− 1.69	192.17	13	0	1	6	5	40.11	118.22	68.29

^aMolecular weight^bMolar refractivity^cTopological polar surface area^dPercentage of absorption**Table 4** Best-binding affinity scores (kcal/mol) of phenolic compounds in the catalytic sites of the enzymes

Compounds	Docking Scores			
	GST	α-Gly	AChE	BChE
Chlorogenic acid	− 7.39	− 5.18	− 8.98	− 7.70
Cynaroside	− 8.36	− 6.81	− 8.39	− 7.42
Luteolin	− 5.93	− 5.56	− 8.49	− 8.17
p-Coumaric acid	− 5.40	− 4.77	− 5.69	− 6.31
Quinic acid	− 7.10	− 6.44	− 6.60	− 6.29

Molecular docking of five major phenolic compounds of the plant with AChE, BChE, GST, and α-Gly target enzymes were calculated to predict the binding affinities and binding interactions at the active sites. Totally, 20 separate docking results were obtained from docking analyses. These structures were placed in the catalytic active region of the enzyme and the placement results were analyzed. In the docking of the most active compounds with the highest binding affinity, it was chosen as the best poses for ligand-enzyme interactions. According to the result scores, chlorogenic acid exhibited the highest binding affinity with AChE enzyme (Table 4).

Molecular docking with GST and α-Gly enzymes

After the best binding pose selection for all of the ligand-enzyme docking studies, the binding modes were analyzed to understand the inhibition mechanisms. Figure 4a, b represent 3D and 2D interactions of the quinic acid-GST docking. Glide score of quinic acid in GST binding affinity was calculated as − 7.10 kcal/mol. Here, hydrogen bond has played an important role in protein docking. Effective coupling occurred in regions of the protein such as GLN53, VAL54, GLU66, and SER67.

In the binding affinity with α-Gly, the Glide score of cynaroside was determined as − 6.81 kcal/mol and it was calculated as the highest score. Among the ligand enzymes in the active site, the hydrogen bond, π-cation, π-anion, π-π stacked, carbon-hydrogen bonds have significant bonds with ASP307, ASP352, ARG442, PHE303, and HIS280 residues, respectively. Figure 4c shows the 3D view of the aromatic surface on the receptor and Fig. 4d shows 2D view of cynaroside-α-Gly enzyme interactions. Cynaroside exhibited the best interactions with AChE and GST enzymes. Cynaroside exhibited the best interactions with AChE and GST among enzymes. A previous molecular docking study showed that cynaroside (luteolin-7-glucoside) strongly interacted to α-glucosidase cavity by establishing six hydrogen bonds (Uysal et al. 2018).

