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ORIGINAL PAPER

Phytochemical, phenolic profile, antioxidant, anticholinergic and antibacterial properties of *Epilobium angustifolium* (Onagraceae)

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Abstract

Epilobium angustifolium is widely used in medicine for disease treatments, as well as in the cosmetic and food industries. The aim of this research was to investigate the antioxidant, and anticholinergic properties, phenolics profile and antibacterial activities of the *E. angustifolium* ethanol extract. The analysis of phenolic compounds was performed with LC–MS/MS. The antioxidant capacity (radical scavenging, metal-reducing power and total antioxidant activity) was assessed by DPPH, ABTS, Cu^{2+}–Cu^{+} reducing (CUPRAC), Fe^{3+}–Fe^{2+} reducing and ferric thiocyanate methods. The antibacterial activity was determined by disc diffusion and MIC (Minimum inhibitory concentration) methods and the anticholinergic property was predicted by inhibition of acetylcholinesterase (AChE). The major phenolic compounds, founding in the plant extract were luteolin, fumaric acid, vanillic acid, and caffeic acid. The ethanol extract of the plant showed DPPH free radical scavenging value of 11.3%, while the ABTS radical scavenging activity was 19.4% and showed moderately metal-reducing power. Also, the extract had 39.3% inhibition on lipid peroxidation of linoleic acid emulsion and showed an inhibition effect on the AChE with IC_{50} values (0.14 mg mL^{-1}). The ethanol extract of the plant showed antibacterial effect on *Staphylococcus aureus*, *Escherichia coli,* and *Salmonella Typhimurium* at different levels. These results suggested that *E. angustifolium* extract might be a suitable natural antioxidant in the preservation of foods by preventing the oxidation of polyunsaturated fatty acids, and might play a role in the treatment of some diseases with its antioxidant, anticholinergic, and antibacterial activity.

Keywords *Epilobium angustifolium* · Antioxidant · Phytochemical analysis · Acetylcholinesterase · Lipid peroxidation · Antibacterial

Introduction

Oxidative stress occurs when free radicals and reactive oxygen species dominate the cellular antioxidant defense system. Oxidative stress plays an important role in many pathologies such as premature aging, Alzheimer's disease (AD), atherosclerosis, Parkinson's disease, diabetes, especially by causing lipid oxidation, DNA damage and protein modification [1]. To reduce this oxidative stress, it is important to know naturally sourced products with bioactive content that have antioxidant properties [1, 2]. Many medicinal plants contain pharmacologically bioactive substances. Phenolic compounds among the substances in plant content play an important role in extending the shelf life of foodstuff as they reduce lipid peroxidation [3–5].

Onagraceae family has very rich, widespread genera and species in the world. *Epilobium* species live in temperate climates and high altitudes ranges, presented in all continents except Antarctica [6]. In Turkey, it grows

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on the edges of streams and lakes, forests, rocky places, meadows, and swamps [7]. The concentrations of bioactive compounds and their biological activities depend on phenotype geographic origin of the plant, climatic conditions, or genotype [6, 7].

The *E. angustifolium* is rich in terms of some bioactive substances especially, hydrolyzable tannins, polyphenolic and flavonoid compounds, steroids, fatty acids, and triterpenes [8–10]. Also, it includes myricetin, quercetin, kaempferol, ellagic acid, valoneic acid, protocatechuic acid, dilactone, chlorogenic acid, gallic acid, ferulic acid, cinnamic acid, and caffeic acid [8–11]. Approximately, 250 different metabolites and 170 substances have been identified in this plant in the last 10 years [9, 12]. Polyphenols are the most abundant components in *E. angustifolium* especially, flavonoids ellagitannins, and phenolic acids [13]. *E. angustifolium* contains ellagitannins at high levels [8, 12, 14, 15].

This plant has various properties, including antioxidant, anticancer, immunomodulatory, anti-inflammatory, antiandrogenic, and antiproliferative properties [16, 17]. These species have been traditionally used in the treatment of important disorders such as urinary tract, prostatitis, prostate cancer cell apoptosis dependent on the hormone, diarrhea, bladder, and gastrointestinal diseases, irritation, skin and mucosa diseases, inflammation, and epilepsy [10, 16–18]. Also, this plant shows analgesic, antimicrobial and antiaging activities [19–21].

