Received: 14 February 2019 | Revised: 6 April 2019 | Accepted: 5 May 2019

DOI: 10.1111/jfbc.12908

FULL ARTICLE

Journal of Food Biochemistry WILEY

Preliminary phytochemical analysis and evaluation of in vitro antioxidant, antiproliferative, antidiabetic, and anticholinergics effects of endemic *Gypsophila* taxa from Turkey

Ahmet Altay[1] | Hatice Tohma[1] | Lokman Durmaz[2] | Parham Taslimi[3,4] | Mustafa Korkmaz[5] | Ilhami Gulcin[4] | Ekrem Koksal[1]

[1]Faculty of Science and Arts, Department of Chemistry, Erzincan Binali Yıldırım University, Erzincan, Turkey

[2]Cayirli Vocational School, Department of Medical Services and Technology, Erzincan Binali Yıldırım University, Erzincan, Turkey

[3]Faculty of Science, Department of Biotechnology, Bartin University, Bartin, Turkey

[4]Faculty of Science, Department of Chemistry, Ataturk University, Erzurum, Turkey

[5]Faculty of Science and Arts, Department of Biology, Erzincan Binali Yıldırım University, Erzincan, Turkey

Correspondence
Ahmet Altay, Faculty of Science and Arts, Department of Chemistry, Erzincan Binali Yıldırım University, Erzincan, Turkey.
Email: aaltay2013@gmail.com

Abstract

The phenolic contents and antioxidant, anticancer, antidiabetic, and anticholinergic potentials of four endemic *Gysophila* taxa (G. *pallida*, G. *arrosti*, G. *tuberculosa*, and G. *eriocalyx*) were investigated. The HPLC analysis showed that methanol extracts of all the tested species were richer in phenolics than water extracts. 3,4-dihydroxybenzoic acid, p-hydroxybenzoic acid, vanillin, syringic acid, and p-coumaric acid were detected in all extracts. In parallel to the phenolic contents, methanol extracts displayed comparatively higher antioxidant activity than water extracts. Additionally, all extracts exhibited dose-dependent antiproliferative activity on the cancer cell lines with lower IC_{50} values changing from 0.170 to 1.805 mg/ml. Moreover, the extracts impressively inhibited the acetylcholinesterase (0.63–26.04), butyrylcholinesterase (3.66–10.73), and α-glycosidase (98.52–235.55) enzymes with very low IC_{50} (mg/ml) values. Together, the present results indicate that *Gysophila* taxa have various biological activities together with higher phenolic contents. Hence, these species hold good potential for use in the pharmaceutical industry.

Practical applications

Gypsophila taxa having numerous biological activities have been used for different purpose in folk medicine as well as their use in the food industry. The obtained results of the current study indicated that the extracts of *Gypsophila* taxa are rich in phenolics and flavonoids with powerful antioxidant and antiproliferative activity against different type of cancer cell lines. In addition, the extracts obtained from these taxa showed notable antidiabetic and anticholinergics effects. *Gypsophila* taxa could be used as a natural material to develop anticancer, antidiabetic, and anticholinergic drugs.

KEYWORDS

anticholinergic, antidiabetic antioxidant, antiproliferative, *Gysophila* taxa, phenolic composition

1 | INTRODUCTION

Many health problems and diseases including aging, cancer, and alzheimer have been associated with oxidative macromolecule damage caused by reactive oxygen species (ROS). This pathogenic phenomenon is known as oxidative stress which is implicated in a wide range of chronic and acute disease. Plant-based phenolic compounds have been suggested as a valuable source as antioxidants which are capable of scavenging ROS or chelating of metals involved in ROS formation (Ashraf, Sarfraz, & Mahmood, 2017; Köksal et al., 2017). In addition, plants with high phenolic compounds have been shown to have a wide variety of other biological activities such as antibacterial, antiviral, antitumor, antidiabetic, and anticancer activity (Altay & Bozoğlu, 2017; Nazia et al., 2016; Wu et al., 2018). Consequently, identification of the major phenolic compounds in medicinal plants and their biological action mechanisms has become the topic of many researchers. Their action mechanism as anticancer agent could be via different pathways such as promoting cell death, inhibiting cell growth, or suppression of angiogenesis, all of which are related to the alteration of cellular pathways (Kunnumakkara, Anand, & Aggarwal, 2008).

As mentioned, endemic medicinal plant species are the focus of pharmaceutical research since they could be used for the preparation of raw materials for medicines containing phytochemicals with biological activity, which might be useful for treating diseases. *Gypsophila* is the genus of mostly perennial plants that widely grows in Asia and Europe. Various *Gypsophila* taxa have been utilized for different purpose in folk medicine (Zheleva-Dimitrova et al., 2018). For example, *G. oldhamiana* and *G. paniculata* have been widely used to lowering fever, treat consumptive disease, malnutrition syndrome, and diabetes (Chen, Luo, & Kong, 2010). *G. elegans* was reported to be used in immune disorders as well as liver diseases (Huang et al., 2012). Besides biological activities, the extracts from different types of *Gypsophila* taxa are widely used to make liqueur, herbal cheese, ice cream, and some foods (Korkmaz & Özçelik, 2011). Additionally, the root extracts of *Gypsophila* taxa have been commonly used to present characteristic properties to halva in Turkey. For instance, the roots of the *Gypsophila* taxa provide whitening to halva, act as an emulsifier, improve the textural properties and increase the volume of the halva (Korkmaz & Özçelik, 2011). Moreover, root extracts have been used to provide softness and a desirable texture to the Turkish delight products (Ozdikicierler, Dirim, & Pazir, 2014).

Different biological activities from different *Gypsophila* taxa have been reported. For example, saponarin isolated from *G. trichotoma* was shown to have hepatoprotective potential and antioxidant activity as well as reducing lipid peroxidation on paracetamol-induced liver damage in rats (Simeonova et al., 2013). Isoorientin isolated from *G. elegans* was shown to have strong anticancer activity against hepatocarcinoma HepG2 cells (Lin et al., 2016). *G. oldhamiana* was also shown to α-glucosidase inhibitory activity.

Butyrylcholinesterase (BChE) has been studied by pharmacologists due to its responsibility for the hydrolysis of succinylcholine (SCh), a drug utilized in operation as a short-acting blocker of the acetylcholine (ACh) receptor. Some patients experiment prolonged apnea due to the slow hydrolysis of SCh which can be relevant to genetic alteration of the BChE (Gulçin & Taslimi, 2018). The significant role of Acetylcholinesterase (AChE) enzyme in some tissues, as for instance the red blood cell membrane, early myotendinous junction, and migrating neuro crest cells, is not explicit. During embryonic extension, a pattern of succession or organization of AChE and BChE has been reported, leading to the hypothesis that BChE functions as an embryonic AChE (Dufresne, Dagenais, & Shevell, 2014). Dietary carbohydrates are absorbed by various types of α-glycosidases such as sucrase, maltase, isomaltase, and glucoamylase present in the intestine. Therefore, inhibition of these enzymes could be a key role in the management of diabetes mellitus (DM). The α-glycosidase inhibitors (AGIs) are rich in natural sources including foodstuffs and plants (Zengin et al., 2018).