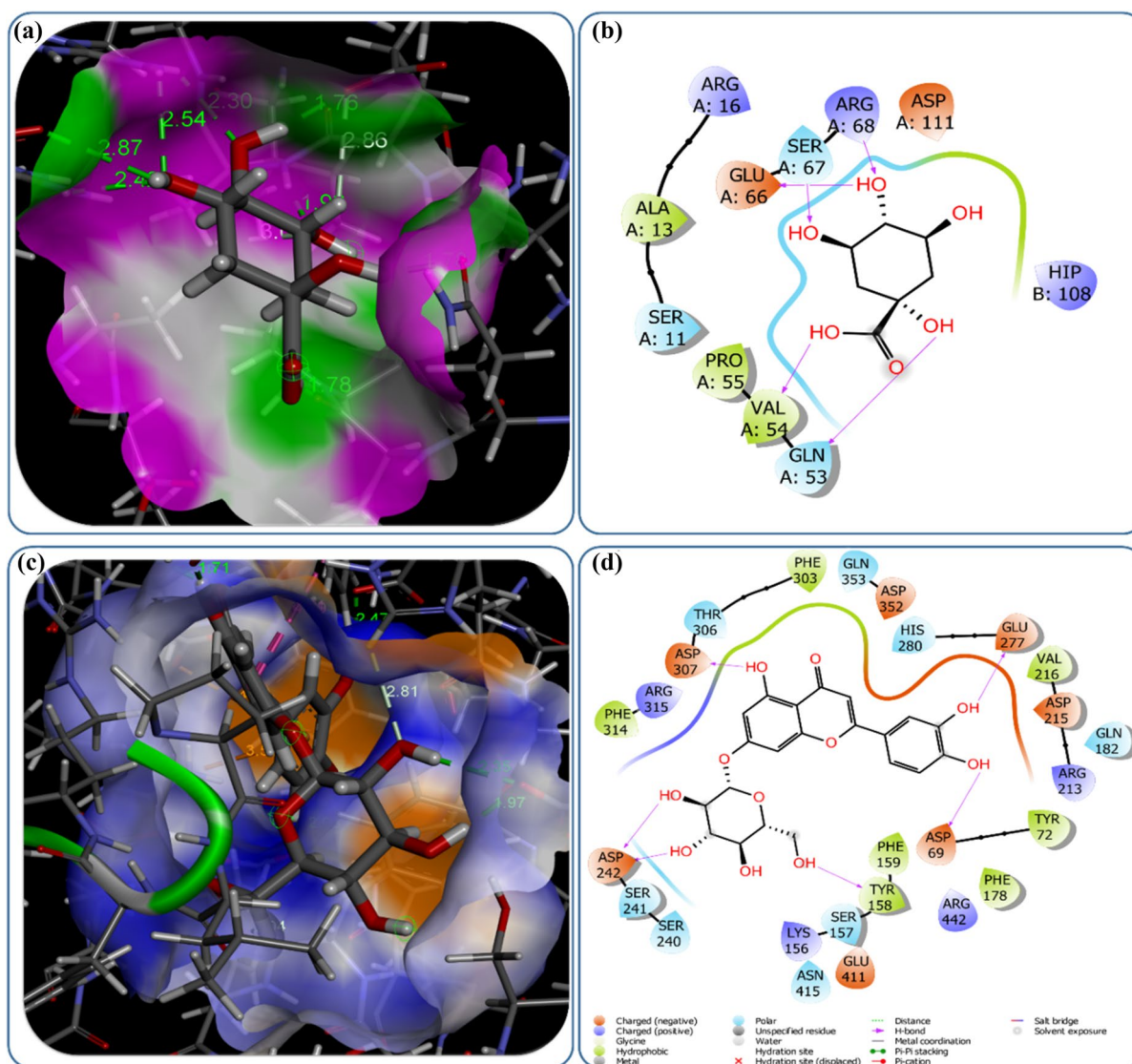


Fig. 4 The interactions of quinic acid-GST and cynaroside- α -Gly

Molecular docking with BChE enzyme

Figure 5a, b represent 3D and 2D interactions of luteolin-BChE docking study. The Glide score of luteolin with BChE (-8.17 kcal/mol) was detected as the highest score. GLU197 hydrogen bond, GLY439 carbon-hydrogen bond, TRP82 π - π stacked of the first benzene ring, TRP82 oxygen-containing ring π - π stacked, ALA328 hydrogen bond are the example ones.

The 3D and 2D interactions of p-coumaric acid with BChE enzyme were showed in Fig. 5c, d. The binding affinity was detected as -6.31 kcal/mol in the binding mechanism of the compound with the BChE enzyme. HIS438 in the active site of the enzyme, TYR128 hydrogen bond, TRP82 π - π stacked on the benzene ring, GIY121 carbon-hydrogen bond interactions were found. All these

observations investigated a good binding of p-coumaric acid with BChE enzyme. Similar to the result of the present study, a previous molecular docking study reported high binding of p-coumaric acid with BChE enzyme (Yener et al. 2020). According to a recent study, molecular docking of p-coumaric acid derivatives against tyrosinase enzyme showed a good binding of tyrosinases and inhibits their activities on melanin synthesis (Varela et al. 2020).