Medicinal plants have been used by humans for the treatment of various diseases for centuries. Although technology and medicine have been developed in recent years, people have preferred to use natural plants and foods due to their harmless and beneficial properties on health. Due to the current lack of development of new antibiotics by pharmaceutical companies and poor economical returns, new and alternative resources need to be found including natural plant-based sources with reported antibacterial properties [22]. Ethanolic extracts of *E. angustifolium* have shown bactericidal activity against both bacteria and cytocidal activity against fungus [23]. In recent studies, ethanolic and alcoholic extracts of *E. angustifolium* have been found an inhibition effect on the growth of *Staphylococcus aureus, S. albus*, *Pseudomonas pyocyanea*, and *Candida albicans* [24].

There is no study on phenolic compound analysis and evaluation of some bioactive properties of *E. angustifolium* grown in the Giresun region. In this study, we focused on the phenolic compound profiles, antioxidant, anticholinergic, and antibacterial properties of *E. angustifolium*. The identification and quantification of phenolic compounds in this plant ethanol extract were performed using LC–MS/MS. The antioxidant properties, anticholinergic and antibacterial effects of the extract were investigated by various methods. In addition, we aimed to bring together much innovative information about *E. angustifolium* with this study.

Material and methods

Material

The aerial parts of *E. angustifolium* were collected during the flowering season (June–August 2020) from Giresun (Bulancak), Turkey (*Latitude*: 40°54'N. *Longitude*: 38°23'E, *Altitude*: 37 m). The plants were properly and botanically identified by the Faculty of Pharmacy, Istanbul University (Herbarium code number; ISTE 83,909) [12, 25]. The obtained aerial parts (2.30 kg) of plant material from the same area was washed with deionized water and dried for 5 to 7 days in the shade at room temperature and milled using a grinder (Lavion Herb Spice Grinder Mill Machine) and stored in flasks at − 4 °C until analyzed [25]. Compounds such as Trolox, BHT and BHA were used as a reference in antioxidant methods, and all other chemicals were purchased commercially [7, 12, 25]. All analyses were performed in triplicate.

Plant extract preparation

The extraction was carried out using the method described previously with minor modifications [26]. The dried sample: solvent (ethanol) ratio was mixed (1:10) and the extraction process was carried out in a shaker for 24 h. The extract was filtered through filter paper (Whatman No.1), and then it was evaporated. The dry matter was kept in a closed container at 4 °C until analysis. LC–MS/MS analysis results were determined considering the total dry matter ratio.

LC–MS/MS analysis

Phenolic content of *E. angustifolium* was determined by LC–MS/MS, using a Nexera model Shimadzu UHPLC attached to a tandem MS device. The validation studies of the method developed for 20 phenolic substances were carried out in the Harran University Central Research Laboratory. The LC-30AD dual pumps, DGU-20A3R degasser, CTO-10ASVP column furnace, and SIL-30AC autosampler were used for the analyses. Chromatographic separation was performed with C18 Inertsil ODS-4 (3.0 mm × 100 mm, 2 µM) analytical column. The column temperature was fixed at 40 °C. The elution gradient was created from mobile phase A (water and 0.1% formic acid) and mobile phase B (methanol and 0.1% formic acid). The injection volume was set to 4 µL and the solvent flow rate was kept at 0.5 mL min^{-1}. MS detection was done using a mass spectrometer equipped with Shimadzu LC–MS 8040 model triple, quadrupole and ESI source operation in both positive and negative ionization modes. LC–MS/MS data calculations were made with

Lab Solutions software (Shimadzu, Kyoto, Japan). Multiple reaction tracking (MRM) mode was used to measure analysis [27]. In the experiment, three applications were made for each compound analysis, averaged and the results were presented quantitatively.

Antioxidant activity

Total reduction capability

The metal reduction capability of *E. angustifolium* extracts was done in a modified form of the method reported by Elmastaş et al. [28]. Different weights of plant extracts (10, 20, 40 µg mL^{-1}) were mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixtures were incubated for 20 min at 50 °C. Then, trichloroacetic acid (2.5 mL, 10%) and $FeCl_3$ (0.25 mL, 0.1%) were added to each mixture and centrifuged at 3,000 rpm for 10 min. The absorbance values of the mixture were measured at 700 nm. The reducing power values were expressed as absorbance and the obtained results were compared to standard antioxidants.

Cu^{++} reduction capacity (CUPRAC)

The CUPRAC method based on the reduction of Cu (II) -Nc to Cu (I) -Nc chelate was applied [29]. 1 mL of $CuCl_2$ (0.01 M) solution, 1 mL of neocuprin (2,9-dimethyl-1,10-phenanthroline), and 1 mL of ammonium acetate (NH_4Ac) buffer solution were added to the test tube and mixed using a vortex. Then the different amounts of extracts (10, 20, 40 µg mL^{-1}) were added and the total volume was adjusted to 4 mL using ultrapure water. The absorbance was recorded at 450 nm after the incubation at room temperature for 30 min. The increased absorbance with the reaction in the mixture indicates that the Cu ion reduction capacity increases. The results were given as absorbance and compared to standard antioxidants.