The present study was performed to determine the phenolic profile and investigate the antioxidant, antiproliferative, antidiabetic, and anticholinergics activities of four endemic *Gysophila* species; *G. pallida*, *G. arrosti*, *G. tuberculosa*, and *G. eriocalyx*, which could be ethnopharmacological importance. Folin–Ciocalteu and aluminum chloride colorimetric methods were used to determine the total phenolic and flavonoid content of the species, respectively. Additionally, Reverse Phase-High Performance Liquide Chromatography (RP-HPLC) analysis was performed to identify and quantify the individual phenolic compounds. For antioxidant activities, four assays were employed; 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Cupric Reducing Antioxidant Capacity (CUPRAC), and Fe^{2+} chelating tests. Antiproliferative potentials of the species were evaluated by XTT assay using HepG2, HT-29, and MCF-7 cell lines. Antidiabetic and anticholinergics activities were evaluated on the inhibition of AChE, BChE, and α-glycosidase enzymes.

2 | MATERIALS AND METHODS

2.1 | Chemicals

The chemicals, 2,9-dimethyl-1,10-phenanthroline, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid, 5,5′-dithiobis(2-nitrobenzoic acid), butyrylcholine iodide, butyrylcholinesterase from equine serum: 4-nitrophenyl α-D-glucopyranoside, α-glucosidase from *Saccharomyces cerevisiae*; acetylcholinesterase from Electrophorus electricus; butylated, hydroxyanisole, butylated hydroxytoluene, 1,1-diphenyl-2-picryl-hydrazyl, 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine, and α tocopherol were obtained from Sigma (Sigma-Aldrich, Germany). (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide) was purchased from Biological Industries (BI, Israil). The other chemicals were purchased from Sigma-Aldrich and/or Merck.

2.2 | Plant samples

The endemic *Gypsophila* taxa were obtained from their natural habitats, *G. eriocalyx* (GE) and *G. pallida* (GP), were obtained from gypsy slopes of Kuruçay-İliç (Erzincan, Turkey), on 27 June 2017, and

enumerated as Korkmaz: 4353 and 4354, respectively. *G. tuberculosa* (GT) was collected from rocky slopes of Üzümlü (Erzincan, Turkey), on 27 June 2017, and enumerated as Korkmaz: 4365 and 4362, respectively. *G. arrostii var. nebulosa* (GA) was collected from field side, Isparta–Konya Highway, on 14 July 2017, and enumerated as Korkmaz 4349. The voucher specimens were prepared according to the herbarium techniques and deposited at the herbarium of the Department of Biology, Erzincan Binali Yıldırım University, Erzincan, Turkey.

2.3 | Extract preparation

The aerial parts of the dried plant species were pounded by liquid nitrogen and then extracted with methanol and water, respectively. First, obtained powders were mixed with methanol in a ratio of 1:5 (w:v), and exposed to shaking overnight. This process was repeated 3 times and obtained solvents, including phenolic compounds, were combined. For preparation of water extract, distilled water (1:5 ratio) was added on the remaining pulp and stirred for 24 hr at room temperature. This step was also repeated 3 times and the solvents were combined. After that, the combined solvents containing phenolic substances were filtered through Whatman filter paper (No. 1). The methanol extract was concentrated by removing methanol with rotary evaporator (Heidolph, Germany). On the other hand, the water extract was lyophilized using a freeze-dryer (Cool-Safe 55, Lynge, Denmark). The obtained extracts were stored in brown bottle for use.

2.4 | Total phenolic content

Total phenolic content (TPC) of the plant species was determined using the method of Singleton and Rossi (1965) with slight modifications. Accordingly, at a certain concentration of the plant extracts (20 µl) was added into the 96-well plate including 100 µl of Folin's reagent (20%). After that, 80 µl of Na_2CO_3 solution (10%) was added and mixed. After incubation for 30 min at room temperature, the absorbance values were recorded at 750 nm using Elisa microplate reader (Epoch, BioTek, USA). Results were reported in terms of gallic acid equivalents (GAE). Each test was performed at least in three replicates.

2.5 | Total flavonoid content

Total flavonoid content (TFC) of the plant species was determined by aluminum chloride colorimetric method Chang, Yang, and Wen (2002) with slight modifications. Accordingly, the plant extracts at a certain concentration (20 µl) was added into the 96-well plate containing 6 µl of sodium nitrite (5%). Then, 80 µl of distilled water was added, and the mixture was incubated for 5 min. After the incubation time, 6 µl of aluminum chloride solution (10%) was added and incubated further 6 min. After that, 40 µl of sodium hydroxide (1 M) was added. Finally, the total volume was completed to 200 µl with distilled water. The changes in the absorbance of the reaction mixture were monitored at 510 nm by Elisa microplate reader. The results were represented as quercetin equivalents (QE). Each experiment was performed at least three replicates.

2.6 | HPLC instrumentation and chromatographic condition

The certain phenolic compounds present in the plant extracts were analyzed by Thermo 3000 HPLC system (Scientific™ Dionex™ UltiMate™, Ulti-Mate 3000), which is equipped with a pump, an autosampler column compartment, a photodiode array detector and a software (Chromeleon). The analysis was performed on an Agilent Zorbax SB-C18 column (250 mm × 4.6 mm × 5 µm) at 30°C during the analysis. The wavelength was scanned between 190 and 400 nm for PDA analysis. The flow rate of the HPLC was 1.0 ml/min. The mobile phase started with A (50% methanol: 50% water) and B (98% water: 2% acetic acid). The gradient elution system during the first 57 min, was changed from A:B (92:8) to A:B (28:72). The solvent ratio was changed back to the initial conditions at final 3 min. Standards of 3,4-dihydroxybenzoic acid, p-hyroxybenzoic acid, catechin, vanillin, syringic acid, p-coumaric acid, rutin, and rosmarinic acid were injected to the system separately and co-injected with samples (10 µl, 10 mg/ml). The presence or absence of the phenolics in the plant extracts were confirmed by comparing the retention time of the standard phenolics and ultraviolet spectra as well as spiking result analysis. All plant extracts and standard phenolic compounds were filtered through a 0.45-µm membrane filter (Millipore, Milford, MA) and 20 µl of sample was injected into the system. The injections were carried out at least three replicates.

2.7 | Antioxidant activity

2.7.1 | DPPH assay

DPPH radical scavenging capacity of the species was evaluated according to the method of Blois with some modifications (Blois, 1958). Briefly, 10 µl of plant extract at various concentrations was mixed with 140 µl of DPPH solution (0.05 mg/ml) into the 96-well plate and incubated for 30 min. After the incubation, the decrease in absorbance of the reaction mixture was monitored spectrophotometrically at 517 using Elisa microplate reader. The antioxidant capacity of the extracts was represented as IC_{50} value which is defined as the concentrations of the extracts scavenging 50% DPPH radicals. α-Tocopherol, trolox, BHA, and BHT were used as positive control.