Molecular docking with AChE enzyme

The interactions of chlorogenic acid-AChE are shown in Fig. 6a, d. The Glide score was calculated as -8.98 kcal/mol. For the attachment mechanism TRP286 benzene ring π - π stacked, TYR72 benzene ring π - π stacked, TYR341

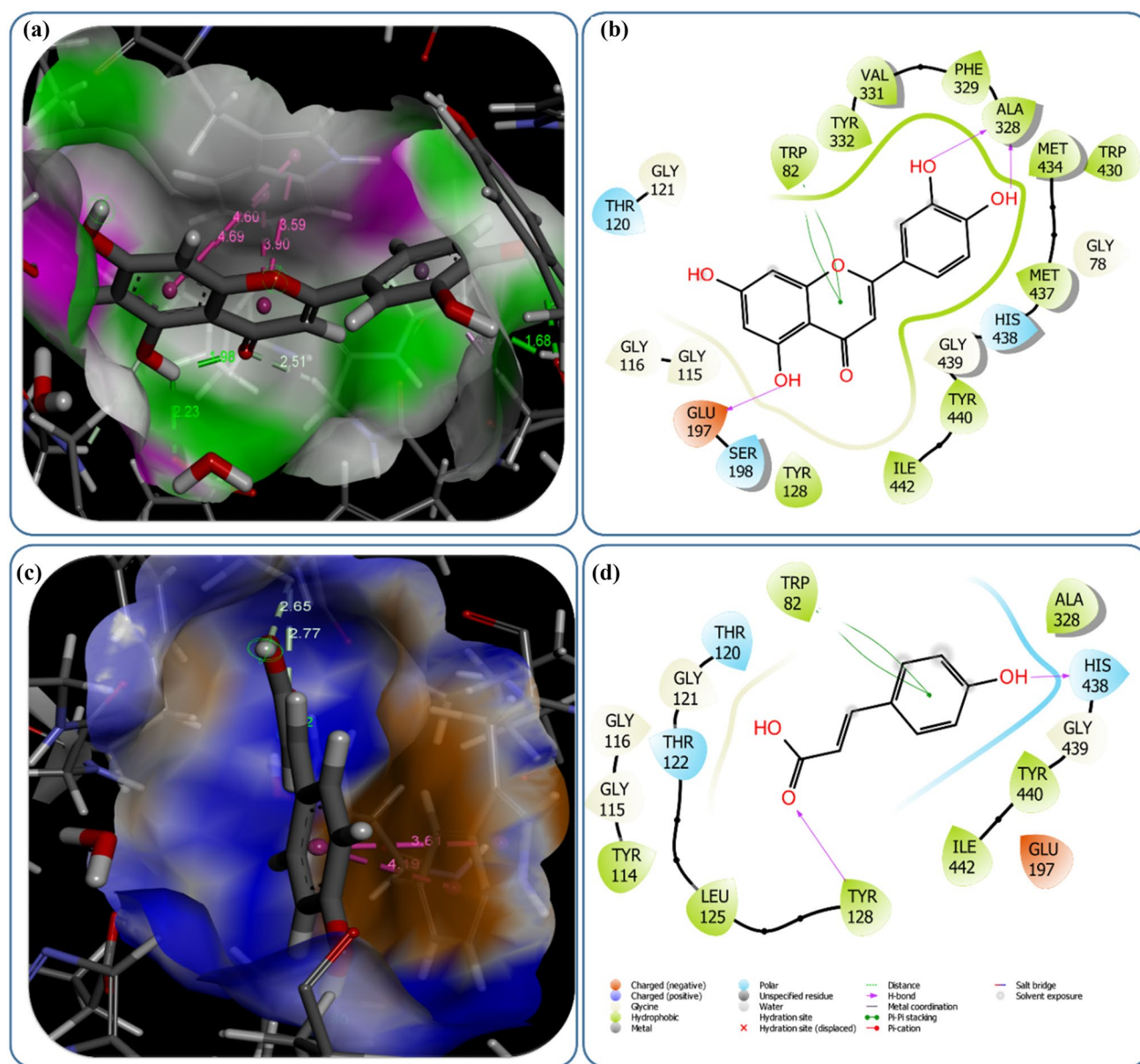


Fig. 5 The interactions of chlorogenic acid-AChE and luteolin-BChE

π -sigma bond, VAL294 carbon–hydrogen bond, TYR124, and VAL282 are the examples of hydrogen bonding.

