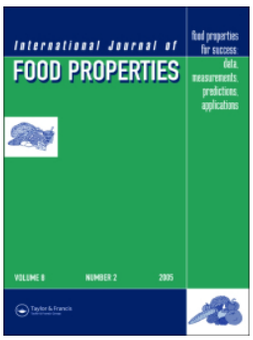


International Journal of Food Properties

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ljfp20

Anticholinergic and antioxidant activities of avocado (*Folium perseae*) leaves – phytochemical content by LC-MS/MS analysis

Leyla Polat Kose, Zeynebe Bingol, Ruya Kaya, Ahmet C. Goren, Hulya Akincioglu, Lokman Durmaz, Ekrem Koksal, Saleh H. Alwasel & İlhami Gülçin

To cite this article: Leyla Polat Kose, Zeynebe Bingol, Ruya Kaya, Ahmet C. Goren, Hulya Akincioglu, Lokman Durmaz, Ekrem Koksal, Saleh H. Alwasel & İlhami Gülçin (2020) Anticholinergic and antioxidant activities of avocado (*Folium perseae*) leaves – phytochemical content by LC-MS/MS analysis, International Journal of Food Properties, 23:1, 878-893, DOI: 10.1080/10942912.2020.1761829

To link to this article: https://doi.org/10.1080/10942912.2020.1761829

Published online: 16 Jun 2020.

INTERNATIONAL JOURNAL OF FOOD PROPERTIES
2020, VOL. 23, NO. 1, 878–893
https://doi.org/10.1080/10942912.2020.1761829

Anticholinergic and antioxidant activities of avocado (*Folium perseae*) leaves – phytochemical content by LC-MS/MS analysis

Leyla Polat Kose[a], Zeynebe Bingol[b], Ruya Kaya[b,c], Ahmet C. Goren[d,e], Hulya Akincioglu[f], Lokman Durmaz[g], Ekrem Koksal[h], Saleh H. Alwasel[i], and İlhami Gülçin[b]

[a]Vocational School, Department of Pharmacy Services, Beykent University, Buyukcekmece, Istanbul, Turkey; [b]Faculty of Sciences, Department of Chemistry, Atatürk University, Erzurum, Turkey; [c]Central Research and Application Laboratory, Agri Ibrahim Cecen University, Agri, Turkey; [d]Department of Analytical Chemistry, Faculty of Pharmacy, Bezmialem Vakif University, Istanbul, Turkey; [e]Drug Application and Research Center, Bezmialem Vakif University, Istanbul, Turkey; [f]Department of Chemistry, Faculty of Sciences and Arts, Agri Ibrahim Cecen University, Agri, Turkey; [g]Department of Medical Services and Technology, Cayirli Vocational School, Erzincan Binali Yildirim University, Cayirli, Erzincan, Turkey; [h]Faculty of Sciences and Arts, Department of Chemistry, Erzincan Binali Yildirim University, Erzincan, Turkey; [i]King Saud University, Department of Zoology, College of Science, Saudi Arabia

ABSTRACT

In the first stage of the manuscript, we aimed to examine antioxidant capacity and anticholinergic properties of avocado (*Folium perseae*) leaves. Avocado leaf was extracted by water (WEFP) and ethyl alcohol (EEFP) and antioxidant activity was determined using by several antioxidant assays including DPPH· and $ABTS^{\bullet+}$ radical scavenging assays, Cu^{2+}-Cu^{+} reducing, Fe^{3+}-Fe^{2+} reducing, and FRAP reducing activities. Avocado leaf extracts demonstrated antioxidant activity and anticholinergic activities, while α-tocopherol, BHT, trolox, and BHA were used as positive antioxidant controls. In the second part of this study, the inhibition effects of WEFP and EEFP were valuated against acetylcholinesterase and butyrylcholinesterase enzymes, which catalyze the breakdown of choline esters (i.e. neurotransmitters). This study obviously showed that avocado leaf extracts had effective antioxidant, antiradical, and anticholinergic influences.

ARTICLE HISTORY
Received 10 January 2020
Revised 21 April 2020
Accepted 22 April 2020

KEYWORDS
Avocado; *Folium perseae*; antioxidant activity; radical scavenging; acetylcholinesterase; butyrylcholinesterase

Introduction

Avocado (*F. perseae*) belongs to Lauraceae family. It was cultivated in the Mediterranean region and Eastern Black Sea region in Turkey. Homeland of avocado is Puebla in Mexico. Both leaves and fruit of avocado have many benefits. Among them, fruits are the richest with regard to protein. It also contains plenty of vitamin E, tannins, and essential oils. Avocado leaf had also blood pressure-lowering effect due to its potassium content.[1] Recently, it was reported that the risk of some disorders including cancer, cataract, and cardiovascular disease was prevented by foods containing natural antioxidants.[2,3]

Oxidation is a biological processes that performing electron transfer between electron receiver and transmitter atoms. Although oxygen is too essential for human life, it has the potential intensive damage to the body. Some reactive oxygen species (ROS) were produced during normal daily metabolism. Compared to normal oxygen molecule with ROS formed by free radicals, which had higher chemical reactivity.[4,5] Free radicals are unstable compounds with high-energy comprising one or more pair of electrons in outer atomic orbitals. Oxidative stress is associated with many diseases including cancer and cardiovascular diseases. Plants contain phenolic compounds, which possessed strong antioxidant activity. So, plant phytochemicals can prevent many chronic diseases.[6–8]

CONTACT İlhami Gülçin igulcin@atauni.edu.tr Faculty of Sciences, Department of Chemistry, Atatürk University, -Erzurum, Turkey

ROS are reactive chemical species of oxygen, which contain free radicals and non-free radicals. They may occur in the human body when exposure to radiation, stress, environmental pollution, cigarette smoke, and toxic chemicals.[9–11] A normal cell already has sufficient antioxidant-prooxidant balance. However, this balance may vary towards the oxidant in case of excessive ROS production or insufficient antioxidants. Oxidative stress can easily occurs at this stage.[12,13] In recent decades, the interest has been enhanced considerable in the plants and fruits, which are the sources of antioxidants.[14–16] In addition to, the restriction of synthetic antioxidants has been increased the interest and demands in natural antioxidants.[17–19]

Up to now, many health benefits of avocado have been identified. It has too effective strength against constipation and the immune system. Avocado contains polyunsaturated fatty acids and prevents the cholesterol level in the blood.[20] In this way, it may delay aging process and plays an important role in preventing of some chronicle diseases. Its extract has been identified and used as a potential treatment anti-diabetes mellitus and hypertension by researchers.[21] Also, different parts of avocado are prevalent using in traditional folk medicines to treat, management, or control of some human diseases. It was reported that aqueous extract of the avocado leaf had anticonvulsant property. Also, it had rich ingredient medium in terms of vitamins, potassium, phytosterols, lutein, and zeaxathin.[22]

Cholinesterases (ChEs) are enzymes having a wide distribution at the cholinergic or not cholinergic tissues available to in plasma and other body fluids. [23,24] A strong pharmacological effect of acetylcholine (ACh) was found in 1906. ChEs also play a role of cell renewal and differentiation against stress.[25] ChEs were determined according to substrate specificity, behaviours in saturated substrate concentration and affinity of inhibitors. These enzymes are called acetylcholinesterase (AChE) and butyrylcholinesterase (BChE).[26–28] AChE presents in the brain, muscle, liver, spleen, and erythrocytes. The most important function of AChE is hydrolysis of ACh, which a neurotransmitter used at the neuromuscular junction. AChE hydrolysis ACh to the choline (Ch) and acetate ions.[29,30] High concentrations of ACh cause Parkinson's disease (PD). On the other hand, BChE found in the serum, heart, pancreas, central nervous system, and liver.[31,32] Also, it makes hydrolysis and regulation of butrylcholine (BCh). AChE inhibitors (AChEIs) have been used as drugs for treatment of some neurological disorders including myasthenia gravis, glaucoma, postural tachycardia syndrome, and Alzheimer's disease (AD).[33–35]

The aim of this study was to determine both anticholinergic and antioxidant properties of water and ethanol extracts of avocado (*F. perseae*) leaves using some bioanalytical methods including DPPH·, $ABTS^{\bullet+}$ radicals scavenging, Cu^{2+} reducing (CUPRAC), Fe^{3+} reducing and FRAP reducing activities.

Materials and methods

Chemicals

5,5′-Dithio-bis(2-nitro-benzoic acid), butyrylthiocholine, acetylthiocholine iodide, BHA, BHT, DPPH, ABTS, and α-tocopherol were commercially obtained from Sigma-Aldrich. The other chemicals were used as analytical grade. Gallic acid (97.5–102.5%, Sigma-Aldrich), herniarin (>98%, Carl Roth GMBH), kaempferol (>90%, Sigma-Aldrich), quercitrin (>97%, TRC Canada), fumaric acid (≥99%, Sigma-Aldrich), pyrogallol (≥98%, Sigma-Aldrich), caffeic acid (98%, Sigma-Aldrich), quercetin (≥95%, Sigma-Aldrich), ellagic acid (>97%, TRC Canada), chlorogenic acid (≥95%, Sigma-Aldrich), rosmarinic acid (>96%, Sigma-Aldrich), luteolin-7-glucoside (98%, Carbosynth limited), luteolin-5-glucoside (>96%, Carbosynth limited), kaempferol-3-O-rutinoside (≥98%, Sigma-Aldrich), rutin (≥99%, Sigma-Aldrich). Curcumin (97%) isolated and purified from *Curcuma longa* by our lab.