DPPH removal activity

Blois method [30] was used for the determination of DPPH free radical removal activity of *E. angustifolium* extracts and standard antioxidants. For this analysis, 0.1 mM DPPH solution was prepared in methanol. The obtained samples (10, 20, 40 µg mL^{-1}) from stock solutions were mixed with 1 mL of this solution and were filled to 3 mL with ethanol. These solutions were thoroughly vortexed and incubated for 30 min in dark. The absorbance of samples was measured using a spectrophotometer at 517 nm. The results were reported as a percent of radical scavenging activity.

ABTS radical removal activity

This method is based on the principle of color change as a result of the treatment of colored $ABTS^+$ cation radical with an extract [31]. ABTS (2 mmol L^{-1}) solution was mixed with 2.45 mmol L^{-1} potassium persulfate ($K_2S_2O_8$) solution. The obtained solution was incubated for 14 h at room temperature in dark. Firstly, $ABTS^+$ radical solution was diluted with sodium phosphate buffer (0.1 mol L^{-1}, pH 7.4) until obtained an absorbance of 0.750 ± 0.025 at 734 nm. Then, 10, 20, 40 µg mL^{-1} of extracted stock solutions were taken and phosphate buffer was added until its volume was filled up to 3 mL. The prepared 1 mL $ABTS^+$ solution was stirred with the extract samples and vortexed. The absorbance was measured using a spectrophotometer at 734 nm. The results were reported as percent of radical scavenging activity.

Acetylcholinesterase (AChE) activity

The inhibitory effect of *E. angustifolium* extract on the AChE enzyme was determined with Ellman spectrophotometric method [32]. Reaction solution containing 50 µL5,5′-dithiobis (2-nitro-benzoic) acid (DTNB), 100 µL Tris – HCl buffer (1 M, pH 8.0) and 50 µL AChE (5.32×10^{-3} U) incubated at 30 °C and stirred for 15 min. Consequently, the reaction was started with the addition of 50 µL of acetylthiocholine iodide (AChI) used as a substrate. Enzymatic hydrolysis of the substrate was detected by spectrophotometry at 412 nm [33]. The effect of *E. angustifolium* ethanol extract in different concentration ranges (0.8–0.25 mg mL^{-1}) on the AChE was screened. The IC_{50} values were calculated from activity (%)-[Ligand] graphs for extract [33–35].

Inhibition of linoleic acid peroxidation

Percent inhibition of linoleic acid peroxidation of the *E. angustifolium* extract was measured according to the ferric thiocyanate method [36]. This method was based on measuring the hydroperoxide formed by linoleic acid oxidation spectrophotometrically at 500 nm. Obtained high absorbance values showed that the excess amount of peroxide formed as a result of peroxidation. The formed hydroperoxide oxidized Fe^{2+} to Fe^{3+}. After, Fe^{3+} formed a complex with the added thiocyanate and this complex gave a maximum absorbance at 500 nm. The concentrations reported to the desired amounts were transferred from stock solutions (1 mg mL^{-1}) to vials by an automatic pipette and volume was completed with buffer solution (2.5 mL, 0.04 M, pH 7.0). Then 2.5 mL of the linoleic acid emulsion was added to each vial. 2.5 mL buffer solution and 2.5 mL linoleic acid emulsion were used as control. Incubation was made at 37 °C. Every six hours, 100 µL of the vials were taken and placed in test tubes containing 4.7 mL of ethanol. 100 µL Fe^{2+} solution and then 100 µL

SCN^- solution were added to them. The blind sample was prepared by adding 100 µL Fe^{2+} and 100 µL SCN^- solutions to the test tube containing 4.8 mL ethanol. Absorbances of the samples at 500 nm were measured against the blind sample. The inhibition effect of samples on lipid peroxidation was determined as percent scavenging activity [37, 38].

Determination of the antibacterial activity of *E. angustifolium* extract

Preparation of cultures

The *Escherichia coli* (ATCC 25,922), *Staphylococcus aureus* (ATCC 25,923), and *Salmonella* Typhimurium (ATCC 14,028) cultures were used for the determination of the antibacterial effects of *E. angustifolium* extracts and stored at -80 °C until analyses. These cultures were activated in Trypticase Soy Yeast Extract (TSYE) for 24 h at 35–37 °C. The densities of cultures were brought to 1×10^8 CFU mL^{-1} with 0.5 McFarland standard [39].