Chlorogenic acid is a polyphenol derivative found in many plant species and a well-known antioxidant compound. A previous study reported the high binding affinity of chlorogenic acid against four selected matrix metalloproteinases that can help to promote wound healing activity (Govindharaj et al. 2020). According to another study, chlorogenic acid had higher inhibitory potential than other tested compounds against the AChE enzyme (Işık and Beydemir 2020).

Overall, all of the ligand–receptor interactions are detailed in Table 5. Totally, interactions of five major

phenolic compounds of *T. migricus* with four enzyme were evaluated, separately.

Conclusions

In this research, we investigated some biological activities and phenolic content of endemic plant *T. migricus* extracts. The results of antioxidant activity methods indicated that *T. migricus* extracts are able to scavenge radical oxygen species. Also, the extracts have significant enzyme inhibition effects considering to the enzyme methods. Molecular docking of the compounds with enzymes demonstrated

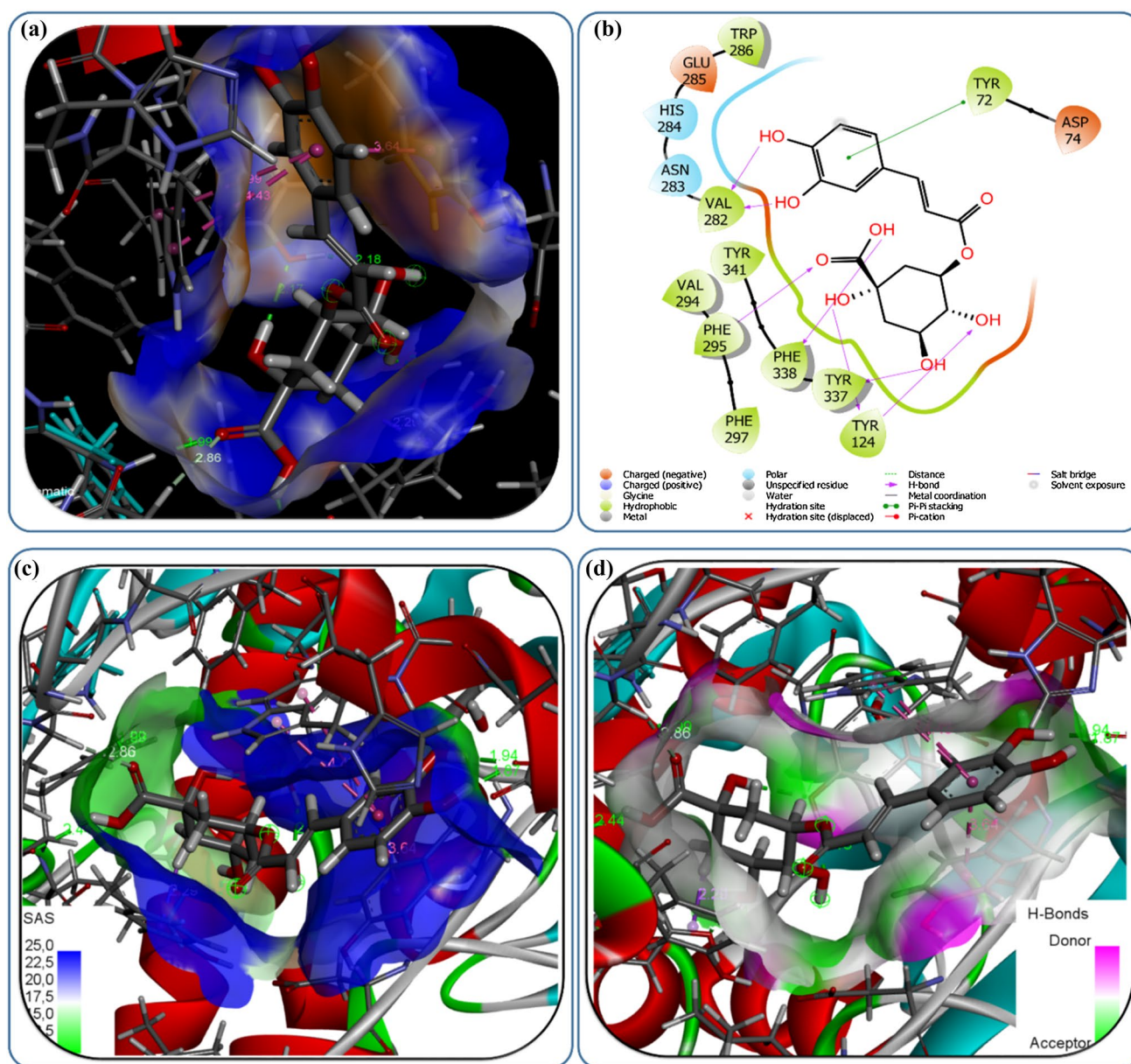


Fig. 6 The interactions of chlorogenic acid-AChE

that chlorogenic acid-AChE, cynaroside- α -Gly, luteolin-BChE, *p*-coumaric acid-BChE, and quinic acid-GST crystal structures had higher affinity scores and more effective in binding energy than the standards. In docking studies, the interactions of the ligands with AChE as well as the inhibition studies showed a relatively better correlation