Water extract of avocado (Folium perseae) leaves

The lyophilized water extract of avocado *(F. perseae)* leaves (WEFP) was performed according to our previous studies.[36] For this purpose, 50 g of dried aerial parts of avocado *(F. perseae)* leaves was powdered, mixed with boiling water (500 mL) and stirred for half hour. The water extract was filtered over cheesecloth and Whatman paper (No. 1), respectively. Then, the residue was frozen in a freezer at −84°C (Sanyo, Japan). Finally, the frozen water extract was lyophilized (50°C, 5 mm-Hg). The WEFP was transferred to a flask and stored until use (−20°C).[37]

Ethanol extracts of avocado (Folium perseae) leaves

Evaporated ethanol extract of avocado *(F. perseae)* leaves (EEFP) was realized according to the previous study.[38] For this purpose, 50 g dried aerial parts of avocado *(F. perseae)* leaves was powdered in a mill and mixed with 500 mL of ethanol and extracted over 1 h. Then, the extracted sample was filtered through paper (Whatman No. 1) and evaporated at 40°C.[39] The ethanolic residue of the avocado *(F. perseae)* leaves was re-extracted under similar extraction conditions until the methanol became colourless. EEFP was transferred to a bottle and stored until use (−20°C).

Total phenolic content of avocado (Folium perseae) leaves

Total phenolic content of WEFP and EEFP were determined using Folin–Ciocalteau methods.[40] This specific Folin–Ciocalteau reagent forms a blue complex with polyphenols, which can be spectrophotometrically quantified at 760 nm.[41] For this purpose, 1 g of WEFP and EEFP or standard solution was taken into test tube and 23 mL of final volume was achieved with distilled water. Then, 0.5 mL of Folin–Ciocalteau reagent was transferred to test tube and after 5 min, 1.5 mL of Na_2CO_3 solution (2%) was added. After being vortexed and keeping in room temperature in darkness for half hour, the absorbance of the samples was spectrophotometrically recorded. Various concentrations of gallic acid ranging from 0 to 500 µg were used as a standard phenolic compound along with the samples and the amount of total phenols were calculated by using gallic acids calibration curve.

Total flavonoid content of avocado (Folium perseae) leaves

Total flavonoid content of WEFP and EEFP was determined by a colorimetric assay.[42] In this assay, 1 mL of WEFP or EEFP was taken into a test tube and 100 µL potassium acetate (1.0 M), 0.1 mL of 10% $Al(NO_3)_3$ in 4.3 mL of ethanol solution were transferred to the samples. Then the mixtures were vortexed and stand at room temperature for half hour. The absorbance of the samples was taken in triplicate at 415 nm by using a UV-vis spectrophotometer. The standard curve of quercetin at different concentrations ranging from 0 to 100 µg was used for determination of flavonoid contents of WEFP and EEFP. The results are recorded as µg quercetin equivalents per g extract.[43]

Preparation of standards and test solution for LC-MS/MS

Stock solutions of standards were prepared as 200 mg/L in ethanol-water. Calibration solutions were prepared in ethanol-water (50:50, v/v) in a linear range (0.1, 0.2, 0.5, 1, 5 and 10 mg/L). About 30 µL of curcumin (500 mg/L in methanol) was used as an Internal Standard (IS) in all LC-MS/MS experiments. About 50–70 mg of each extract weighed in a round bottom flask and 3 mL of the ethanol-water mixture (50:50 v/v) was added. In order to obtain a good solubility, the flask was gently heated at 60°C on an ultrasonic bath until a clear solution was obtained. It takes approximately 15 min. The solutions were then transferred into a 5 mL of volumetric flask,

rinsed with a 200 µL ethanol-water mixture (50:50 v/v) for three times and diluted to volume with a mobile phase. A portion of 1 mL of this stock solution was transferred into 5 mL of another volumetric flask, and 30 µL of curcumin (500 mg/L in methanol) solution was added as internal standard and diluted to the volume with mobile phase. The solution was filtered through a 0.45 µm Millipore Millex-HV filter and the final solution (1 mL) was transferred into a capped auto sampler vial and 10 mL of sample was injected to LC for each run. The samples in the auto sampler were kept at 150° C during the experiment.[44,45]

HPLC-MS conditions

Measurements of secondary metabolites were conducted by a Zivak® HPLC (high performance liquid chromatography) and Zivak® Tandem Gold Triple quadrupole (Istanbul, Turkey) mass spectrometry equipped with a C18 (150 × 3 mm; 3 µm) column (Fortis Technologies, UK). CID gas pressure of 2.40 mTorr, 5000 V ESI needle voltage, 600 V ESI (electrospray ionization) shield voltage, 300°C drying gas temperature, 50°C API housing temperature, 55 psi nebulizer gas pressure, and 40 psi drying gas pressure were determined as optimum ESI parameters.[46,47] LC-MS/MS parameters of secondary metabolites and internal standard are given in Table 1. The LODs were determined to be three times bigger than while LOQs were determined to be 10 times bigger than standard deviation (Table 2). The validation and uncertainty evaluation procedure and the results of validation are discussed in former studies.[40,46,47]

Biological activities

Reducing assays

The first method for determination of reducing ability of avocado leaf was $Fe^{3+}(CN^{-})_6$ reduction method, which had maximum absorbance at 700 nm.[48–50] Cu^{2+} reducing ability was used as important antioxidant-reducing assay for both extracts of avocado leaf. This method was exerted according to the method of Gulcin[51] as described in details.[52] The absorbances were registered at 450 nm after half hour.[29] FRAP assay, which the last used reducing method, is based on degradation of Fe^{3+}-TPTZ complicated.[53] The increased absorbance of Fe^{2+}-TPTZ was spectrometrically reported at 593 nm as described in our study.[54]

Table 1. LC-MS/MS parameter of selected compounds in water and ethanol extracts of avocado (*Folium perseae*) leaves [EEFP: ethanol extract of avocado (*F. perseae*) leaves, WEFP: water extract of avocado (*F. perseae*) leaves].

	Compounds	Parent ion	Daughter ion	Collision energy (V)
1	Gallic acid	168.6	124	13
2	Herniarin	177	121	12
3	Kaempferol	287	152.3	30
4	Quercitrin	471.9	309.9	16
5	Fumaric acid	115	71	8
6	Pyrogallol	125	80	16
7	Caffeic acid	179	135	10
8	Quercetin	301	178.5	16
9	Ellagic acid	301	228.3	25
10	Chlorogenic acid	353	191	14
11	Rosmarinic acid	359.2	160.5	15
12	Luteolin-7-glucoside	447	284.5	14
13	Luteolin-5-glucoside	447	289.5	20
14	Kaempferon-3-O-rutinoside	593	284.4	18
15	Rutin	609	301	16
16	Curcumin*	369.3	176.9	20

Notes: * Used as internal standard.

Table 2. Validation and uncertainty parameters of selected compounds.

Compounds	Linear regression equation	r^2	LOD (mg/L)	LOQ (mg/L)	RSD (%)
Gallic acid	$y = 0.0569x + 0.0177$	0.991	0.002	0.008	4.85
Herniarin	$y = 0.0325x + 0.0107$	0.9917	0.370	1.233	9.47
Kaempferol	$y = 0.0230x + 0.0116$	0.984	0.002	0.008	5.47
Quercitrin	$y = 0.0290x + 0.0058$	0.9918	0.001	0.002	4.28
Fumaric acid	$y = 0.0569x + 0.0177$	0.991	0.003	0.01	5.44
Pyrogallol	$y = 0.0438x + 0.0073$	0.980	0.001	0.002	5.47
Caffeic acid	$y = 0.3300x + 0.0036$	0.992	0.028	0.093	8.04
Quercetin	$y = 0.1150x + 0.0078$	0.993	0.001	0.002	0.11
Ellagic acid	$y = 0.2358x + 0.005$	0.9950	0.020	0.068	0.11
Chlorogenic acid	$y = 0.2620x + 0.0674$	0.998	0.445	1.483	5.45
Rosmarinic acid	$y = 0.1960x + 0.0043$	0.998	0.022	0.072	3.73
Luteolin-7-glucoside	$y = 0.1350x + 0.0246$	0.995	0.022	0.072	8.56
Luteolin-5-glucoside	$y = 0.2300x + 0.0413$	0.992	0.01	0.034	1.12
Kaempferon-3-O-rutinoside	$y = 0.1080x + 0.0135$	0.997	0.014	0.045	8.15
Rutin	$y = 0.0232x + 0.0008$	0.996	0.01	0.034	7.90

Radical scavenging assays

The DPPH· solution was daily prepared and kept in a glass flask in the dark (4°C). An aliquot (1.5 mL) of avocado leaf extracts dissolved in ethanol and transferred to fresh 500 µL of DPPH· solution (0.1 M). These mixtures were strongly stirred and incubated in the dark (30 min). Then, their absorbances were spectrophotometrically registered at 517 nm.[55] $ABTS^{\bullet+}$ was obtained by reacting of ABTS (7.0 mM) to $K_2S_2O_8$ (2.5 mM). $ABTS^{\bullet+}$ scavenging ability of both extracts of avocado leaf was done according to the spectroscopic method defined previously.[56–59] Radicals scavenging activity was calculated from the equation of RSI (%) = [1 - (*As*/*Ac*)] × 100. Where RSI is radical scavenging influences, *As* and *Ac* are the absorbance values of samples and control, respectively.[60]

Anticholinergic assay

Inhibition influences for avocado extracts on AChE/BChE enzymes were calculated by Ellman's method[61] as described in our previous studies.[62–64] DTNB and acetylcholine iodide (AChI)/ butrylcholine iodide (BChI) were used for the assigned of the AChE/BChE activities. Measurements were recorded at the maximum absorption at the wavelength of 412 nm. Half maximal inhibition concentration (IC_{50}) was obtained from activity (%) towards for the extract plots.[65]

Statistical analysis

The experiment regarding antioxidant activity was carried out at triplicate. The values were expressed as mean ± standard deviation and analyzed by SPSS (version 11.5 for Windows 98, SPSS Inc.). A one-way ANOVA was performed to determine the significance of difference. The significant differences between the means were determined by LSD tests. $p < 0.05$ was accepted as significant while $p < 0.01$ was regarded as being substantially significant.