Disc diffusion method

The sterile and 9 cm diameter of petri dishes were prepared using 20 mL of Mueller–Hinton Agar. The standard quantities of each bacterial suspension (10^8 CFU mL^{-1}) were transferred to the prepared petries. Then, 20 µL of EMS extract impregnated sterile paper discs (6 mm) were placed on the media. The prepared plates were kept at 4 °C for 1 h and then incubated for 24 to 48 h at 37 °C. Diameters of the bactericidal areas (mm) including the control disk were evaluated. Ciprofloxacin (5 µg $disk^{-1}$) was used as a positive control for this analysis [40].

Minimum inhibitory concentration (MIC) method

For this analysis, *E. angustifolium* extracts were prepared in 35% Dimethyl sulfoxide (DMSO) at the concentration of 312 mg mL^{-1} (w/v). The obtained mix was sterilized by 0.45 µm Millipore filters (France). The sterile samples were kept in 1.5 mL of Eppendorf tubes at 4 °C until antibacterial activity tests. The 10 µL of each bacterial inoculum was added to the microplates and the obtained plant extract was diluted to 312, 156, 78, 39, 19.5, 9.75 mg mL^{-1} using nutrient broth (NB). The sterilized DMSO solution was used in one well as a negative control. The prepared microplates were incubated at 35–37 °C for 24 h. The turbidity and growth in the plates were interpreted as positive situations. At the end of the incubation period, the plates were evaluated for the presence or absence of growth. Each test was repeated at least three times [40].

Statistical analysis

The obtained data were evaluated using GraphPad Prism version 6 (GraphPad Software, La Jolla, California, USA). The results were showed as mean ± standard deviation (95% confidence intervals). Differences between data sets were determined statistically significant at a p value ≤ 0.05. All analyzes were done in 2 parallel and 3 replicates.

Results and discussion

Analysis of phytochemical phenolic compound by LC–MS/MS

Linear regression quotations and linearity ranges of standard compounds available in the instrument library are given in Table 1. Correlation coefficients (R^2) of the standard curves were found to be higher than 0.99. In the analytical method used, LOD; 0.5–206.8 µg L^{-1}, LOQ; It varied in the range of 0.1 µg mL^{-1}–214.3 µg L^{-1}. Recovery of phenolic compounds; ranges from 96.6% to 101.1%. When the method validation studies performed for the determination of phenolic compounds in the literature were examined, it was seen that they were in parallel with the data in this study. In addition, the obtained R^2 values showed that they were within the limits of the data available in the literature. Zhu et al. [41] determined in their study that the correlation coefficients were higher than 0.99 and the recoveries varied between 95.9% and 106%. Moreover, in the LC–MS/MS validation study conducted by Agar et al. [42] for *Achillea* species; and they reported that the LOD ranged from 0.05–25.8 µg L^{-1} and LOQ: 0.17 to 85.9 µg L^{-1}, and the recovery of phenolic compounds ranged from 96.9% to 106.2%. These results supported our quantitative phenolic analysis.

The phenolic compounds (flavonoids and phenolic acids) composition of *E. angustifolium* was detected compared to standards. According to Table 1, major components of *E. angustifolium* was determined in luteolin (2716.7 µg L^{-1}) and it was followed by fumaric acid, vanillic acid, acetohydroxamic acid, quercetin, resveratrol, alizarin, salicylic acid, 4-hydroxybenzoic acid, kaempferol, bütein, gallic acid, oleuropein, and ellagic acid, respectively in the range of 942.9 µg L^{-1} down to 2.8 µg L^{-1}. The compounds in the study consisted mainly of phenolic flavonoid glycosides, phenolic acids, and derivatives. In a study, the rosebay willowherb (*Chamaenerion angustifolium* (L.) Scop. syn. *Epilobium angustifolium* L.) from the Onagraceae family was collected at different harvest times (flowering period in June and September and after) and their metabolites were identified using advanced LC–MS systems. As a result of the analysis, 45 phenolic metabolites, mainly gallic acid, oenothein B and quercetin 3-O-arabinoside were determined

Table 1 Quantitative determination of phytochemicals content of *Epilobium angustifolium* by LC–MS/MS method