The percent radical scavenging activity was calculated as follow:

$$\text{RSA}\ (\%) = \left[\left(A_0 - A_1\right) / A_0\right] \times 100.$$

2.7.2 | ABTS assay

ABTS cation radical scavenging capacity of the species was performed to the method described by (Re et al., 1999). First, ABTS radical was produced by mixing 2 mM ABTS solution with 2.45 mM $K_2S_2O_8$ solution for 6 hr. and adjusted the absorbance to 0.75 at 734 nm. Then, 250 µl of ABTS radical solution was added into the 96-well plate containing 2.5 µl of extract solution at various

concentrations and the changes in the absorbance of the reaction mixture was monitored at 734 nm using Elisa microplate reader. The results were represented as IC_{50} value (the concentrations of the plant extracts scavenging 50% ABTS radicals). α-Tocopherol, trolox, BHA, and BHT were used as positive controls. All measurements were performed at least three replicates. Radical scavenging activity was calculated using the following equation:

RSA (%) = $\left[\left(A_0 - A_1\right) / A_0\right]$ x 100, where A_0 is the absorbance of the control reaction and A_1 is the absorbance of the samples.

2.7.3 | Metal chelating assay

The iron chelating ability of all the plant extracts was estimated by the method of Dinis (Dinis, Madeira, & Almeida, 1994). Briefly, the extract solutions at various concentrations (50 μl) were mixed with 5 μl of $FeCl_2$ solution (2 mM) into the 96-well plate which contains 185 μl of distilled water. After 5-min incubation, 10 μl of ferrozine solution (5 mM) was added into each well and incubated further 10 min. The changes in the absorbance of the reaction mixture were measured at 562 nm. The metal chelating activity results of the plant extracts were expressed as IC_{50} value. EDTA was employed as reference control. All measurements were performed at least three replicates. The percent of bounded ferrous ions was calculated using the following equation:

Bounded ferrous ions (%) = $\left[\left(A_0 - A_1\right) / A_0\right]$ X 100.

2.7.4 | CUPRAC assay

Cupric ions reducing capacity of the plant species was carried out according to the method of Apak (Apak, Güçlü, Özyürek, & Çelik, 2008). Briefly, different plant extract solutions at various concentrations were added to the reaction tubes containing 125 μl of ammonium buffer solution (1.0 M), 125 μl of ethanolic neocuproine solution (7.5×10^{-3} M) and 125 μl of $CuCl_2$ solution (0.01 M). Finally, the final volumes were adjusted to 1 ml with distilled water. After 30-min incubation time, the absorbances were monitored at 450 nm. An increase in absorbance indicates a high reducing capacity. All measurements were applied at least three replicates. α-Tocopherol, trolox, BHA, and BHT were used as positive control.

2.8 | Antiproliferative bioassay

2.8.1 | Cell lines and culture

HT-29, HepG2, and MCF-7 cell lines were purchased from the ATCC (American Type Culture Collection, LGC Promochem, UK). HepG2 and MCF-7 cells were cultured in EMEM medium which includes L-Glutamine and HEPES buffer (25 mM) (BI, USA). HT-29 cells were grown in McCoy's 5A medium, including L-Glutamine and HEPES buffer (25 mM). The complete medium was formed by supplemented with 10% heat-inactivated fetal bovine serum, 1% penicillin–streptomycin and 1% Na-pyruvate (BI, USA). Incubation of the cell cultures were done at 37°C in incubator an incubator supplied with 5% CO_2 and 95% humidity (NUVE, Turkey). The cell culture studies were maintained in Class II Safety Cabinet.

2.8.2 | Determination of cell survival by XTT assay

XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay was performed to evaluate the antiproliferative ability of the plant extracts according to the manufacturer's instructions. Briefly, 100 μl of the cell suspension (10,000 cells/well) was added into 96-well culture plates and incubated overnight in a CO_2 incubator. The other day, the medium was removed and the cell culture plate was washed with PBS buffer. After removing the PBS, 100 μl of plant extracts at various concentration (5–1,000 μg/ml) cells were added into each well and incubated for 48 hr. After the incubation time, 50 μl of XTT reagent was added and incubated for 5 hr. Then, the cellular metabolic activity was measured at 450 nm spectrophotometrically by Epoch Microplate Reader (BioTek, USA). Antiproliferative potential of the plant extracts were represented as IC_{50} value which is expressed the concentration of the plant extract inhibiting 50% cell proliferation. IC_{50} values were estimated by regression analysis via dose-response curves. All measurements were performed at least 3 times.

2.9 | Enzymes studies

2.9.1 | AChE/BChE activity assay

The inhibitory action of four *Gypsophila* taxa on AChE/BChE enzymes activities were determined according to the procedure of Ellman, Courtney, Andres, and Featherstone (1961). In this part, acetylthiocholine iodide and butyrylthiocholine iodide (AChI/BChI) were utilized as substrates in both reactions. Briefly, 750 μl of sample solution at various concentrations was added into test tubes containing 100 μl of Tris/HCl buffer (1 M, pH 8.0). Then, 50 μl of BChE/AChE (5.32×10^{-3} U) solution was added into the test tubes and incubated for 8 min at 20°C. After incubation time, 50 μl of DTNB (0.5 mM) was added to the reaction tubes. The reaction was initiated by adding 50 μl of BChI/AChI. The reduction in BChI/AchI substrates was monitored at a wavelength of 412 nm, spectrophotometrically.

2.9.2 | α-Glycosidase activity assay

α-Glycosidase inhibitory activity of the plant extracts was performed according to the method of Tao, Zhang, Cheng, and Wang (2013). Briefly, the samples (10–100 μl) at various concentrations prepared in ethanol:water mixture were added into the test tubes containing 100 μL of phosphate buffer and 20 μl of the enzyme solution (0.15 U/ml, pH 7.4). Then, the mixture was pre-incubated at 35°C for 12 min. After that, 50 μl of *p*-NPG (5 mM, pH 7.4) was added into the test tubes and further incubated at 37°C. The changes in absorbance values were spectrophotometricaly monitored at 405 nm.

The IC50 values were calculated from percent activity versus sample concentration plots.

2.10 | Statistical analysis

For statistical analyses of antioxidant and anticancer activity results, unpaired Student's *t*-test was performed using GraphPad Prism 6 (GraphPad, La Jolla, CA) Software 7.0). Statistically significance was chosen at the $p < 0.05$ level.

3 | RESULTS AND DISSCUSSION

3.1 | Total phenolic and flavonoid content

In the present study, our first purpose was to determine the total phenolic and flavonoid contents of four endemic *Gypsophila* taxa since it has been well established that there is a strict correlation between the biological activity potential and phenolic contents of the medicinal plant extracts.