Results and discussion

Antioxidant compounds from natural resources are the safer ones and they are only alternative against synthetic antioxidants. So, they were discovered sources of antioxidants found in nature. At the same time, various methods have been developed and used for determination of natural antioxidants capacities.[66–68] For this aim, the antioxidant capacities of both extracts of avocado leaf were carried out by some antioxidant experiments.

Table 3. Determination of reducing power by potassium ferricyanide reduction method and, CUPRAC and FRAP methods of avocado (*Folium perseae*) leaves at the same concentration (30 µg/mL) [EEFP: ethanol extract of avocado (*F. perseae*) leaves, WEFP: water extract of avocado (*F. perseae*) leaves].

	Fe^{3+}-Fe^{2+} reducing		Cu^{+}-Cu^{+} reducing		Fe^{3+}-TPTZ reducing	
Antioxidants	λ_{700}	r^2	λ_{450}	r^2	λ_{593}	r^2
BHA	2.170 ± 0.005	0.9616	2.396 ± 0.018	0.9107	2.853 ± 0.003	0.8282
BHT	1.490 ± 0.002	0.9950	2.020 ± 0.004	0.9206	2.026 ± 0.002	0.8870
Trolox	1.170 ± 0.001	0.9955	1.452 ± 0.050	0.9970	2.102 ± 0.003	0.9201
α-Tocopherol	1.101 ± 0.006	0.9631	1.262 ± 0.018	0.9920	1.855 ± 0.001	0.9175
EEFP	0.606 ± 0.002	0.9691	0.690 ± 0.001	0.9592	1.316 ± 0.001	0.8102
WEFP	0.401 ± 0.002	0.9538	0.309 ± 0.002	0.9926	0.982 ± 0.002	0.8945

The first method used for this purpose was Fe^{3+} reduction assay (Figure 2). In general, the reduction characteristics depend on the presence of reductive agents, which possessed antioxidant and radical scavenging capability with a hydrogen atom donor.[69,70] $Fe^{3+}(CN^{-})_6$ reducing assay have been utilized for capacity of any molecule and extract. $Fe^{3+}(CN^{-})_6$ reducing ability of both avocado leaf extracts were compared to the standards including BHT, BHA, trolox, and α-tocopherol. [71,72] As seen in Fig. 2a and Table 3, EEFP (0.606, r^2: 0.9691) and WEFP (0.401, r^2: 0.9538) showed marked Fe^{3+} reducing ability and these differences were statistically found important ($p < 0.01$).

Reducing power of 30 µg/mL of EEFP (r^2: 0.9691), WEFP (r^2: 0.9538), and standards were given as the follows: BHA (2.170; r^2: 0.9616) > BHT (1.490; r^2: 0.9950) > trolox (1.170; r^2: 0.9955) > α-tocopherol (1.101; r^2: 0.96631) > EEFP (0.606; r^2: 0.9691) > WEFP (0.401; r^2: 0.9538). These results showed that both avocado leaf extracts had effective Fe^{3+} reducing ability. It also shows the ability to create stable products by provide electron to neutralizing free radicals.

Reduction reactions that occur in metabolic conditions can cause devastating effects to the cell. Reduction capacity of the plant extract can be evaluated by reducing Fe^{3+} ions. This assay was frequently used for reduction of Fe^{3+} ions in the presence of the plant extracts.[73,74] Fe^{3+} reduction assay, which consisting of the ferric salt used as an oxidant, is advantageous for the electron chain reaction.[75] The Cuprac method is easy, fast, selective, inexpensive, and steady to the very different antioxidant system can be applied. This assay is easily complete within half hour.[76] Both avocado leaf extracts and positive controls showed effective Cu^{2+} reducing capacity for in Fig. 2b. Between Cu^{2+} reduced power and avocado leaf extract in different concentrations, there was a positive relevance. It was found that Cu^{2+} reducing ability of avocado leaf extracts was addicted to three different concentrations (10–30 µg/mL, Table 3). Cu^{2+} reducing ability of avocado leaf extracts and standards at the concentration of 30 µg/mL showed the following order: BHA (2.396; r^2: 0.9107) > BHT (2.020; r^2: 0.9206) > trolox (1.452; r^2: 0.9970) > α-tocopherol (1.262; r^2: 0.9920) > EEFP (0.690; r^2: 0.9592) > WEFP (0.309; r^2: 0.9926).

There was a positive correlation between Fe^{3+} and Cu^{2+} reducing powers. Most effective reducing power was found in BHA and comparatively the lowest powerful reductive power was viewed in WEFP for both methods. Fe^{2+} ions can be defined spectrophotometrically owing to its coloured complex with TPTZ. This complex demonstrated absorbance at 593 nm.[77] As can see in Fig. 2c and Table 3, Cu^{2+} reducing effect of avocado leaf extracts and standards at the 30 µg/mL concentration ordered as following: BHA (2.853; r^2: 0.8282) > trolox (2.102; r^2: 0.9201) > BHT (2.026; r^2: 0.870) > α-tocopherol (1.855; r^2: 0.9175) > EEFP (1.316; r^2: 0.8945) > WEFP (0.982; r^2: 0.8102). The FRAP method is used to determine the total reduction capacity of pure antioxidant molecules or plant extracts. FRAP assay was chosen for assessment of the reducing

Table 4. Determination of half maximal scavenging concentrations (IC_{50}, μg/mL) of DPPH· and $ABTS^{\bullet+}$ scavenging, and AChE/BChE inhibition assays for EEFP and WEFP [EEFP: ethanol extract of avocado (*F. perseae*) leaves, WEFP: water extract of avocado (*F. perseae*) leaves].

	DPPH· scavenging		$ABTS^{\bullet+}$ scavenging		AChE inhibition		BChE inhibition	
Antioxidants	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2
BHA	45.6456	0.9482	18.8416	0.7539	–	–	–	–
BHT	87.9514	0.8916	16.9169	0.9159	–	–	–	–
α-Tocopherol	85.8573	0.9781	125.8622	0.9217	–	–	–	–
Trolox	58.1614	0.9585	17.0084	0.7263	–	–	–	–
EEFP	240.400	0.9989	286.0504	0.9829	0.0168	0.9803	0.0214	0.9912
WEFP	601.001	0.9876	524.4258	0.9883	0.0171	0.9911	0.0226	0.9945
Tacrine	–	–	–	–	1.0376	0.9922	0.0870	0.9862

effects of avocado leaf extracts for some reasons. This method is relatively easy and basic to be standardized.[78] At the same time, this reduction assay has been frequently used for a quick assignation of the antioxidant capability of various foods, pharmaceuticals and medicinal plants.[79]

DPPH radical scavenging method is often used to diagnose the removal of free radicals. In this assay, solution of non-radical DPPH-H in alcohol is provided formation in time DPPH that radical source and then by antioxidant agents of one hydrogen donor is performed efficient scavenging of DPPH radicals.[80–82] Both avocado leaf extracts perform scavenging of DPPH radicals and reduces the colour intensity of the test solution. Then, it made the measurement of absorbance at 517 nm.[83,84] As shown in Fig. 3b and Table 4, avocado leaf extracts had potent ABTS radical scavenger as concentration-dependently (10–30 μg/mL). A lower IC_{50} value of a sample shows a higher $ABTS^{\bullet+}$ scavenging ability.[85] Table 4 and Fig. 3 show a crucial scavenging decrement ($p < 0.01$) in presence of both avocado leaf extracts. Their IC_{50} values were calculated as 240.40 μg/mL (0.9989) for EEFP, 601.0 μg/mL (0.9876) for WEFP, 58.16 μg/mL (0.9585) for trolox, 85.86 μg/mL (0.9781) for α-tocopherol, 87.95 μg/mL (0.8916) for BHT, and 45.64 μg/mL (0.9482) for BHA and increased in the order of BHA > trolox > α-tocopherol ≈ BHT > EEFP > WEFP. A lower IC_{50} indicated a higher DPPH· scavenging profile.[86] Also another radical scavenging method is determined by ABTS removal activity.[87,88]

Table 5. The quantity (mg/kg) of secondary metabolites in avocado (*F. perseae*) leaves extracts [EEFP: ethanol extract of avocado (*F. perseae*) leaves, WEFP: water extract of avocado (*F. perseae*) leaves].