compared to the other enzymes. In vivo and in silico studies obtained values demonstrated that this plant can be taken into consideration in drug design processes used in DM and AD treatment. Altogether, this study highlighted the impressive pharmacological activity of *T. migricus* as a source of potential bioactive components.

Table 5 The parameters of the interaction modes between the major phenolic compounds of the *T. migricus* and enzymes

Important interactions	Quinic acid-GST ID: 1GNW		Cynaroside- α -Gly ID: 3A4A		Chlorogenic acid-AChE (ID: 5HF9)		Luteolin-BChE ID: 5NN0		p-Coumaric acid-BChE ID: 5NN0	
	Residues	Bond length (Å)	Residues	Bond length (Å)	Residues	Bond length (Å)	Residues	Bond length (Å)	Residues	Bond length (Å)
Conventional Hydrogen Bond	GLN 53	1.78	ASP 242	1.97, 235	PHE 338	2.44	ALA 328	1.68, 2.22	TYR 128	1.82
	VAL 54	1.78	ASP 307	1.71	PHE 295	1.99	GLU 197	2.23	HIS 438	1.80
	SER 67	1.96	GLN 353	2.16	TYR 337	2.14				
	GLU 66	1.76	GLU 277	2.47	TYR 124	2.17, 2.18				
	ARG 68	2.30, 2.54	ASP 69	2.63	VAL 282	1.87, 1.94				
	ASP 111	2.87	TYR 158	1.75						
Carbon Hydrogen Bond	ARG 16	2.42								
	GLU 66	2.86	HIS 280	2.81	VAL 294	2.86	GLY 439	2.51	GLY 121	2.65, 2.77
π - π Stacked			TYR 158	3.04			GLY 115	2.66		
			PHE 303	4.51, 4.79	TYR 72	3.64	TRP 82	4.02, 4.07	TRP 82	3.78
π -Alkyl					TRP 286	4.06				
							ALA 328	4.04		
π -Anion			ASP 352	4.71				N.B		
			ARG 442	3.59				N.B		
π -Cation								N.B		
					TYR 341	2.29		N.B		
π -Sigma										

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Compliance with ethical standards

Conflict of interest No potential conflict of interest was reported by the authors.