The obtained data regarding TPC and TFC of both extracts from four *Gypsophila* taxa were tabulated in Table 1. It was observed that TPC and TFC showed significant differences among the species. Methanol extracts were observed to richer in TPC and TFC than water extracts in all tested species. *G. arrosti* indicated the highest phenolic content in both methanol (188.4 mg GAE/g extract) and water (139.6 mg GAE/g extract) extracts, whereas *G. pallida* displayed the lowest phenolic content in both extracts (112.06 mg GAE/g extract for methanol and 104.44 mg GAE/g extract for water). Total flavonoid content was parallel to the phenolic content that is the methanol extracts were richer than water extracts except for *G. arrosti*. The highest flavonoid content was determined in water (114.21 mg QE/g extract) and methanol (89.77 mg QE/g extract) extracts of *G. arrosti*, while the lowest flavon content was in water (16.82 mg QE/g extract) and methanol (32.52 mg QE/g extract) extracts of *G. tuberculosa*.

TPC and TFC from various *Gysophila* species were previously reported (Zheleva-Dimitrova et al., 2018). Accordingly, TPC of *G. glomerata*, *G. trichotoma*, and *G. perfoliata* were 20.59, 17.07, and 21.60 mg GAE/g extract, respectively, and TFC of the same species were 33.00, 35.58, and 25.07 mg RE/g extract, respectively. These results indicate that our investigated four *Gypsophila* taxa have higher phenolic composition compared to three other *Gypsophila* taxa.

3.2 | HPLC analysis of the solvent extracts

Considering the remarkable effects of phenolic compounds on human health, many researches have focused on various analytical techniques to quantify phenolic compounds from different natural sources. HPLC is a widely used technique to detect phenolic compounds in various types of food and beverages. The phenolic compounds which are quantified in the different extracts of *Gypsophila* taxa were shown in Table 2.

As shown in Table 2, all of the extracts were found to include 3,4-dihydroxybenzoic acid, p-hydroxybenzoic acid, vanillin, syringic acid, and p-coumaric acid. The other scanned standard phenolics were not detected in both extracts. The highest phenolic compound detected in the methanol extracts was 3,4-dihydroxybenzoic acid in the following order: GE (1.15 μg/g extract) > GT (0.71 μg/g extract) > GP (0.47 μg/g extract) > GA (0.24 μg/g extract). While it was not detected in methanol extract of GA, rutin was the second phenolic compound measured at high level in methanol extracts of GT (0.601 μg/g extract) > GE (0.416 μg/g extract) > GA (0.361 μg/g extract). p-Hydroxybenzoic acid detected in methanol extracts except GT was in the following order: GE (0.06 μg/g extract)> GA (0.006 μg/g extract) > GP (0.004 μg/g extract). Catechin was detected only in methanol extract of GT (0.542 μg/g extract). Vanillin, except GT was found in the following order: GE (0.367 μg/g extract)> GA (0.068 μg/g extract) > GP (0.065 μg/g extract). Syringic acid was measured only GP (0.111 μg/g extract) and GE (0.490 μg/g extract). Methanol and water extracts of GE was the only species that contain rosmarinic acid as 0.293 and 0.436 μg/g extract, respectively. Considering the water extracts, 3,4-dihydroxybenzoic acid was the phenolic compound detected at the highest level in GP (1.573 μg/g extract) and

TABLE 1 Total phenolic and flavonoid contents of different extracts from four *Gypsophila* taxa

Extract	Species	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg QE/g extract)
Methanol[a]	*G. pallida*	112.06 ± 1.06 B[b]	48.76 ± 0.85 D[b]
	G. arrosti	188.4 ± 1.02 G	89.77 ± 0.54 F
	G. tuberculosa	120.65 ± 0.88 C	32.52 ± 0.45 C
	G. eriocalyx	143.82 ± 1.05 F	55.41 ± 0.64 E
Water[a]	*G. pallida*	104.44 ± 0.98 A	25.55 ± 0.45 B
	G. arrosti	139.63 ± 0.78 E	114.21 ± 1.35 G
	G. tuberculosa	108.12 ± 1.03 A	16.82 ± 0.97 A
	G. eriocalyx	127.45 ± 1.33 D	36.15 ± 0.46 C

[a]Extract.
[b]Values followed by different capital letters in the same column differ significantly at $p < 0.05$.

TABLE 2 Identification and quantification of phenolic compounds from four *Gypsophila* taxa by RP-HPLC

	Analytical parameters					*Gypsophila* taxa							
						Water extract (µg/g extract)				MeOH extract (µg/g extract)			
Standard phenolics	RT (min	Range (µg/L)	R^2	*LOD* (mg/L)	*LOQ*(mg/L) (mg/L)	GP	GA	GT	GE	GP	GA	GT	GE
3,4-Dihyroxybenzoic acid	5.60	0.125–10	0.999	0.0003	0.001	1.573	0.426	nd	nd	0.472	0.240	0.717	1.155
p-Hyroxybenzoic acid	9.37	0.5–10	0.999	0.003	0.001	0.0186	0.036	nd	nd	0.0042	0.0062	nd	0.0621
Catechin	11.3	1–10	0.997	0.003	0.009	nd	nd	nd	nd	nd	nd	0.542	nd
Vanillin	23.28	0.1–1	0.995	0.002	0.008	nd	0.103	nd	nd	0.0687	0.0656	nd	0.367
Syringic acid	23.97	0.1–1	0.996	0.002	0.009	0.199	0.170	0.1502	0.0582	0.111	nd	nd	0.490
p-Coumaric acid	28.16	0.050–8	0.998	0.004	0.001	0.0490	nd	0.0205	nd	nd	nd	nd	nd
Rutin	50.06	0.5–8	0.997	0.001	0.05	nd	0.176	0.393	0.4384	nd	0.361	0.601	0.416
Rosmarinic acid	51.88	5–80	0.998	0.001	0.005	nd	nd	nd	0.4360	nd	nd	nd	0.293

Abbreviations: GA, G. arrostii var. nebulosa; GE, G. eriocalyx; GP, G. pallid; GT, G. tuberculosa; LOD, Limit of detection; LOQ, Limit of quantification; nd, Not determined; RT, Retention time.

GA (0.426 µg/g extract), while not detected in GT and GE. In parallel to 3,4-dihydroxybenzoic acid, p-Hydroxybenzoic acid was measured only in GP (0.018 µg/g extract) and GA (0.036 µg/g extract). Vanillin was found only in GA (0.103 µg/g extract). Syringic acid was in the following order: GP (0.199 µg/g extract) > GA (0.170 µg/g extract) > GT (0.150 µg/g extract)> GE (0.058 µg/g extract). p-Coumaric acid was detected only in GP (0.049 µg/g extract) and GT (0.020 µg/g extract). Rutin except GP was measured in the following order: GE (0.438 µg/g extract)> GT (0.393 µg/g extract) > GA (0.176 µg/g extract). Considering all standard phenolic compounds in the species, 3,4-dihydroxybenzoic acid comes to the forefront. These results were supported by our previous study indicating that 3,4-dihydroxybenzoic acid was the most found phenolic detected in the extracts of *Gypsophila* species changing from 0.41 to 0.72 µg/g extract (Altay, Degirmenci, Korkmaz, Cankaya, & Koksal, 2018).