Compounds	EEFP	WEFP
Gallic acid	5.41 ± 0.38	–
Herniarin	17.98 ± 1.81	0.60 ± 0.06
Kaempferol	663.54 ± 46.83	50.15 ± 3.54
Quercitrin	58.61 ± 3.74	15.10 ± 0.96
Fumaric acid	214.32 ± 14.86	59.18 ± 4.10
Pyrogallol	–	122.25 ± 8.14
Caffeic acid	32.74 ± 6.48	10.83 ± 2.14
Quercetin-3-O-arabinoside	253.18 ± 33.66	–
Quercetin	16.20 ± 2.15	–
Ellagic acid	5.56 ± 0.37	–
Chlorogenic acid	852.81 ± 118.09	28.83 ± 3.99
Rosmarinic acid	5.46 ± 0.42	–
Luteolin-7-glucoside	43.15 ± 4.39	1.08 ± 0.11
Luteolin-5-glucoside	18.44 ± 1.19	–
Kaempferon-3-O-rutinoside	25.45 ± 2.30	9.45 ± 0.85
Rutin	68.41 ± 4.48	26.05 ± 1.71
Isorhamnetin	34.37 ± 9.33	1.84 ± 0.12

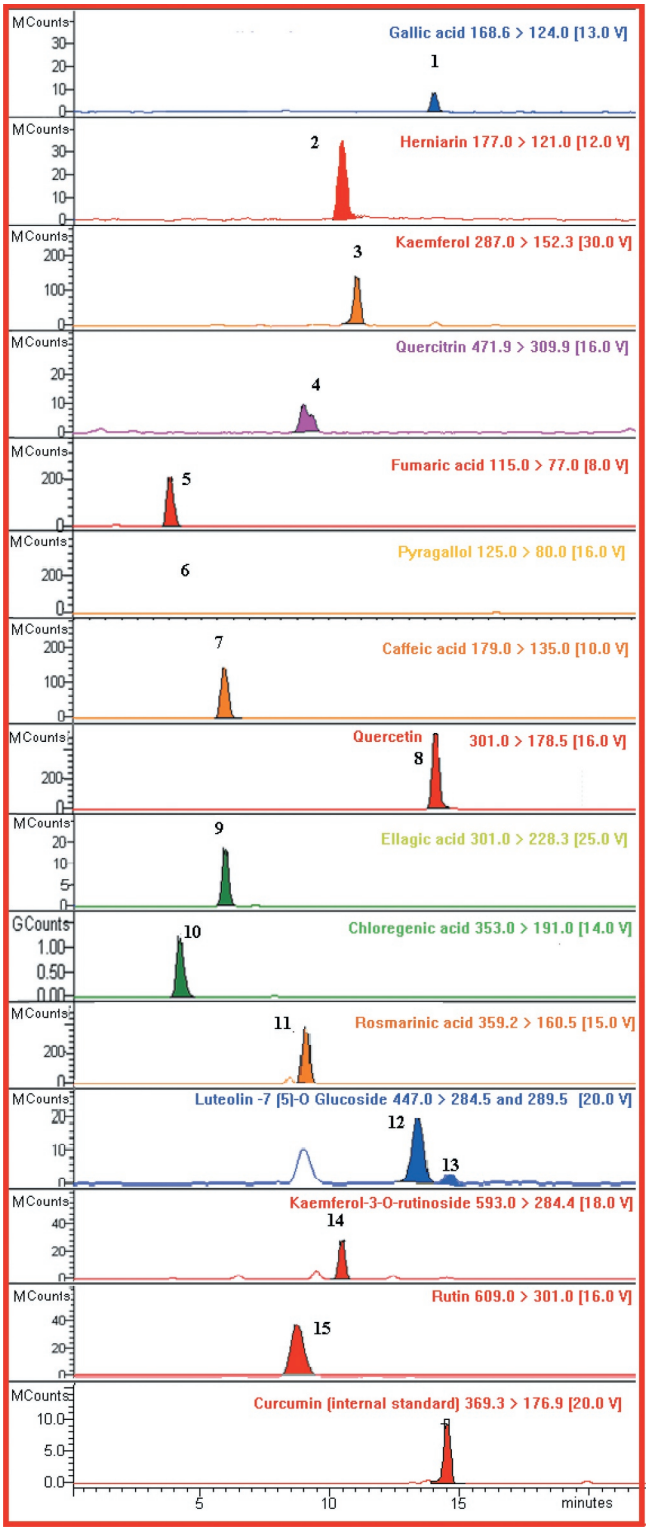
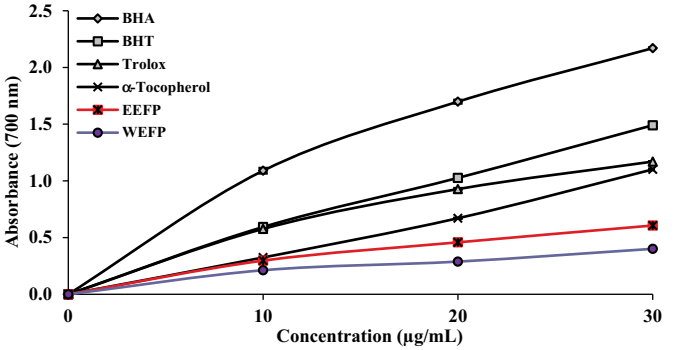
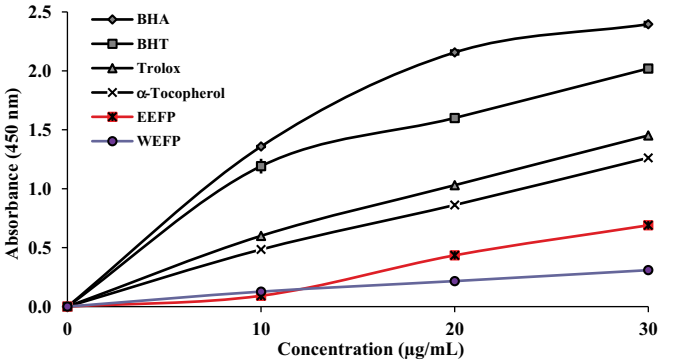


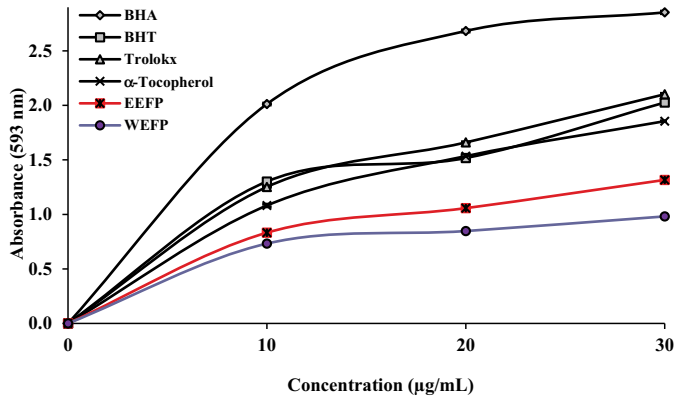
Figure 1. Chromatogram of secondary metabolites of avocado leaf (*F. perseae*) in EEFP [EEFP: ethanol extract of avocado (*F. perseae*) leaves].



a



b



c

Figure 2. (a) Fe^{3+}-Fe^{2+} reductive potential of different concentrations (10–30 µg/mL) of avocado leaf extracts and reference antioxidants. (b) Cu^{2+} reducing ability of different concentrations (10–30 µg/mL) of avocado leaf extracts and reference antioxidants. (c) Fe^{3+}-TPTZ-Fe^{2+}-TPTZ reducing ability of different concentrations (10–30 µg/mL) of avocado leaf extracts and reference antioxidants [EEFP: ethanol extract of avocado (*Folium perseae*) leaves, WEFP: water extract of avocado (*F. perseae*) leaves, BHA, butylated hydroxyanisole, BHT, butylated hydroxytoluene].

The IC_{50} values for EEFP and WEFP in this analysis were 286.05 µg/mL for EEFP (r^2: 0.9829) and 524.42 µg/mL for WEFP (r^2: 0.9883). With avocado leaf extract seen decline in the $ABTS^{\bullet+}$ radical amount. Furthermore, IC_{50} values for trolox, α-tocopherol, BHT, and BHA were found as 17.01 µg/mL