References

- Alisi IO, Uzairu A, Abechi SE, Idris SO (2018) Evaluation of the antioxidant properties of curcumin derivatives by genetic function algorithm. *J Adv Res* 12:47–54
- Aouam I, Atki YE, Taleb M, Taroq A, Kamari FE, Lyoussi B, Abdellaoui A (2019) Antioxidant capacities and total phenolic contents of *Thymus riatarum*. *Mater Today Proc* 13:579–586
- Apak R, Güçlü K, Özyürek M, Esin Karademir S, Erçağ E (2006) The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. *Int J Food Sci Nutr* 57:292–304
- Aras A, Silinsin M, Bingol MN, Bursal E (2017) Identification of bioactive polyphenolic compounds and assessment of antioxidant activity of *Origanum acutidens*. *ILNS* 66:1–8
- Aras A, Bursal E, Alan Y, Türkan F, Alkan H, Kılıç Ö (2018) polyphenolic content, antioxidant potential and antimicrobial activity of *Satureja boissieri*. *Iran J Chem Chem Eng Res* 37:209–219
- Aras A, Bursal E, Türkan F, Tohma H, Kılıç Ö, Gülçin İ, Köksal E (2019) Phytochemical content, antidiabetic, anticholinergic, and antioxidant activities of endemic *Lecokia cretica* extracts. *Chem Biodiv* 16:e1900341
- Aslan HE, Demir Y, Özarslan MS, Türkan F, Beydemir Ş, Küfrevioğlu Öİ (2018) The behavior of some chalcones on acetylcholinesterase and carbonic anhydrase activity. *Drug Chem Toxicol* 42:1–7
- Baharfar R, Azimi R, Mohseni M (2015) Antioxidant and antibacterial activity of flavonoid-, polyphenol- and anthocyanin-rich extracts from *Thymus kotschyanus* boiss & hohen aerial parts. *J Food Sci Technol* 52:6777–6783
- Başer KHC, Demirci B, Ne K, Satil F, Tümen G (2002) The essential oils of *Thymus migricus* and *T. fedtschenkoi* var. *handellii* from Turkey. *Flavour Fragrance J* 17:41–45
- Burley SK et al (2019) RCSB Protein Data Bank: biological macromolecular structures enabling research and education in fundamental biology, biomedicine, biotechnology and energy. *Nucleic acids Res* 47:D464–D474
- Burmaoglu S, Yilmaz AO, Polat MF, Kaya R, Gulcin İ, Algul O (2019) Synthesis and biological evaluation of novel tris-chalcones as potent carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase and α -glycosidase inhibitors. *Bioorg Chem* 85:191–197
- Bursal E, Aras A, Kılıç Ö (2019a) Evaluation of antioxidant capacity of endemic plant *Marrubium astracanicum* subsp. *macrodon*: identification of its phenolic contents by using HPLC-MS/MS. *Nat Prod Res* 33:1975–1979
- Bursal E, Aras A, Kılıç Ö, Taslimi P, Gören AC, Gülçin İ (2019b) Phytochemical content, antioxidant activity, and enzyme inhibition effect of *Salvia eriophora* Boiss. & Kotschy against acetylcholinesterase, α -amylase, butyrylcholinesterase, and α -glycosidase enzymes. *J Food Biochem* 43:e12776
- Bursal E, Taslimi P, Gören AC, Gülçin İ (2020) Assessments of anticholinergic, antidiabetic, antioxidant activities and phenolic content of *Stachys annua*. *Biocatal Agric Biotechnol* 28:101711