It was reported that 3,4-dihydroxybenzoic in diet at very low level could effectively inhibit the proliferation of many cancer types including skin (Tseng et al., 1998), colon (Tanaka, Kojima, Suzui, & Mori, 1993), and pancreatic (Pour, Salmasi, & Runge, 1978). Of course, connecting the antiproliferative effect of *G. eriocalyx* to one single polyphenol could be misleading, since the composition and synergistic effect of polyphenols found in the whole extract could be responsible for the observed effect.

For instance, a possible synergism was suggested in a study where chemopreventive potential of EGCG found in green tea was tested alone as well as in combination with NS398 against three different prostate cancer cells; LNCaP, PC-3, and CWR22Rν1 (Adhami et al., 2007). The result showed that the combination of EGCG and NS-398 had more effect on cell growth and apoptosis compared with two agents alone. In the same study, antiproliferation effect of the combination of green tea polyphenol GTP and celecoxib was compared with the effects of these compounds alone on CWR22Rn1 tumors in athymic nude mice in vivo. It was shown that the percent inhibition of the tumor growth by the combined compounds (81%) much higher than those of the compounds alone (42 and 57%) (Adhami et al., 2007). Therefore, combination of polyphenols could synergistically enhance their biological actions.

3.3 | Antioxidant activity

Phenolic compounds are a group of natural substances synthesized in plant as a secondary metabolism byproduct. Many studies revealed that phenolic substances may exhibit different biological actions, stability, and physiological action depending on different subgroups and structures. Although most of the evidence for antioxidant properties of phenolic compounds comes from the in vitro studies, increasing evidence suggests that they may in ways act beyond antioxidant action. Nonetheless, antioxidant assays are still widely used tools to assess biological functions of phenolic compounds.

In this study, antioxidant capacity of four endemic *Gypsophila* taxa were evaluated by DPPH and ABTS radical scavenging, CUPRAC, and Fe^{2+} chelating assays. The results were summarized in Table 3. In parallel to TPC, the methanol solvent extracts

TABLE 3 IC_{50} (μg/ml) values of methanol (MeOH) and water (WT) extracts of four *Gypsophila* taxa and standard antioxidants

	Radical scavenging				Metal chelating	
Assays	**ABTS IC_{50} (μg/ml)**		**DPPH IC_{50} (μg/ml)**		**Fe^{2+} chelating IC_{50} (μg/ml)**	
BHA	15.6 ± 0.85		8.2 ± 0.52		nt	
BHT	7.2 ± 0.56		21.6 ± 0.12		nt	
Trolox	12.4 ± 0.52		18.8 ± 0.75		nt	
α-Tocopherol	18.7 ± 0.78		28.4 ± 0.45		nt	
EDTA	nt		nt		34.7 ± 0,27	
Species	**MeOH**	**WT**	**MeOH**	**WT**	**MeOH**	**WT**
G.pallida	97.4 ± 1.4 B[a]	198 ± 1.7 C[a]	365 ± 1.7 D[a]	446 ± 1.75 C[a]	541 ± 3.5 C	1,489 ± 9.4 B[a]
G.arrosti	59 ± 1.25 A	68 ± 1.2 A	281 ± 1.2 B	301 ± 1.42 B	631 ± 4.2 D[a]	1,030 ± 7.8 A
G.tuberculosa	107 ± 1.8 C	139 ± 2.5 B	337 ± 2.5 C	556 ± 3.6 D	507 ± 3.6 B	1510 ± 5.7 B
G.eriocalyx	57 ± 1.2 A	69.5 ± 1.8 A	255 ± 1.8 A	279 ± 3.4 A	366 ± 4.4 A	1,224 ± 4.8 A

Abbreviations: BHA, Butylated Hydroxyanisole; BHT, Butylated hydroxytoluene; EDTA, Ethylenediaminetetraacetic acid; nt, not tested.
[a]Data marked with different superscripts (A, B, C, and D) within the same column indicate a significant difference statistically ($p < 0.05$).

showed stronger antioxidant activity than water extracts in all the assays.

DPPH radical scavenging activity results revealed that *G. eriocalyx* was the most powerful scavenger with its lower IC_{50} values in methanol (255 μg/ml) and water (279 μg/ml) extracts, respectively, while the lowest activity was exhibited by water extract of *G. tuberculosa* with an IC_{50} value of 556 μg/ml. Nevertheless, all the species exhibited moderate DPPH radical scavenging capacity than the tested standard antioxidants.

The methanol extract of *G. arrosti* displayed the highest ABTS cation radical scavenging capacity with an IC_{50} value of 59 μg/ml. The lowest efficiency, but still considerable, was observed in water extract of *G. pallida* with IC_{50} value of 198 μg/ml. Overall, methanol extracts of the species indicated better ABTS radical screening activity as compared to water extracts. In comparison with the DPPH method, the species, especially methanol extracts, exhibited closer activity to standard antioxidant compounds in the ABTS method.

We also examined the metal chelating activity of the species. As shown in Table 3, the chelating capacities of Fe^{2+} ions increased in parallel to the concentrations of the plant extracts. The IC_{50} values of the extracts were ranged from 366 μg/ml to 631 μg/ml for methanol solvent extracts and from 1,030 to 1,510 μg/ml for water solvent extracts. *G. eriocalyx* exhibited the highest chelating activity, while *G. arrosti* showed the lowest activity among the methanol extracts. As for water extracts, *G. arrosti* displayed the highest activity in all species. Nonetheless, metal chelating activity of EDTA was superior to the tested species.

CUPRAC assay is a commonly used antioxidant method for evaluation of the plant extracts and individual phenolic compounds (Karaman, Tütem, Sözgen Başkan, & Apak, 2010). The advantages of this assay are to measure thiol-type antioxidants including glutathione. This method depends on the reduction of Cu^{2+} ion to Cu^{+}in the presence of neocuproine. The higher absorbance is the indication of better reducing activity. As shown in Figure 1, reducing power of the

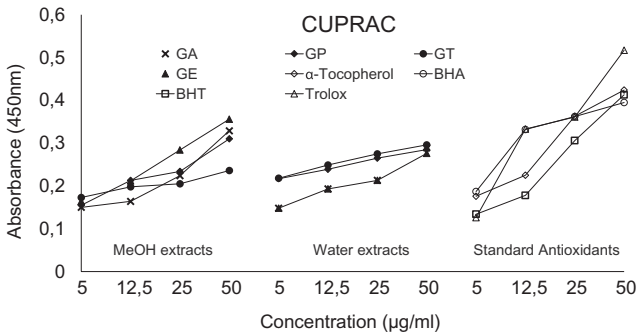

FIGURE 1 Reducing power of endemic *Gypsophila* taxa and standard antioxidants by CUPRAC assay

species were dose-dependent manner and similar to the previous antioxidant assays that are methanol extracts displayed better-reducing activity than water extracts. Among the methanol extracts, *G.eriocalyx and G.arrosti* exhibited the highest and lowest activity at 50 μg/ml with the absorbance values of 0.356 and 0.329, respectively. Moreover, the species showed close activity to the standard antioxidants at low concentrations. However, the concentration increasing up to 50 μg/ml, our species displayed moderate activity.