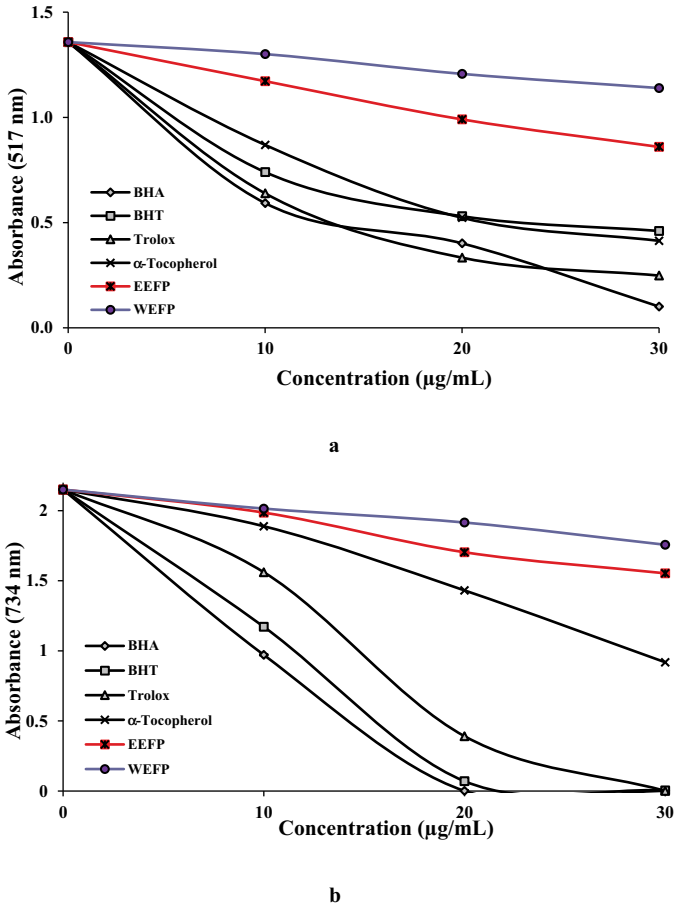


Figure 3. (a) DPPH free radical scavenging activity of different concentrations (10–30 µg/mL) of avocado leaf (*Folium perseae*) extracts and reference antioxidants. (b) ABTS radical scavenging activity of different concentrations (10–30 µg/mL) of avocado leaf extracts and standard antioxidant compounds [EEFP: ethanol extract of avocado (*F. perseae*) leaves, WEFP: water extract of avocado (*F. perseae*) leaves, BHA, butylated hydroxyanisole, BHT, butylated hydroxytoluene].

(r^2: 0.7263), 125.86 µg/mL (r^2: 0.9217), 16.92 µg/mL (r^2: 0.9159), and 18.84 µg/mL (r^2: 0.7539), respectively. The $ABTS^{\bullet+}$ scavenging ability of avocado leaf extracts and standards increased as following order: BHA ≈ trolox ≈ BHT > α-tocopherol > EEFP > WEFP.

Avocado leaf is a plant sources in terms of phenolics compounds. Total phenolic compound in the avocado leaf extract was calculated using the Folin–Ciocalteu reagent. Gallic acid graph was drawn as a standard graph (r^2: 0.9840) as previously described.[89] Fruits and vegetables, which including polyphenols, are good sources of human diet.[90] Accordingly, the consumption of food that is sources of polyphenols exhibits very great importance for the purchase of natural antioxidants.[91] Plant polyphenols have attracted great attention in terms of human health.[92] The quantity of total phenolics in avocado leaf extracts was defined from the equation obtained from standard graph as gallic acid equivalents (GAE/mg extract). In this sense, 0.218 and 0.092 µg of GAE of phenolics were calculated from 1 mg of EEFP and WEFP, respectively (Fig. 4a). Also, to determine the total flavonoids content of both extracts, a standard chart of quercetin was used. Consequently, the total quantity of flavonoid was

calculated with the equation obtained by using standard graph. It was shown that 1.480 and 0.328 µg of QE of flavonoids were found from 1 mg of EEFP and WEFP, respectively (Fig. 4b). Also, the standard chromatogram for phenolic compounds by LC-MS/MS (mg/mL) is given in Fig. 1. In accordance with LC-MS/MS analysis, the main phenolic compounds identified in 1 mg of EEFP are chlorogenic acid (852.81 ± 118.09 mg/kg), kaempferol (663.54 ± 46.83 mg/kg) of and quercetin-3-O-arabinoside (253.18 ± 33.66 mg/kg). On the other hand, pyrogallol (122.25 ± 8.14 mg/kg), kaempferol (50.15 ± 3.54 mg/kg), and chlorogenic acid (28.83 ± 3.99 mg/kg) are the most abundant phenolics in 1 mg of WEFP (Table 5).

In our study, we determined relationship between the inhibition effects of AChE/BChE enzymes and avocado leaf extracts. In accordance with our data, we have identified the effect of avocado leaf extracts on the AChE/BChE inhibition. Both extracts of avocado leaf had considerable higher AChE and BChE inhibition activities than that it was assumed standard AChE/BChE inhibitor like tacrine[93,94] as can be seen in Table 4. Tacrine is widely used as a reference AChE and BChE inhibitor.[95,96] For the AChE, IC_{50} values were calculated as 0.0168 mg/mL (r^2: 0.980) for EEFP, 0.0171 mg/mL (r^2: 0.991) for WEFP. Furthermore, it was declared that tacrine as a standard compound showed IC_{50}: 1.038 µg/mL value for the AChE. Also, IC_{50} was found as 0.0214 mg/mL (r^2: 0.9911) for BChE, for EEFP, 0.0226 mg/mL (r^2: 0.994) for WEFP. It was declared that tacrine showed IC_{50}: 0.0870 µg/mL against BChE. The results demonstrated that all avocado leaf extracts had powerful AChE and BChE inhibition effects (Table 4). It was reported that both cholinergic enzyme inhibitors are primarily used to treat some neurodegenerative conditions including cognitive, Parkinson's disease, and symptoms of dementia.[97–99]

Conclusion

In this study, for both extracts of avocado had effective antioxidant and antiradical properties. As mentioned earlier, avocado extracts should be used as protective agents in food industry through the providing bioavailability. Acetylcholine and butrylcholine are molecules that performing important functions for the brain. Being as balanced of these molecules is too important for neurological diseases. Both extracts of avocado leaf showed efficacy as potential inhibitors of AChE and BChE.

Acknowledgments

S. Alwasel would like to extend his sincere appreciation to the Researchers Supporting Project (RSP-2019/59), King Saud University, Saudi Arabia, for support. No conflict of interest associated with this work.

Funding

This work was supported by the King Saud University [RSP-2019/59].

ORCID

İlhami Gülçin http://orcid.org/0000-0001-5993-1668

References

[1] Chen, H.; Morrell, P. L.; Ashworth, V. E. T. M.; De La Cruz, M.; Clegg, M. T. Tracing the Geographic Origins of Major Avocado Cultivars. *J. Hered.* 2009, *100*, 56–65.
[2] Gulcin, I. Antioxidant Activity of Food Constituents: An Overview. *Arch. Toxicol.* 2012, *86*, 345–396.