Standard compounds	[a]MRM	[b]RSD %	[c]LOD/LOQ (μg L^{-1})	Recovery (%)	[d]RT	[e]R^2	Equation	Concentration (μg L^{-1})
Quercetin	301.1 > 151	0.0136	22.5/25.7	1.001	3.891	0.999	Y = (13.7831)X + (− 146.951)	280.83
Acetohydroxamic Acid	76.10 > 43.10	0.0082	2.8/8.2	1.000	0.406	0.999	Y = (150.982)X + (23.1833)	423.43
Catechin hydrate	291.10 > 139.00	0.0236	8.2/11.4	0.994	2.532	0.999	Y = (79.2933)X + (− 2406.22)	N.D
Vanillic Acid	168.80 > 93.00	0.0062	125.5/142.2	1.001	2.762	0.998	Y = (48.0522)X + (− 876.904)	676.00
Resveratrol	229.10 > 135.00	0.0131	9.0/13.6	0.998	3.606	0.998	Y = (46.4361)X + (− 1314.61)	218.65
Fumaric Acid	115.20 > 71.00	0.0047	25.2/31.3	0.997	0.809	0.999	Y = (20.2986)X + (− 762.592)	942.90
Gallic acid	169.20 > 125.00	0.0136	0.90/1.6	1.000	1.278	0.999	Y = (65.3835)X + (− 2699.84)	37.01
Caffeic Acid	179.20 > 135.00	0.0137	6.3/10.7	1.009	2.836	0.996	Y = (124.785)X + (− 487.132)	466.83
Phloridzin dihydrate	435.00 > 273.10	0.0564	61.0/207.0	1.000	3.594	0.999	Y = (33.4069)X + (− 1396.90)	N.D
Oleuropein	539.10 > 377.20	0.0694	0.05/1.0	0.997	3.567	0.999	Y = (25.9240)X + (− 558.916)	22.19
Ellagic Acid	300.90 > 145.10	0.0856	0.101/0.333	1.002	3.681	1.000	Y = (5.25903)X + (− 1167.31)	2.78
Myricetin	317.10 > 150.90	0.0079	55.4/59.6	0.999	3.644	0.999	Y = (37.0934)X + (2684.23)	N.D
Protocatechuic acid	181.20 > 108.00	0.0129	30.3/35.4	1.011	3.556	0.994	Y = (526.954)X + (23,026.1)	N.D
Bütein	271.10 > 135.00	0.0145	22.7/28.6	0.096	3.935	0.999	Y = (49.3543)X + (367.917)	53.90
Naringenin	271.10 > 150.90	0.0205	5.4/6.4	0.998	3.952	0.996	Y = (317.241)X + (33,733.3)	N.D
Luteolin	285.20 > 132.90	0.0057	0.5/2.5	1.007	4.069	0.998	Y = (34.6668)X + (3721.79)	2716.72
Kaempferol	285.10 > 116.90	0.0144	206.6/214.3	0.999	4.298	0.999	Y = (2.63905)X + (− 206.494)	72.53
Alizarin	239.20 > 210.90	0.0351	65.2/77.5	0.966	4.594	0.998	Y = (3.97487)X + (1614.23)	115.42
4-Hydroxybenzoic Acid	137.20 > 93.00	0.0154	30.5/40.25	0.996	3.664	0.999	Y = (735.804)X + (− 498.102)	74.92
Salicylic acid	137.20 > 93.00	0.0124	4.2/7.6	1.009	3.558	0.999	Y = (746.369)X + (6072.41)	77.66

[a]MRM: Multiple Reaction Monitoring. [b]RSD: Relative standard deviation. [c]LOD/LOQ (μg L^{-1}): limit of detection/ limit of quantitation. [d]RT: Retention time. [e]R^2: Determination coefficient. N.D: Not detected

in plant samples [43] Hevesi et al. [11] reported that 50 different flavonoids and their derivatives were determined in *E. angustifolium* plant extracts. Especially, these extracts contained flavonol aglycones including myricetin, kaempferol, and quercetin which contain a single sugar moiety of glucose, rhamnose, arabinose, galactose, or glucuronic acid [44]. The principal metabolites found in this plant were gallic acid, oenothein B and quercetin glucuronide. Also, glycosides of 3-methylmyricetin, kaempferol were identified for the first time in this genus, while myricetin, quercetin was already well-known phytochemicals of this plant species [45]. Ellagitannins and flavonoids, especially oenothein B, were among the compounds reported being the primary biologically active components in *E. angustifolium* extracts [8]. Also, the obtained results displayed that this plant extract had the richest phenolic profile from both quantitative and qualitative points of view. The richness of *E. angustifolium* in terms of phenolic compounds can be considered as a key factor preventing the development of benign prostatic hyperplasia (BPH) [8, 45].

Antioxidant properties of *E. angustifolium*

The antioxidant properties of a phenolic compound are originated from their free radical scavenging ability, chelate metal ions or donate hydrogen atom electron [25, 46]. The structure of phenolic compounds has key properties for their metal chelating activity and radical scavenging activity [46]. Due to the high DNA binding capacity and rich chemical composition, *E. angustifolium* can be considered as an excellent obstructing prodrug for potential cancer and bacterial diseases [25].