Regarding the antioxidant activity of *Gysophila* species, literature has limited information. Previously, it was reported that *G. trichotoma* displayed better activity by ABTS and DPPH assays compared to *G. perfoliata*. In addition, it was reported that *G. pilulifera* is capable of scavenging DPPH radical and verbascoside is the main compound responsible for this action in methanol extract (Chima, Nahar, Majinda, Celik, & Sarker, 2014).

3.4 | Antiproliferative activity

To evaluate the inhibition potential of the *Gypsophila* taxa on the cell viability, XTT tests were carried out. The treatment of the

TABLE 4 IC_{50} (mg/ml) values of four *Gypsophila* taxa against human-derived cancer cell lines

Treatment	Species	HepG2	HT–29	MCF–7
Methanol[a]	*G. pallida*	0.513 ± 0.028 C[b]	0.384 ± 0.022 C[b]	0.615 ± 0.045 C[b]
	G. arrosti	0.423 ± 0.019 B	0.227 ± 0.031 B	0.530 ± 0.029 B
	G. tuberculosa	0.599 ± 0.033 D	0.661 ± 0.051 D	0.379 ± 0.022 A
	G. eriocalyx	0.269 ± 0.064 A	0.170 ± 0.056 A	0.381 ± 0.048 A
Water[a]	*G. pallida*	1.502 ± 0.056 G	1.148 ± 0.074 G	1.002 ± 0.062 F
	G. arrosti	1.040 ± 0.096 F	0.839 ± 0.046 E	0.941 ± 0.073 E
	G. tuberculosa	1.805 ± 0.094 H	0.971 ± 0.04 F	1.306 ± 0.075 G
	G. eriocalyx	0.856 ± 0.12 E	0.644 ± 0.080 D	0.814 ± 0.075 D
5-FU		0.047 ± 0.002	0.023 ± 0.006	0.018 ± 0.004

Abbreviation: 5-FU, 5-Flourouracil (positive control).
[a]Extract.
[b]Values followed by different capital letters in the same column differ significantly at $p < 0.05$.

cell lines with both extracts from *Gypsophila* taxa against HepG2, HT-29, and MCF-7 cell lines has resulted in a notable cell growth inhibition (Table 4). The antiproliferative activity results of the species were similar to the antioxidant activity results, that is, the methanol extracts were more effective than water extracts. As shown in Table 4, *G. eriocalyx* was the most effective inhibitor of cell growth with the lowest IC_{50} values in both methanol and water extracts against all three cell lines. Thus, our data suggest that the *Gysophila* species, especially methanol extracts, could induce antiproliferation in HepG2, HT29, and MCF-7 cell lines. On the other hand, the extracts from the tested *Gypsophila* taxa showed moderate antiproliferative activity compared to standard chemotherapeutic drug 5-FU. Nevertheless, further studies are needed to elucidate the exact molecular mechanisms of this activity.

Numerous studies showed that the biological activity of Gypsohila taxa could be attributed to the presence of triterpenoids. For instance, saponins which are isolated from *Gypsophila oldhamiana* was reported to have inhibitory activity on HT29, SGC7901, and PLC/PRF/5 cell lines (Bai et al., 2007). Isoorientin from *Gypsophila elegans* was shown to have cytotoxic effect on HepG2 cells through the regulation of cell cycle-related genes and inducing apoptosis by arresting the cell cycle and increasing proteolytic activities of caspases. Evaluation of triterpenoid saponins from *Gypsophila arrostii* against SW480 cells showed that it has cytotoxic effect (Arslan, Celik, & Melzig, 2013). Two new triterpenoids from *Gypsophila oldhamiana* was reported to have significant cytotoxicity on H460 tumor cell lines (Xie et al., 2016). Moreover, two compounds were isolated from *Gypsophila trichotoma*, one of which showed strong cytotoxic effects against seven human cancer cell lines (Yotova, Krasteva, Jenett-Siems, Zdraveva, & Nikolov, 2012). A remarkable study showed that cytotoxic effects and the action mechanism of the root extract of *Gypsophila* vary depending on the type of cell lines. For example, the extract from *Gypsophila* species preferentially was reported to induce apoptosis in human hepatoma SMMC-7721 cells through activating caspase-3 as well as increasing the phosphorylation of the extracellular signal-regulated kinase and c-Jun N-terminal kinase, whereas these changes were not observed in hepatic L02 cell line (Zhang, Luo, Zhang, & Kong, 2013).

3.5 | Anticholinergics and antidiabetic activities

It has been reported numerous documents about the biological effects of natural dietary supplements that have been used for treating Alzheimer disease (AD) and diabetes in traditional medicine. Inhibition of AChE and BChE enzymes which hydrolysis Ach and BCh is the most established approach for the treatment AD. AChE is exists in all excitable cells, while BChE enzyme is present more commonly in the central neural system, plasma, and liver (Gulçin et al., 2017).

As shown in Table 5, all of the tested *Gypsophila* extracts could efficiently inhibit AChE, BChE, and α-Glycosidase enzymes. 9-Amino-1,2,3,4-tetrahydroacridine (TAC) compound is known as a reversible inhibitor for BChE and AChE enzymes as well as the first drug used as placative treatment in AD. The IC_{50} values for these enzymes were obtained in the range 0.63–26.04 μg/ml for AChE, 3.66–10.73 μg/ml for BChE, and 98.52–235.55 μg/ml for α-glycosidase. In addition, Tacrine (TAC) which is used as standard AChE and BChE inhibitors was found to have IC_{50} values 23.64 μmol/L and 33.74 μmol/L, respectively, against these enzymes. The IC_{50} values belonging to the extracts and TAC for AChE inhibition was in the following order: *G. tuberculosa* (0.63 μg/ml, r^2:0.972) (methanol) < *G. tuberculosa* (1.23 /ml, r^2:0.973) (water) < *G. pallida* (4.84 μg/ml, r^2: 0.963) (methanol) < *G. pallida* (6.04 μg/ml, r^2: 0.988) (water) < *G. arrosti* (13.70 μg/ml, r^2: 0.989) (methanol) < *G. arrosti* (16.90 μg/ml, r^2: 0.993) (water) < *G. arrosti* (25.06 μg/ml, r^2: 0.990) (water) < *G. eriocalyx* (26.04 μg/ml, r^2: 0.930) (methanol) < *tacrine* (33.74 μg/ml, r^2: 0.973).