[3] Çetin Çakmak, K.; Gulcin, I. Anticholinergic and Antioxidant Activities of Usnic acid-An Activity-structure Insight. *Toxicol. Rep.* 2019, *6*, 1273–1280.
[4] Bursal, E.; Koksal, E.; Gulcin, I.; Bilsel, G.; Goren, A. C. Antioxidant Activity and Polyphenol Content of Cherry Stem (*Cerasus Avium* L.) Determined by LC-MS/MS. *Food Res. Int.* 2013, *51*(1), 66–74.
[5] Polat Kose, L.; Gulcin, I.; Gören, A. C.; Namiesnik, J.; Martinez-Ayala, A. L.; Gorinstein, S. LC-MS/MS Analysis, Antioxidant and Anticholinergic Properties of Galanga (*Alpinia Officinarum* Hance) Rhizomes. *Ind. Crop Prod.* 2015, *74*, 712–721.
[6] Gulcin, I.; Goren, A. C.; Taslimi, P.; Akyuz, B.; Tuzun, B. Anticholinergic, Antidiabetic and Antioxidant Activities of Anatolian Pennyroyal (*Mentha pulegium*)-Analysis of Its Polyphenol Contents by LC-MS/MS. *Biocat. Agric. Biotechnol.* 2020, *23*, 101441.
[7] Gulcin, I. Antioxidant Activity of Eugenol-a Structure and Activity Relationship Study. *J. Med. Food.* 2011, *14*, 975–985.
[8] Aras, A.; Bursal, E.; Tohma, H.; Kılıc, O.; Alan, Y.; Gulcin, I.; Köksal, E. Evaluation of Antioxidant and Antimicrobial Activities of *Lecokia Cretica* (Lam.) DC and Determination of Its Phenolic Compounds by HPLC-MS/MS. *Chem. Biodiver.* 2019, *16*(10), e1900341.
[9] Gulcin, I. Antioxidant Activity of Caffeic Acid (3,4-dihydroxycinnamic Acid). *Toxicology.* 2006, *217*(2–3), 213–220.
[10] Gulcin, I.;. Antioxidant and Antiradical Activities of L-Carnitine. *Life Sci.* 2006, *78*(8), 803–811.
[11] Bursal, E.; Gulcin, I. Polyphenol Contents and in Vitro Antioxidant Activities of Lyophilized Aqueous Extract of Kiwifruit (*Actinidia Deliciosa*). *Food Res. Int.* 2011, *44*, 1482–1489.
[12] Turkan, F.; Atalar, M. N.; Aras, A.; Gulcin, I.; Bursal, E. ICP-MS and HPLC Analyses, Enzyme Inhibition and Antioxidant Potential of *Achillea Schischkinii* Sosn. *Bioorg. Chem.* 2019, *93*, 103333.
[13] Elmastas, M.; Gulcin, I.; Beydemir, Ş.; Kufrevioglu, O. I.; Aboul-Enein, H. Y. A Study on the in Vitro Antioxidant Activity of Juniper (*Juniperus Communis* L.) Seeds Extracts. *Anal. Lett.* 2006, *39*(1), 47–65. DOI: 10.1080/00032710500423385.
[14] Gulcin, I.; Topal, F.; Cakmakcı, R.; Goren, A. C.; Bilsel, M.; Erdogan, U. Pomological Features, Nutritional Quality, Polyphenol Content Analysis and Antioxidant Properties of Domesticated and Three Wild Ecotype Forms of Raspberries (*Rubus Idaeus* L.). *J. Food Sci.* 2011, *76*(4), C585–C593. DOI: 10.1111/j.1750-3841.2011.02142.x.
[15] Gulcin, I. Antioxidants and Antioxidant methods-An Updated Overview. *Arch. Toxicol.* 2020, *94*(3), 651–715.
[16] Topal, F.; Topal, M.; Gocer, H.; Kalın, P.; Koçyigit, U. M.; Gulcin, I.; Alwasel, S. H. Antioxidant Activity of Taxifolin: An Activity-structure Relationship. *J. Enzyme Inhib. Med. Chem.* 2016, *31*, 674–683. DOI: 10.3109/14756366.2015.1057723.
[17] Gulcin, I. In Vitro Prooxidant Effect of Caffeine. *J. Enzyme Inhib. Med. Chem.* 2008, *23*,149–152.
[18] Gulcin, I. Antioxidant Activity of L-Adrenaline: An Activity-structure Insight. *Chem. Biol. Int.* 2009, *179*, 71–80. DOI: 10.1016/j.cbi.2008.09.023.
[19] Gulcin, I.; Kaya, R.; Goren, A. C.; Akıncıoglu, H.; Topal, M.; Bingol, Z.; Cetin Cakmak, K.; Ozturk Sarikaya, S. B.; Durmaz, L.; Alwasel, S. Anticholinergic, Antidiabetic and Antioxidant Activities of Cinnamon (*Cinnamomum Verum*) Bark Extracts: Polyphenol Contents Analysis by LC-MS/MS. *Int. J. Food Prop.* 2019, *22*(1), 1511–1526. DOI: 10.1080/10942912.2019.1656232.
[20] Alicia, O. M.; Lidia, D.; Juvencio, G.; Rosa, I. G. Effect of Different Extraction Methods on Fatty Acids, Volatile Compounds, and Physical and Chemical Properties of Avocado (*Persea Americana* Mill) Oil. *J. Agric. Food Chem.* 2003, *51*, 2216–2221. DOI: 10.1021/jf0207934.
[21] Gondwe, M.; Kamadyaapa, D. R.; Tufts, M. A.; Chuturgoon, A. A.; Ojewole, J. A.; Musabayane, C. T. Effects of *Persea Americana* Mill (Lauraceae) (Avocado) Ethanolic Leaf Extract on Blood Glucose and Kidney Function in Streptozotocin-induced Diabetic Rats and on Kidney Cell Lines of the Proximal (LLCPK1) and Distal Tubules (MDBK). *Method. Find. Exp. Clin.* 2008, *30*, 25–35. DOI: 10.1358/mf.2008.30.1.1147769.
[22] Dreher, M. L.; Davenport, A. J. Hass Avocado Composition and Potential Health Effects. *Crit. Rev. Food Sci. Nutr.* 2013, *53*, 738–750. DOI: 10.1080/10408398.2011.556759.
[23] Bayrak, Ç.; Taslimi, P.; Gulcin, I.; Menzek, A. The First Synthesis of 4-phenylbutenone Derivative Bromophenols Including Natural Products and Their Inhibition Profiles for Carbonic Anhydrase, Acetylcholinesterase and Butyrylcholinesterase Enzymes. *Bioorg. Chem.* 2017, *72*, 359–366. DOI: 10.1016/j.bioorg.2017.03.001.
[24] Gocer, H.; Topal, F.; Topal, M.; Kucuk, M.; Teke, D.; Gulcin, I.; Alwasel, S. H.; Supuran, C. T. Acetylcholinesterase and Carbonic Anhydrase Isoenzymes I and II Inhibition Profiles of Taxifolin. *J. Enzyme Inhib. Med. Chem.* 2016, *31*(3), 441–447. DOI: 10.3109/14756366.2015.1036051.
[25] Oztaskin, N.; Taslimi, P.; Maras, A.; Goksu, S.; Gulcin, I. Novel Antioxidant Bromophenols with Acetylcholinesterase, Butyrylcholinesterase and Carbonic Anhydrase Inhibitory Actions. *Bioorg. Chem.* 2017, *74*, 104–114. DOI: 10.1016/j.bioorg.2017.07.010.

[26] Biçer, A.; Kaya, R.; Yakali, G.; Gültekin, M. S.; Turgut Cin, G.; Gulcin, I. Synthesis of Novel β-amino Carbonyl Derivatives and Their Inhibition Effects on Some Metabolic Enzymes. *J. Mol. Struct.* 2020, *1204*, 127453. DOI: 10.1016/j.molstruc.2019.127453.
[27] Bilginer, S.; Gonder, B.; Gul, H. I.; Kaya, R.; Gulcin, I.; Anil, B.; Supuran, C. T. Novel Sulfonamides Incorporating Triazene Moieties Show Powerful Carbonic Anhydrase I and II Inhibitory Properties. *J. Enzyme Inhib. Med. Chem.* 2020, *35*(1), 325–329. DOI: 10.1080/14756366.2019.1700240.
[28] Bytyqi-Damoni, A.; Kestane, A.; Taslimi, P.; Tüzün, B.; Zengin, M.; Genç Bilgiçli, H.; Gulcin, I. Novel Carvacrol Based New Oxypropanolamine Derivatives: Design, Synthesis, Characterization, Biological Evaluation, and Molecular Docking Studies. *J. Mol. Struct.* 2020, *1202*, 127297.
[29] Gocer, H.; Akıncıoglu, A.; Oztaskin, N.; Goksu, S.; Gulcin, I. Synthesis, Antioxidant and Antiacetylcholinesterase Activities of Sulfonamide Derivatives of Dopamine Related Compounds. *Arch. Pharm.* 2013, *346*(11), 783–792.
[30] Mamedova, G.; Mahmudova, A.; Mamedov, S.; Erden, Y.; Taslimi, P.; Tüzün, B.; Taş, R.; Farzaliyev, V.; Sujayev, A.; Alwasel, S. H.; et al. Novel Tribenzylaminobenzolsulphonylimine Based on Their Pyrazine and Pyridazines: Synthesis, Characterization, Antidiabetic, Anticancer, Anticholinergics, and Molecular Docking Studies. *Bioorg. Chem.* 2019, *93*, 103313.
[31] Ozbey, F.; Taslimi, P.; Gulcin, I.; Maras, A.; Goksu, S.; Supuran, C. T. Synthesis, Acetylcholinesterase, Butyrilcholinesterase, Carbonic Anhydrase Inhibitory and Metal Chelating Properties of Some Novel Diaryl Ether. *J. Enzyme Inhib. Med. Chem.* 2016, *31*(S2), 79–85.
[32] Al-Sayed, N. A. E.; Farag, A. E. S.; Ezzat, M. A. F.; Akincioglu, H.; Gulcin, I.; Abou-Seri, S. M. Design, Synthesis, in Vitro and in Vivo Evaluation of Novel Pyrrolizine-based Compounds with Potential Activity as Cholinesterase Inhibitors and anti-Alzheimer's Agents. *Bioorg. Chem.* 2019, *93*, 103312.
[33] Garibov, E.; Taslimi, P.; Sujayev, A.; Bingol, Z.; Cetinkaya, S.; Gulcin, I.; Beydemir, S.; Farzaliyev, V.; Alwasel, S. H.; Supuran, C. T. Synthesis of 4,5-disubstituted-2-thioxo-1,2,3,4-tetrahydropyrimidines and Investigation of Their Acetylcholinesterase, Butyrylcholinesterase, Carbonic Anhydrase I/II Inhibitory and Antioxidant Activities. *J. Enzyme Inhib. Med. Chem.* 2016, *31*(S3), 1–9.
[34] Taslimi, P.; Turkan, F.; Cetin, A.; Burhan, H.; Karaman, M.; Bildirici, I.; Gulcin, I.; Sen, F. Pyrazole[3,4-d] pyridazine Derivatives: Molecular Docking and Explore of Acetylcholinesterase and Carbonic Anhydrase Enzymes Inhibitors as Anticholinergics Potentials. *Bioorg. Chem.* 2019, *93*, 103213.
[35] Gulcin, I.; Tel, A. Z.; Goren, A. C.; Taslimi, P.; Alwasel, S. Sage (*Salvia Pilifera*): Determination Its Polyphenol Contents, Anticholinergic, Antidiabetic and Antioxidant Activities. *J. Food Measure.* 2019, *13*(3), 2062–2074.
[36] Koksal, E.; Bursal, E.; Dikici, E.; Tozoğlu, F.; Gulcin, I. Antioxidant Activity of *Melissa Officinalis* Leaves. *J. Med. Plants Res.* 2011, *5*(2), 217–222.
[37] Koksal, E.; Bursal, E.; Gulcin, I.; Korkmaz, M.; Çaglayan, C.; Goren, A. C.; Alwasel, S. H. Antioxidant Activity and Polyphenol Content of Turkish Thyme (*Thymus Vulgaris*) Monitored by LC-MS/MS. *Int. J. Food Prop.* 2017, *20*(3), 514–525.
[38] Isik, M.; Korkmaz, M.; Bursal, E.; Gulcin, I.; Koksal, E.; Tohma, H. Determination of Antioxidant Properties of *Gypsophila Bitlisensis*. *Int. J. Pharmacol.* 2015, *11*(4), 366–371.
[39] Elmastas, M.; Celik, S. M.; Genc, N.; Aksit, H.; Erenler, R.; Gulcin, I. Antioxidant Activity of an Anatolian Herbal tea-Origanum Minutiflorum: Isolation and Characterization of Its Secondary Metabolites. *Int. J. Food Prop.* 2018, *21*(1), 374–384.
[40] Han, H.; Yılmaz, H.; Gulcin, I. Antioxidant Activity of Flaxseed (*Linum Usitatissimum* L.) And Analysis of Its Polyphenol Contents by LC-MS/MS. *Rec. Nat. Prod.* 2018, *12*(4), 397–402.
[41] Gulcin, I.; Elmastas, M.; Aboul-Enein, H. Y. Determination of Antioxidant and Radical Scavenging Activity of Basil (*Ocimum Basilicum*) Assayed by Different Methodologies. *Phytother. Res.* 2017, *21*(4), 354–361.
[42] Gulcin, I.;. The Antioxidant and Radical Scavenging Activities of Black Pepper (*Piper Nigrum*) Seeds. *Int. J. Food Sci. Nutr.* 2005, *56*(7), 491–499.
[43] Elmastas, M.; Gulcin, I.; Isıldak, Ö.; Kufrevioglu, O. I.; Ibaoglu, K.; Aboul-Enein, H. Y. Antioxidant Capacity of Bay (*Laurus Nobilis* L.) Leaves Extracts. *J. Iran. Chem. Soc.* 2006, *3*(3), 258–266.
[44] Goren, A. C.; Cıkrıkcı, S.; Cergel, M.; Bilsel, G. Rapid Quantitation of Curcumin in Turmeric via NMR and LC-Tandem Mass Spectrometry. *Food Chem.* 2009, *113*, 1239–1242.
[45] Goren, A. C.; Bilsel, G.; Bilsel, M. Rapid and Simultaneous Determination of 25-OH-vitamin D2 and D3 in Human Serum by LC/MS/MS: Validation and Uncertainty Assesment. *J. Chem. Metrol.* 2007, *1*, 1–10.
[46] Baki, S.; Tufan, A. N.; Altun, M.; Özgökçe, F.; Güçlü, K.; Özyürek, M. Microwave-assisted Extraction of Polyphenolics from Some Selected Medicinal Herbs Grown in Turkey. *Rec. Nat. Prod.* 2018, *12*, 29–39.
[47] Li, M.; Pare, P. W.; Zhang, J.; Kang, T.; Zhang, Z.; Delong, Y.; Wang, K.; Xing, H. Antioxidant Capacity Connection with Phenolic and Flavonoid Content in Chinese Medicinal Herbs. *Rec. Nat. Prod.* 2018, *12*, 239–250.