The free radical scavenging efficacy of the extracts is an important assessment and these properties have been determined using DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical scavenging value and ABTS (% 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical removal activity methods. The free radicals serve as oxidizable substrate and can be reduced using different methods [47].

The ABTS radical cation was obtained by oxidation of ABTS with potassium persulfate ($K_2S_2O_8$) [48]. The *E. angustifolium* extract had 11.3 ± 0.19% DPPH radical scavenging activity and 19.4 ± 0.40% ABTS radical scavenging activity at a concentration of 0.2 mg mL^{-1} (Table 2). The highest antioxidant activity was found in the standard of Trolox (81.2 ± 5.63%) and it was followed by BHA (71.8 ± 2.65%), BHT (46.3 ± 2.00%), and *E. angustifolium* (11.31 ± 0.19%), respectively. The lower antioxidant activities of the *E. angustifolium* plant than standard antioxidants might be stemmed from the presence of a low amount of

Table 2 The antioxidant activity and inhibition effect on AChE of *Epilobium angustifolium*

Antioxidants	DPPH[a] (0.2 mg mL^{-1})	ABTS[a] (0.2 mg mL^{-1})	FRAP Assay[b] (0.2 mg mL^{-1})	CUPRAC Assay[b] (0.2 mg mL^{-1})	AChE	
					IC_{50} (mg mL^{-1})	R^2
Epilobium angustifolium	11.31 ± 0.19	19.36 ± 1.36	0.17 ± 0.01	0.29 ± 0.01	0.14 ± 0.01	0.982 ± 0.02
BHA	71.82 ± 4.86	83.67 ± 5.41	0.45 ± 0.02	0.58 ± 0.02		
BHT	46.33 ± 2.64	48.35 ± 3.20	0.62 ± 0.02	0.64 ± 0.02		
Trolox	81.19 ± 5.63	80.06 ± 6.32	0.25 ± 0.01	0.52 ± 0.02		

Standard antioxidants (BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene, trolox)

[a]Values are expressed as percent radical scavenging activity

[b]Values are expressed as absorbance. High absorbance indicates high metal reduction capacity

antioxidant compounds. Similar findings were reported by Sayık et al. [25] for wild-growing *E. angustifolium* extracts. Shikov et al. [49] analyzed commercially available water-soluble extracts of *E. angustifolium* leaves and they found significant antioxidant activity in *in-vitro* assays. The DPPH scavenging results showed that the *E. angustifolium* extract is capable of free radicals scavenging. Also, it might have the ability to prevent the initiation and propagation of some free radical-mediated chain reactions. The antioxidant capacity of *E. angustifolium* species was attributed to the high ellagitannin content and especially to oenothein B [11, 17, 50].

The CUPRAC assay is a simple, rapid, effective, steady, and selective antioxidant measurement method for a wide variety of polyphenols [29]. An important property of antioxidant activity is the chelating/deactivation of transition metals which possess can catalyze Fenton-type reactions and hydroperoxide decomposition [51]. As can be seen in Table 2, the highest Fe^{3+}–Fe^{2+} reducing activity value and metal-reducing activity in CUPRAC assay were found for BTH and it was followed by BHA, Trolox, and *E. angustifolium*, respectively. The obtained results revealed that the *E. angustifolium* extract had lower copper and iron-reducing activity than standard antioxidants (BHA, BHT and Trolox). From these results, it might be said that some phenolic compounds found in *E. angustifolium* had significantly radical removal and metal reduction capacity. This plant might play a role in reducing oxidative stress due to the effect of phenolic compounds in its content for removing free radicals.

Anticholinergic effect

The increase in the hydrolysis of the neurotransmitter acetylcholine leads to the development of Alzheimer's disease (AD)**.** For this purpose, AChE inhibitors are widely used in the treatment of this disease. It is also known that AChE inhibitors used in the symptomatic treatment of AD increase antioxidant production and protect cells from oxidative damage [52]. Table 2 showed that the ethanolic extract of *E. angustifolium* had an inhibition effect on the AChE (IC_{50}: 0.14 ± 0.01 mg mL^{-1}).

The neuroprotective effects of phenolic compounds are stemmed from their important role in the treatment of AD. There are a lot of important approaches for the treatment of the disease (stemmed from raise to the acetylcholine ratio in the brain) with the use of AChE inhibitors [33]. Many studies have reported that flavonoid and phenolic compounds have anti-acetylcholinesterase activity. For example, a study reported that ellagic acid had a strong inhibitory effect on tyrosinase and AChE [33]. It is known that donepezil, which is widely used today, has an AChE inhibitory effect approximately 10,000 times stronger than ellagic acid [53, 54]. However, due to the side effects of synthetic reference inhibitors, it is especially important to identify alternative compounds of natural origin [55]. In this study, considering the LS-MS/MS content analysis, it might be said that vanilic acid, silymarin, caffeic acid, resveratrol, luteolin and other phenolic acids contained in *E. angustifolium* could have an inhibitory effect on AChE [53, 55, 56].