Inhibition of the carbohydrate digestion enzymes such as α-glycosidase and α-amylase can cause to reduction of postprandial blood glucose level when fed with a high-carbohydrate diet (Wasai et al., 2018). Therefore, it can be an important strategy for the management of postprandial blood glucose level in type 2 DM as well as borderline patients. As shown in Table 5, the tested *Gypsophila* extracts

TABLE 5 The enzyme inhibition results of four *Gypsohila* species against AChE, BChE, and α-glycosidase enzymes

Treatment	Species	AChE IC_{50} (μg/ml)	r^2	BChE IC_{50} (μg/ml)	r^2	α-Gly IC_{50} (μg/ml)	r^2
Methanol	*G. pallida*	4.84	0.963	8.03	0.952	135.84	0.983
	G. arrosti	13.70	0.989	4.04	0.903	98.52	0.962
	G. tuberculosa	0.63	0.972	3.66	0.974	110.21	0.981
	G. eriocalyx	26.04	0.930	10.73	0.982	205.88	0.927
Water	*G. pallida*	6.04	0.988	9.42	0.951	184.65	0.946
	G. arrosti	16.90	0.993	4.93	0.910	104.82	0.993
	G. tuberculosa	1.23	0.973	4.57	0.942	125.04	0.976
	G. eriocalyx	25.06	0.990	10.52	0.976	235.55	0.917
	TAC[a]	33.74	0.973	23.64	0.917	–	–
	ACR[b]	–	–	–	–	22.8	–

[a]Tacrine (TAC) was used as positive control for AChE and BChE enzymes and determined as μM levels.
[b]Acarbose (ACR) was used as positive control for α-glycosidase enzymes and determined as μM levels.

could effectively inhibit α-glycosidase enzyme, and the IC_{50} values of the *Gypsophila* taxa and ACR for α-glycosidase enzyme were found in the following order: acarbose (22.08 nM) < *G. arrosti* (98.52 μg/ml, r^2:0.962) (methanol) < *G. arrosti* (104.82 μg/ml, r^2:0.993) (water) < *G. tuberculosa* (110.21 μg/ml, r^2: 0.981) (methanol) < *G. tuberculosa* (125.04 μg/ml, r^2: 0.976) (water) < *G. pallida* (135.84 nM, r^2: 0.983) (methanol) < *G. pallida* (184.65 nM, r^2: 0.946) (water) < *G. eriocalyx* (205.88 μg/ml, r^2: 0.927) (methanol) < *G. arrosti* (235.55 μg/ml, r^2: 0.917) (water).

4 | CONCLUSION

Phenolic compounds are a large group of phytochemicals that are widely distributed in plant kingdom. Growing interest in these substances is due to their biological actions in human body, which could prevent obesity, coronary heart disease, colon cancer, gastrointestinal disorders, and can also reduce the risk of diabetes. Therefore, numerous plants and different analytical methods have been used to evaluate potential pharmaceutical value of various sources. This paper provides information on antioxidant, antiproliferative, antidiabetic, and anticholinergics activities as well as phenolic content found in four endemic *Gypsophila* taxa. Taken all together, HPLC data revealed that different extraction solutions altered chromatographic profiles and allowed the identification of different photochemical from the samples. As for the biological activity section of this paper, in general, the methanol extracts of four *Gypsophila* taxa exhibited higher antioxidant, antiproliferative, antidiabetic, and anticholinergics activities with lower IC_{50} values compared to the water extracts. However, the underlying molecular mechanisms of this activity, especially anticancer mechanism, should be further investigated in detail. Additionally, inhibition of AChE, BChE can have a significant role in discovery and drug design as well as in toxicology and medicine. In this context, these plants may be evaluated for their antidiabetic and anticholinergic activity potentials.

ACKNOWLEDGMENTS

This study was financially supported by grants from Erzincan Binali Yıldırım University, Scientific Research Projects Coordination Commission (EU-BAP) (Project No.: FBA-2017-470).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ORCID

Ahmet Altay https://orcid.org/0000-0001-8120-8900
Lokman Durmaz https://orcid.org/0000-0002-3773-5751
Parham Taslimi https://orcid.org/0000-0002-3171-0633
Ilhami Gulcin https://orcid.org/0000-0001-5993-1668
Ekrem Koksal https://orcid.org/0000-0002-1026-972X

REFERENCES

Adhami, V. M., Malik, A., Zaman, N., Sarfaraz, S., Siddiqui, I. A., Syed, D. N., … Mukhtar, H. (2007). Combined inhibitory effects of green tea polyphenols and selective cyclooxygenase-2 inhibitors on the growth of human prostate cancer cells both in vitro and in vivo. *Clinical Cancer Research*, *13*(5), 1611–1619. https://doi.org/10.1158/1078-0432.CCR-06-2269

Altay, A., & Bozoğlu, F. (2017). Salvia fruticosa modulates mRNA expressions and activity levels of xenobiotic metabolizing CYP1A2, CYP2E1, NQO1, GPx, and GST enzymes in human colorectal adenocarcinoma HT-29 Cells. *Nutrition and Cancer*, *69*(6), 892–903.

Altay, A., Degirmenci, S., Korkmaz, M., Cankaya, M., & Koksal, E. (2018). In vitro evaluation of antioxidant and anti-proliferative activities of *Gypsophila sphaerocephala* (*Caryophyllaceae*) extracts together with their phenolic profiles. *Journal of Food Measurement and Characterization*, *12*(4), 2936–2945. https://doi.org/10.1007/s11694-018-9909-y

Apak, R., Güçlü, K., Özyürek, M., & Çelik, S. E. (2008). Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchimica Acta*, *160*(4), 413–419. https://doi.org/10.1007/s00604-007-0777-0

Arslan, I., Celik, A., & Melzig, M. F. (2013). Nebulosides A-B, novel triterpene saponins from under-ground parts of *Gypsophila arrostii Guss*. var. nebulosa. *Bioorganic and Medicinal Chemistry*, *21*(5), 1279–1283. https://doi.org/10.1016/j.bmc.2012.12.036

Ashraf, A., Sarfraz, R. A., & Mahmood, A. (2017). Phenolic compounds' characterization of Artemisia rutifolia spreng from Pakistani flora and their relationships with antioxidant and antimicrobial attributes. *International Journal of Food Properties*, *20*(11), 2538–2549.

Bai, H., Zhong, Y., Xie, Y. Y., Wang, Y. S., Liu, L., Zhou, L., … Zuo, C. X. (2007). A major triterpenoid saponin from *Gypsophila oldhamiana*. *Chemistry and Biodiversity*, *4*(5), 955–960. https://doi.org/10.1002/cbdv.200790085

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*, 1199–1200. https://doi.org/10.1038/181119 9a0

Chang, C., Yang, M., & Wen, H. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric. *Methods. Journal of Food and Drug Analysis*, *10*(3), 178–182.