[48] Gulcin, I. Comparison of in Vitro Antioxidant and Antiradical Activities of L-tyrosine and L-Dopa. *Amino Acids*. 2007, *32*, 431–843.
[49] Gulcin, I. Antioxidant Properties of Resveratrol: A Structure-activity Insight. *Innov. Food Sci. Emerg.* 2010, *11*, 210–218.
[50] Elmastas, M.; Turkekul, İ.; Oztürk, L.; Gulcin, I.; Isıldak, O.; Aboul-Enein, H. Y. The Antioxidant Activity of Two Wild Edible Mushrooms (*Morchella Vulgaris* and *Morchella Esculanta*). *Comb. Chem. High Throughput Screen*. 2006, *9*, 443–448.
[51] Gulcin, I. Measurement of Antioxidant Ability of Melatonin and Serotonin by the DMPD and CUPRAC Methods as Trolox Equivalent. *J. Enzyme Inhib. Med. Chem.* 2006, *23*, 871–876.
[52] Bursal, E.; Aras, A.; Kılıc, Ö.; Taslimi, P.; Goren, A. C.; Gulcin, I. 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.* 2019, *43*(3), e12776.
[53] Gulcin, I.; Beydemir, Ş.; Sat, I. G.; Küfrevioglu, O. I. Evaluation of Antioxidant Activity of Cornelian Cherry (*Cornus mas* L.). *Acta Aliment. Hung.* 2005, *34*(2), 193–202.
[54] Ak, T.; Gulcin, I. Antioxidant and Radical Scavenging Properties of Curcumin. *Chem. Biol. Interact.* 2008, *174*, 27–37.
[55] Balaydin, H. T.; Gulcin, I.; Menzek, A.; Göksu, S.; Sahin, E. Synthesis and Antioxidant Properties of Diphenylmethane Derivative Bromophenols Including a Natural Product. *J. Enzyme Inhib. Med. Chem.* 2010, *25*(5), 685–695.
[56] Talaz, O.; Gulcin, I.; Goksu, S.; Saracoglu, N. Antioxidant Activity of 5,10-dihydroindeno[1,2-b]indoles Containing Substituents on Dihydroindeno Part. *Bioorg. Med. Chem*. 2009, *17*(18), 6583–6589.
[57] Gulcin, I.; Elias, R.; Gepdiremen, A.; Taoubi, K.; Koksal, E. Antioxidant Secoiridoids from Fringe Tree (*Chionanthus Virginicus* L.). *Wood Sci. Technol.* 2009, *43*(3–4), 195–212.
[58] Slinkard, K.; Singleton, V. L. Total Phenol Analyses: Automation and Comparison with Manual Methods. *Am. J. Enol. Viticul.* 1977, *28*, 49–55.
[59] Oktay, M.; Gulcin, I.; Küfrevioglu, O. I. Determination of in Vitro Antioxidant Activity of Fennel (*Foeniculum Vulgare*) Seed Extracts. *Lebensm. Wissen. Technol.* 2003, *36*(2), 263–271.
[60] Gulcin, I.; Kirecci, E.; Akkemik, E.; Topal, F.; Hisar, O. Antioxidant and Antimicrobial Activities of an Aquatic Plant: Duckweed (*Lemna minor* L.). *Turk. J. Biol.* 2010, *34*(2), 175–188.
[61] Ellman, G. L.; Courtney, K. D.; Andres, V.; Featherston, R. M. A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. *Biochem. Pharmacol.* 1961, *7*, 88–95.
[62] Tohma, H.; Altay, A.; Koksal, E.; Goren, A. C.; Gulcin, I. Measurement of Anticancer, Antidiabetic and Anticholinergic Properties of Sumac (*Rhus coriaria*) - Analysis of Its Phenolic Compounds by LC-MS/MS. *J. Food Measure.Charac*. 2019, *13*(2), 1607–1619.
[63] Atmaca, U.; Kaya, R.; Kahraman, H. S.; Celik, M.; Gulcin, I. Synthesis of Oxazolidinone from Enantiomerically Enriched Allylic Alcohols and Determination of Their Molecular Docking and Biologic Activities. *Bioorg. Chem.* 2019, *88*, 102980.
[64] Boztas, M.; Taslimi, P.; Yavari, M. A.; Gulcin, I.; Sahin, E.; Menzek, A. Synthesis and Biological Evaluation of Bromophenol Derivatives with Cyclopropyl Moiety: Ring Opening of Cyclopropane with Monoester. *Bioorg. Chem.* 2019, *89*, 103017.
[65] Yigit, B.; Yigit, M.; Barut Celepci, D.; Gok, Y.; Aktas, A.; Aygun, M.; Taslimi, P.; Gulcin, I. Novel Benzylic Substituted Imidazolinium, Tetrahydropyrimidinium and Tetrahydrodiazepinium Salts-potent Carbonic Anhydrase and Acetylcholinesterase Inhibitors. *ChemistrySelect*. 2018, *3*(27), 7976–7982.
[66] Duh, P. D.;. Antioxidant Activity of Burdock (*Arctium lappa* L.): It's Scavenging Effect on Free Radical and Active Oxygen. *J. Am. Oil Chem. Soc.* 1998, *75*, 455–461.
[67] Rezai, M.; Bayrak, C.; Taslimi, P.; Gulcin, I.; Menzek, A. The First Synthesis, Antioxidant and Anticholinergic Activities of 1-(4,5-dihydroxybenzyl)pyrrolidin-2-one Derivative Bromophenols Including Natural Products. *Turk. J. Chem.* 2018, *42*(3), 808–825.
[68] Sehitoglu, M. H.; Han, H.; Kalin, P.; Gulcin, I.; Ozkan, A.; Aboul-Enein, H. Y. Pistachio (*Pistacia vera* L.) Gum: A Potent Inhibitor of Reactive Oxygen Species. *J. Enzyme Inhib. Med. Chem.* 2015, *30*(2), 264–269.
[69] Gulcin, I.; Mshvildadze, V.; Gepdiremen, A.; Elias, R. Screening of Antioxidant and Antiradical Activity of Monodesmosides and Crude Extract from *Leontice smirnowii* Tuber. *Phytomedicine*. 2006, *13*, 343–351.
[70] Koksal, E.; Gulcin, I. Antioxidant Activity of Cauliflower (*Brassica oleracea* L.). *Turk. J. Agric. For.* 2008, *32*, 65–78.
[71] Buldurun, K.; Turkan, F.; Taslimi, P.; Gulcin, I.; Bursal, E.; Turan, N.; Mantarcı, A. Synthesis, Spectroscopic Properties, Crystal Structures, Antioxidant Activities and Enzyme Inhibition Determination of Co(II) and Fe(II) Complexes of Schiff Base. *Res. Chem. Intermed.* 2019, *16*(8), e1900243.