Linoleic acid peroxidation removal activity of *E. angustifolium*

The linoleic acid peroxidation removal ability using the ferric thiocyanate method is one of the most preferred antioxidant determination parameters [3–5]. This method measures the number of peroxides formed during the lipid peroxidation reaction [4, 5]. The total antioxidant activity of *E. angustifolium* extracts and the reference compounds (Trolox and α-tocopherol) were determined by the ferric thiocyanate method with linoleic acid.

The percentage inhibition effect of plant extracts on lipid peroxidation between 0–36 h at the 20 µg mL^{-1} concentration is given in Fig. 1. The plant extract inhibited the peroxidation of linoleic acid emulsion by 39.3% at the 24th hour, while the standard antioxidants (α-tocopherol and Trolox) exhibited an inhibition effect of 37.8% and 28.3%, respectively. The results showed that *E. angustifolium*

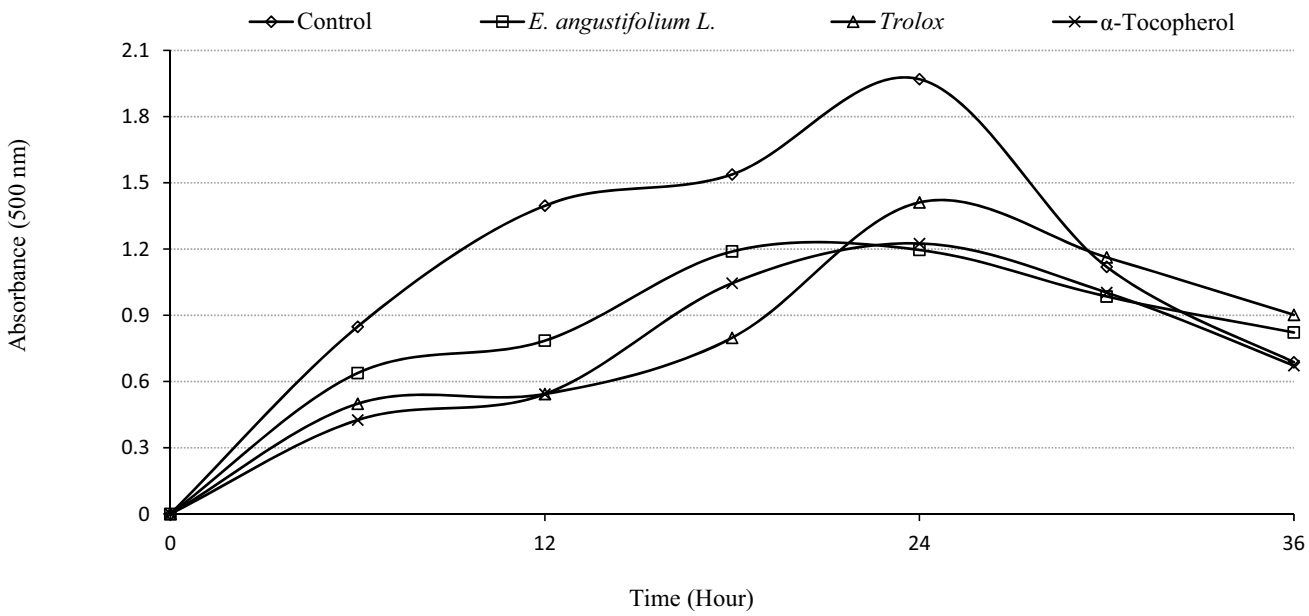

Fig. 1 Inhibition effect on linoleic acid peroxidation of standard antioxidants (α-tocopherol and Trolox) and *Epilobium angustifolium* ethanol extracts (20 µg mL^{-1})

extract had higher total antioxidant activity than Trolox and α-tocopherol.

These results indicate that the phenolic compounds in plant content could prevent lipid peroxidation. The obtained results showed that the high lipid peroxidation inhibitory effect of samples stemmed from the phenolic compounds in the plant content. Gülçin [56] reported that many phenolic and flavonoid compounds had an inhibitory effect on lipid peroxidation. The phenolic compounds found in plant content could be a suitable natural antioxidant for preventing the oxidation of fatty acids and foodstuffs containing them [56]. Also, Ferrante et al. [57] showed that the catechin concentration of *E. angustifolium* extract could reduce lipid peroxidation at high efficiency.