- Chen Y et al (2017) Discovery of new acetylcholinesterase and butyrylcholinesterase inhibitors through structure-based virtual screening. *RSC Adv* 7:3429–3438
- Cheng Z-Q et al (2019) Molecular-docking-guided design and synthesis of new IAA-tacrine hybrids as multifunctional AChE/BChE inhibitors. *Bioorg Chem* 83:277–288
- Dob T, Dahmane D, Benabdelkader T, Chelghoum C (2006) Studies on the essential oil composition and antimicrobial activity of *Thymus algeriensis* Boiss. et Reut. *Int J Aromather* 16:95–100
- Gonçalves M, Cruz M, Cavaleiro C, Lopes M, Salgueiro L (2010) Chemical, antifungal and cytotoxic evaluation of the essential oil of *Thymus zygis* subsp. *sylvestris*. *Ind Crops Prod* 32:70–75
- Govindharaj D, Nachimuthu S, Gonsalves DF, Kothandan R, Dhurai B, Rajamani L, Ramakrishna S (2020) Molecular docking analysis of chlorogenic acid against matrix metalloproteinases (MMPs). *Biointerface Res Appl* 10:6865–6873
- Gülçin İ, Scozzafava A, Supuran CT, Akıncioğlu H, Koksall Z, Turkan F, Alwasel S (2016) The effect of caffeic acid phenethyl ester (CAPE) on metabolic enzymes including acetylcholinesterase, butyrylcholinesterase, glutathione S-transferase, lactoperoxidase, and carbonic anhydrase isoenzymes I II, IX, and XII. *J Enzyme Inhib Med Chem* 31:1095–1101
- Gülçin İ et al (2016) Rosmarinic acid inhibits some metabolic enzymes including glutathione S-transferase, lactoperoxidase, acetylcholinesterase, butyrylcholinesterase and carbonic anhydrase isoenzymes. *J Enzyme Inhib Med Chem* 31:1698–1702
- Gülçin İ, Gören AC, Taslimi P, Alwasel SH, Kılıç O, Bursal E (2020) Anticholinergic, antidiabetic and antioxidant activities of Anatolian pennyroyal (*Mentha pulegium*)-analysis of its polyphenol contents by LC-MS/MS. *Biocatal Agric Biotechnol* 23:101441
- Gülçin İ et al (2018) Antidiabetic and antiparasitic potentials: inhibition effects of some natural antioxidant compounds on α -glycosidase, α -amylase and human glutathione S-transferase enzymes. *Int J Biol Macromol* 119:741–746
- Gulcin İ (2020) Antioxidants and antioxidant methods: an updated overview. *Arch Toxicol* 94:651–715
- Hassan W et al (2017) Oxidative stress and antioxidant potential of one hundred medicinal plants. *Curr Top Med Chem* 17:1336–1370
- Hazzit M, Baaliouamer A, Verissimo A, Faleiro M, Miguel MG (2009) Chemical composition and biological activities of Algerian *Thymus* oils. *Food Chem* 116:714–721
- Işık M, Beydemir Ş (2020) The impact of some phenolic compounds on serum acetylcholinesterase: kinetic analysis of an enzyme/inhibitor interaction and molecular docking study. *J Biomol Struct Dyn*. <https://doi.org/10.1080/07391102.2020.1801509>
- Işık M, Demir Y, Durgun M, Türkerş C, Necip A, Beydemir Ş (2019) Molecular docking and investigation of 4-(benzylideneamino)- and 4-(benzylamino)-benzenesulfonamide derivatives as potent AChE inhibitors. *Chem Papers* 1–11
- Karimov A et al (2020) Novel functionally substituted esters based on sodium diethyldithiocarbamate derivatives: Synthesis, characterization, biological activity and molecular docking studies. *Bioorg Chem* 99:103762
- Kocycigit UM et al (2018) Synthesis of chalcone-imide derivatives and investigation of their anticancer and antimicrobial activities, carbonic anhydrase and acetylcholinesterase enzymes inhibition profiles. *Arch Physiol Biochem* 124:61–68
- Koeduka T et al (2014) Bioactivity of natural O-prenylated phenylpropanes from *Illicium anisatum* leaves and their derivatives against spider mites and fungal pathogens. *Plant Biol* 16:451–456
- Köksal E, Bursal E, Gülçin İ, Korkmaz M, Çağlayan C, Gören AC, Alwasel SH (2017) Antioxidant activity and polyphenol content of Turkish thyme (*Thymus vulgaris*) monitored by liquid chromatography and tandem mass spectrometry. *Int J Food Prop* 20:514–525