[72] Gulcin, I.; Huyut, Z.; Elmastas, M.; Aboul-Enein, H. Y. Radical Scavenging and Antioxidant Activity of Tannic Acid. *Arab. J. Chem.* 2010, *3*, 43–53.
[73] Gulcin, I.; Topal, F.; Ozturk Sarikaya, S. B.; Bursal, E.; Goren, A. C.; Bilsel, M. Polyphenol Contents and Antioxidant Properties of Medlar (*Mespilus germanica* L.). *Rec. Nat. Prod.* 2011, *5*, 158–175.
[74] Gulcin, I.; Elias, R.; Gepdiremen, A.; Boyer, L. Antioxidant Activity of Lignans from Fringe Tree (*Chionanthus virginicus* L.). *Eur. Food Res. Technol.* 2006, *223*, 759–767.
[75] Oztaskin, N.; Kaya, R.; Maras, A.; Sahin, E.; Gulcin, I.; Göksu, S. Synthesis and Characterization of Novel Bromophenols: Determination of Their Anticholinergic, Antidiabetic and Antioxidant Activities. *Bioorg. Chem.* 2019, *87*, 91–102.
[76] Gulcin, I.; Beydemir, S.; Topal, F.; Gagua, N.; Bakuridze, A.; Bayram, R.; Gepdiremen, A. Apoptotic, Antioxidant and Antiradical Effects of Majdine and Isomajdine from *Vinca Herbacea* Waldst. And Kit. *J. Enzyme Inhib. Med. Chem.* 2012, *27*(4), 587–594.
[77] Koksal, E.; Gulcin, I.; Ozturk Sarikaya, S. B.; Bursal, E. On the in Vitro Antioxidant Activity of Silymarin. *J. Enzyme Inhib. Med. Chem.* 2009, *24*(2), 395–405.
[78] Serbetci Tohma, H.; Gulcin, I. Antioxidant and Radical Scavenging Activity of Aerial Parts and Roots of Turkish Liquorice (*Glycyrrhiza Glabra* L. *Int. J. Food Prop.* 2010, *13*(4), 657–671.
[79] Tohma, H.; Gulcin, I.; Bursal, E.; Goren, A. C.; Alwasel, S. H.; Köksal, E. Antioxidant Activity and Phenolic Compounds of Ginger (*Zingiber Officinale* Rosc.) Determined by HPLC-MS/MS. *J. Food Measure. Charact*. 2017, *11*(2), 556–566.
[80] Oztaskın, N.; Cetinkaya, Y.; Taslimi, P.; Goksu, S.; Gulcin, I. Antioxidant and Acetylcholinesterase Inhibition Properties of Novel Bromophenol Derivatives. *Bioorg. Chem.* 2015, *60*, 49–57.
[81] Eruygur, N.; Atas, M.; Tekin, M.; Taslimi, P.; Koçyigit, U. M.; Gulcin, I. In Vitro Antioxidant, Antimicrobial, Anticholinesterase and Antidiabetic Activities of Turkish Endemic *Achillea Cucullata* (Asteraceae) from Ethanol Extract. *S. Afr. J. Bot.* 2019, *120*, 141–145.
[82] Cetinkaya, Y.; Göçer, H.; Menzek, A.; Gulcin, I. Synthesis and Antioxidant Properties of (3,4-dihydroxyphenyl) (2,3,4-trihydroxyphenyl)methanone and Its Derivatives. *Arch. Pharm.* 2012, *345*(4), 323–334.
[83] Aksu, K.; Topal, F.; Gulcin, I.; Tumer, F.; Göksu, S. Acetylcholinesterase Inhibitory and Antioxidant Activities of Novel Symmetric Sulfamides Derived from Phenethylamines. *Arch. Pharm.* 2015, *348*(6), 446–455.
[84] Kalin, P.; Gulcin, I.; Goren, A. C. Antioxidant Activity and Polyphenol Content of Cranberries (*Vaccinium Macrocarpon*). *Rec. Nat. Prod.* 2015, *9*(4), 496–502.
[85] Gulcin, I.; Tel, A. Z.; Kirecci, E. Antioxidant, Antimicrobial, Antifungal and Antiradical Activities of *Cyclotrichium Niveum* (Boiss.) Manden and Scheng. *Int. J. Food Prop.* 2008, *11*(2), 450–471.
[86] Maharramova, G.; Taslimi, P.; Sujayev, A.; Farzaliyev, F.; Durmaz, L.; Gulcin, I. Synthesis, Characterization, Antioxidant, Antidiabetic, Anticholinergic, and Antiepileptic Properties of Novel N-substituted Tetrahydropyrimidines Based on Phenylthiourea. *J. Biochem. Mol. Toxicol.* 2018, *32*(12), e22221.
[87] Taslimi, P.; Koksal, E.; Goren, A. C.; Bursal, E.; Aras, A.; Kilic, O.; Alwasel, S.; Gulcin, I. Anti-Alzheimer, Antidiabetic and Antioxidant Potential of *Satureja cuneifolia* and Analysis of Its Phenolic Contents by LC-MS/MS. *Arab. J. Chem.* 2020, *13*(3), 4528–4537.
[88] Gulcin, I.; Sat, I. G.; Beydemir, Ş.; Kufrevioglu, O. I. Evaluation of the in Vitro Antioxidant Properties of Extracts of Broccoli (*Brassica oleracea* L.). *Ital. J. Food Sci.* 2004, *16*(1), 17–30.
[89] Gulcin, I.; Kufrevioglu, O. I.; Oktay, M.; Buyukokuroglu, M. E. Antioxidant, Antimicrobial, Antiulcer and Analgesic Activities of Nettle (*Urtica dioica* L.). *J. Ethnopharmacol.* 2004, *90*, 205–215.
[90] Gulcin, I.; Elias, R.; Gepdiremen, A.; Boyer, L.; Köksal, E. A Comparative Study on the Antioxidant Activity of Fringe Tree (*Chionanthus virginicus* L.) Extracts. *Afr. J. Biotechnol.* 2007, *6*(4), 410–418.
[91] Taslimi, P.; Gulcin, I. Antioxidant and Anticholinergic Properties of Olivetol. *J. Food Biochem.* 2018, *42*(3), e12516.
[92] Gulcin, I.; Berashvili, D.; Gepdiremen, A. Antiradical and Antioxidant Activity of Total Anthocyanins from *Perilla pankinensis* Decne. *J. Ethnopharmacol.* 2005, *101*, 287–293.
[93] Aktas, A.; Barut Celepci, D.; Gök, Y.; Taslimi, P.; Akıncıoglu, H.; Gulcin, I. A Novel Ag-N-heterocyclic Carbene Complex Bearing the Hydroxyethyl Ligand: Synthesis, Characterization, Crystal and Spectral Structures and Bioactivity Properties. *Crystals*. 2020, *10*, 171.
[94] Yılmaz, S.; Akbaba, Y.; Ozgeris, B.; Polat Kose, L.; Goksu, S.; Gulcin, I.; Alwasel, S. H.; Supuran, C. T. Synthesis and Inhibitory Properties of Some Carbamates on Carbonic Anhydrase and Acetylcholine Esterase. *J. Enzyme Inhib. Med. Chem.* 2012, *31*(6), 1484–1491.
[95] Ozmen Ozgun, D.; Yamali, C.; Gul, H. I.; Taslimi, P.; Gulcin, I.; Yanik, T.; Supuran, C. T. Inhibitory Effects of Isatin Mannich Bases on Carbonic Anhydrases, Acetylcholinesterase and Butyrylcholinesterase. *J. Enzyme Inhib. Med. Chem.* 2016, *31*, 1498–1501.

[96] Burmaoglu, S.; Yılmaz, A. O.; Polat, M. F.; Algul, O.; Kaya, R.; Gulcin, I. Synthesis and Biological Evaluation of Novel Tris-chalcones as Potent Carbonic Anhydrase, Acetylcholinesterase, Butyrylcholinesterase, and α-glycosidase Inhibitors. *Bioorg. Chem.* 2019, *85*,191–197.

[97] Genc Bilgicli, H.; Kestane, A.; Taslimi, P.; Karabay, O.; Bytyqi-Damoni, A.; Zengin, M.; Gulcin, İ. Novel Eugenol Bearing Oxypropanolamines: Synthesis, Characterization, Antibacterial, Antidiabetic, and Anticholinergic Potentials. *Bioorg. Chem.* 2019, *88*, 102931.

[98] Bayindir, S.; Çaglayan, C.; Karaman, M.; Gulcin, I. The Green Synthesis and Molecular Docking of Novel N-substituted Rhodanines as Effective Inhibitors for Carbonic Anhydrase and Acetylcholinesterase Enzymes. *Bioorg. Chem.* 2019, *90*, 103096.

[99] Topal, M.; Gocer, H.; Topal, F.; Kalin, P.; Polat Kose, P.; Gulcin, I.; Cakmak, K. C.; Kucuk, M.; Durmaz, L.; Goren, A. C.; et al. Antioxidant, Antiradical and Anticholinergic Properties of Cynarin Purified from the Illyrian Thistle (*Onopordum illyricum* L.). *J. Enzyme Inhib. Med. Chem.* 2016, *31*(2), 266–275. DOI: 10.3109/14756366.2015.1018244.