Based on our findings, the phenolic compound profiles of the *E. angustifolium* plant showed significant anticholinergic and antioxidant activities (radical and lipid peroxidation removal and metal reduction activity). In addition, this research revealed that the *E. angustifolium* had important natural antioxidants and might prevent many diseases such as Alzheimer's and atherosclerosis. It was thought that *E. angustifolium* extract might have an inhibitory effect on lipid peroxidation owing to the phenolic compounds it contains, and therefore it could be used to extend the shelf life of foods as an alternative to synthetic preservatives.

Antibacterial properties of *E. angustifolium*

The antibacterial activities of the *E. angustifolium* extract were determined against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* Typhimurium (Tables 3, 4).

As seen in Table 3, the 312 µg mL^{-1} *E.angustifolium* extract created the highest inhibition zone diameter on *Escherichia coli* (8.0 ± 0.10 mm), followed by *Staphylococcus aureus* (7.0 ± 0.20 mm) and *Salmonella Typhimurium* (6.0 ± 0.22 mm), respectively. The inhibition effect of *E. angustifolium* extract on bacteria cultures was found to be quite low compared to the ciprofloxacin antibiotic. The obtained MIC (Minimum inhibitory concentration) results showed that, the most effective of 10 µL plant extract inoculum applied at six different concentrations as 312 µg mL^{-1}, 156 µg mL^{-1}, 78 µg mL^{-1}, 39 µg mL^{-1}, 20 µg mL^{-1}, and 10 µg mL^{-1} (Table 4). When the determined results were examined, 312 µg mL^{-1} concentration was found the most

Table 3 Antibacterial effect of *Epilobium angustifolium* extract

Sample (6.24 mg $disk^{-1}$)	Concentration	Inhibition zone diameter (mm) *Staphylococcus aureus*	Inhibition zone diameter (mm) *Escherichia coli*	Inhibition zone diameter (mm) *Salmonella Typhimurium*
Epilobium angustifolium	312 mg mL^{-1}	7.0 ± 0.20	8.0 ± 0.10	6.0 ± 0.22
Control (Ciprofloxacin)	5 µg	19.0 ± 0.12	18.0 ± 0.12	15.0 ± 0.20

Table 4 MIC (Minimum inhibitory concentration) results of *Epilobium angustifolium* extract

Epilobium angustifolium	Concentration (mg mL^{-1})	Inoculum amount (µL)	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella Typhimurium*
	312	10	–	–	–
	156	10	–	–	+
	78	10	+	+	+
	39	10	+	+	+
	19.5	10	+	+	+
	9.75	10	+	+	+
Medium + Inoculum	0	10	+	+	+
Medium + Solvent (DMSO)	0	10	+	+	+
Medium	0	0	–	–	–

(+): growth, (–): no growth

effective on all bacteria. The 156 µg mL^{-1} concentration of plant extract showed an antibacterial effect on the examined bacteria except *Salmonella Typhimurium*. However, the other four concentrations were not effective on any bacteria. The obtained results show that the *E. angustifolium* had slightly antibacterial effects against the studied bacteria. Previous studies have shown that the therapeutic effect of herbs is due to the synergistic effect of multiple compounds, not just one component they have [24]. Battinelli et al. [23] reported in their study that *E. angustifolium* and *E. rosmarinifolium* prevented bacteria, yeasts, and fungi growth. On the other hand, *E. angustifolium* might be used for the preparation of antiseptic and antimycotic medicines to psoriasis, treat eczema, and other skin conditions [23, 58].

Besides their established antioxidant activity, many phenolic compounds may exhibit significant antibacterial activity. Polyphenols can exhibit very different antibacterial activity against the microbial strains that are representative of the foodborne pathogenic and food spoilage bacteria. The same polyphenols may be effective on one type of Gram-positive (or Gram-negative) strain. These inferences were highlighted as an important result in a study conducted by Bouarab-Chibane et al. [59] and Kılıç et al. [60].

Conclusions

Our results reveal that *E. angustifolium* extracts have antioxidant (metal reduction, free radical scavenging and anti-lipid peroxidation), anticholinergic and antibacterial activities. Also, the findings showed that this species might be evaluated for various purposes because of its important properties. Finally, the chemo-diversity of this species is promising for the treatment of important health problems due to the new antibacterial and antioxidant compounds. As a result, *E. angustifolium* might be considered as an important source of natural antioxidants, and partly an antibacterial agent for foods, preventing or minimizing lipid peroxidation in foods, prolonging shelf-life of pharmaceuticals and foodstuff.

Declarations

Conflict of interest All authors declared that they had no conflict of interest.

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