- Küçükbay FZ, Kuyumcu E, Azaz AD, Arabacı T, Yüçetürk SÇ (2014) Chemical composition of the essential oils of three *Thymus* taxa from Turkey with antimicrobial and antioxidant activities. *Rec Nat Prod* 2:110–120
- Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 23:3–25
- Lu X et al (2019) Expansion of the scaffold diversity for the development of highly selective butyrylcholinesterase (BChE) inhibitors: discovery of new hits through the pharmacophore model generation, virtual screening and molecular dynamics simulation. *Bioorg Chem* 85:117–127
- Nirmala C, Bisht MS, Bajwa HK, Santosh O (2018) Bamboo: a rich source of natural antioxidants and its applications in the food and pharmaceutical industry. *Trends Food Sci Technol* 77:91–99
- Release S (2019) 3: Maestro Schrödinger. LLC, New York, p 2019
- Rendra E, Riabov V, Mossel DM, Sevastyanova T, Harmsen MC, Kzhyshkowska J (2019) Reactive oxygen species (ROS) in macrophage activation and function in diabetes. *Immunobiology* 224:242–253
- Rota MC, Herrera A, Martínez RM, Sotomayor JA, Jordán MJ (2008) Antimicrobial activity and chemical composition of *Thymus vulgaris* *Thymus zygis* and *Thymus hyemalis* essential oils. *Food Control* 19:681–687
- Silinsin M, Bursal E (2018) UHPLC-MS/MS phenolic profiling and in vitro antioxidant activities of *Inula graveolens* (L.) Desf. *Nat Prod Res* 32:1467–1471
- Tao Y, Zhang Y, Cheng Y, Wang Y (2013) Rapid screening and identification of α -glucosidase inhibitors from mulberry leaves using enzyme-immobilized magnetic beads coupled with HPLC/MS and NMR. *Biomed Chromatogr* 27:148–155
- Taslimi P et al (2020) Anti-Alzheimer, antidiabetic and antioxidant potential of *Satureja cuneifolia* and analysis of its phenolic contents by LC-MS/MS. *Arab J Chem* 13:4528–4537
- Taslimi P, Turhan K, Türkan F, Karaman HS, Turgut Z, Gulcin I (2020) Cholinesterases, α -glycosidase, and carbonic anhydrase inhibition properties of 1H-pyrazolo [1, 2-b] phthalazine-5, 10-dione derivatives: synthetic analogues for the treatment of Alzheimer's disease and diabetes mellitus. *Bioorg Chem* 97:103647
- Tohidi B, Rahimmalek M, Arzani A (2017) Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of *Thymus* species collected from different regions of Iran. *Food Chem* 220:153–161
- Türkan F, Huyut Z, Taslimi P, Gülçin İ (2019) The effects of some antibiotics from cephalosporin groups on the acetylcholinesterase and butyrylcholinesterase enzymes activities in different tissues of rats. *Arch Physiol Biochem* 125:12–18
- Turrens JF (2003) Mitochondrial formation of reactive oxygen species. *J Physiol* 552:335–344
- Uysal S et al (2018) Novel in vitro and in silico insights of the multi-biological activities and chemical composition of *Bidens tripartita* L. *Food Chem Toxicol* 111:525–536
- Varela MT, Ferrarini M, Mercaldi VG, da Silva SB, Padovani G, Nazato LIS, Fernandes JPS (2020) Coumaric acid derivatives as tyrosinase inhibitors: efficacy studies through in silico, in vitro and ex vivo approaches. *Bioorg Chem* 103:104108–104108
- Visualizer DS (2005) Accelrys software inc Discovery Studio Visualizer 2
- Yates K, Pohl F, Busch M, Mozer A, Watters L, Shiryaev A, Lin PKT (2019) Determination of sinapine in rapeseed pomace extract: its antioxidant and acetylcholinesterase inhibition properties. *Food Chem* 276:768–775

- Yener I et al (2020) Selective in vitro and in silico enzymes inhibitory activities of phenolic acids and flavonoids of food plants: relations with oxidative stress. *Food Chem* 327:127045
- Yilmaz MA (2020) Simultaneous quantitative screening of 53 phytochemicals in 33 species of medicinal and aromatic plants: a

detailed, robust and comprehensive LC–MS/MS method validation. *Ind Crops Prod* 149:112347

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