Chen, Q., Luo, J.-G., & Kong, L.-Y. (2010). Triterpenoid Saponins from *Gypsophila altissima* L. *Chemical & Pharmaceutical Bulletin*, *58*(3), 412–414. https://doi.org/10.1248/cpb.58.412

Chima, N. K., Nahar, L., Majinda, R. R. T., Celik, S., & Sarker, S. D. (2014). Assessment of free-radical scavenging activity of *Gypsophila pilulifera*: Assay-guided isolation of verbascoside as the main active component. *Brazilian Journal of Pharmacognosy*, *24*(1), 38–43. https://doi.org/10.1590/0102-695X20142413391

Dinis, T. P. C., Madeira, V. M. C., & Almeida, L. M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics*, *315*(1), 161–169. https://doi.org/10.1006/abbi.1994.1485

Dufresne, D., Dagenais, L., & Shevell, M. I. (2014). Epidemiology of severe hearing impairment in a population-based cerebral palsy cohort. *Pediatric Neurology*, *51*(5), 641–644. https://doi.org/10.1016/j.pediatrneurol.2014.07.005

Ellman, G. L., Courtney, K. D., Andres, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, *7*(2), 88–95. https://doi.org/10.1016/0006-2952(61)90145-9

Gulçin, İ., Abbasova, M., Taslimi, P., Huyut, Z., Safarova, L., Sujayev, A., … Supuran, C. T. (2017). Synthesis and biological evaluation of aminomethyl and alkoxymethyl derivatives as carbonic anhydrase, acetylcholinesterase and butyrylcholinesterase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *32*(1), 1174–1182. https://doi.org/10.1080/14756366.2017.1368019

Gulçin, İ., & Taslimi, P. (2018). Sulfonamide inhibitors: A patent review 2013-present. *Expert Opinion on Therapeutic Patents*, *28*(7), 541–549. https://doi.org/10.1080/13543776.2018.1487400

Huang, Q. F., Zhang, S. J., Zheng, L., Liao, M., He, M., Huang, R. B., … Lin, X. (2012). Protective effect of isoorientin-2'-O-α-l-arabinopyranosyl isolated from *Gypsophila elegans* on alcohol induced hepatic fibrosis in rats. *Food and Chemical Toxicology*, *50*(6), 1992–2001. https://doi.org/10.1016/j.fct.2012.03.044

Karaman, Ş., Tütem, E., Sözgen Başkan, K., & Apak, R. (2010). Comparison of total antioxidant capacity and phenolic composition of some apple juices with combined HPLC-CUPRAC assay. *Food Chemistry*, *120*(4), 1201–1209. https://doi.org/10.1016/j.foodchem.2009.11.065

Köksal, E., Tohma, H., Kılıç, Ö., Alan, Y., Aras, A., Gülçin, I., & Bursal, E. (2017). Assessment of antimicrobial and antioxidant activities of nepeta trachonitica: Analysis of its phenolic compounds using HPLC-MS/MS. *Scientia Pharmaceutica*, *85*(2), 1–14. https://doi.org/10.3390/scipharm85020024

Korkmaz, M., & Özçelik, H. (2011). Economic importance of *Gypsophila* L., Ankyropetalum Fenzl and *Saponaria* L. (Caryophyllaceae) taxa of Turkey. *African Journal of Biotechnology*, *10*(47), 9533–9541.

Kunnumakkara, A. B., Anand, P., & Aggarwal, B. B. (2008). Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins. *Cancer Letters*, *269*(2), 199–225. https://doi.org/10.1016/j.canlet.2008.03.009

Lin, X., Wei, J., Chen, Y., He, P., Lin, J., Tan, S., … Huang, Q. (2016). Isoorientin from *Gypsophila elegans* induces apoptosis in liver cancer cells via mitochondrial-mediated pathway. *Journal of Ethnopharmacology*, *187*, 187–194. https://doi.org/10.1016/j.jep.2016.04.050

Nazia, A., Syed, A. R. N., Zaib, H., Nasir, R., Zulfiqar, A. K., Sohail, A. S., … Hawa, Z. J. (2016). Compositional difference in antioxidant and antibacterial activity of all parts of the *Carica papaya* using different solvents. *Chemistry Central Journal*, *10*(5). https://doi.org/10.1186/s13065-016-0149-0

Ozdikicierler, O., Dirim, S. N., & Pazir, F. (2014). The effects of spray drying process parameters on the characteristic process indices and rheological powder properties of microencapsulated plant (*Gypsophila*) extract powder. *Powder Technology*, *253*, 474–480. https://doi.org/10.1016/j.powtec.2013.12.004

Pour, P. M., Salmasi, S. Z., & Runge, R. G. (1978). Selective induction of pancreatic ductular tumors by single doses of N-nitrosobis(2-oxopropyl)amine in syrian golden hamsters. *Cancer Letters*, *4*(C), 317–323. https://doi.org/10.1016/S0304-3835(78)95502-7

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*, 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Simeonova, R., Vitcheva, V., Kondeva-Burdina, M., Krasteva, I., Manov, V., & Mitcheva, M. (2013). Hepatoprotective and antioxidant effects of saponarin, isolated from *Gypsophila trichotoma* wend. on paracetamol-induced liver damage in rats. *BioMed Research International*, *2013*(2). https://doi.org/10.1155/2013/757126

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphtungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144–158.

Tanaka, T., Kojima, T., Suzui, M., & Mori, H. (1993). Chemoprevention of colon carcinogenesis by the natural product of a simple phenolic compound protocatechuic acid: Suppressing effects on tumor development and biomarkers expression of colon tumorigenesis. *Cancer Research*, *53*(17), 3908–3913.

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. *Biomedical Chromatography*, *27*(2), 148–155.

Tseng, T. H., Hsu, J. D., Lo, M. H., Chu, C. Y., Chou, F. P., Huang, C. L., & Wang, C. J. (1998). Inhibitory effect of Hibiscus protocatechuic acid on tumor promotion in mouse skin. *Cancer Letters*, *126*(2), 199–207. https://doi.org/10.1016/S0304-3835(98)00010-X

Wasai, M., Nonaka, H., Kitamura, R., Murata, M., Tachibana, H., & Fujimura, Y. (2018). Postprandial glycaemia-lowering effect of a green tea cultivar Sunrouge and cultivar-specific metabolic profiling for determining bioactivity-related ingredients. *Scientific Reports*, *8*(1), 1–9. https://doi.org/10.1038/s41598-018-34316-8

Wu, Y., Bai, J., Liu, X., Liu, L., Zhong, K., Huang, Y., & Gao, H. (2018). Antibacterial effect of 3-p-trans-coumaroyl-2-hydroxyquinic acid, a phenolic compound from needles of *Cedrus deodara*, on cellular functions of *Staphylococcus aureus*. *RSC Advances*, *8*(9), 4969–4975.

Xie, L. X., Zhang, H. C., Wang, H. Y., Wang, Y., Wang, F. L., & Sun, J. Y. (2016). Two new triterpenoids from *Gypsophila oldhamiana*. *Natural Product Research*, *30*(9), 1068–1074.

Yotova, M., Krasteva, I., Jenett-Siems, K., Zdraveva, P., & Nikolov, S. (2012). Triterpenoids in *Gypsophila trichotoma* wend. *Phytochemistry Letters*, *5*(4), 752–755. https://doi.org/10.1016/j.phytol.2012.08.006

Zengin, M., Genc, H., Taslimi, P., Kestane, A., Guclu, E., Ogutlu, A., ... Gulçin, İ. (2018). Novel thymol bearing oxypropanolamine derivatives as potent some metabolic enzyme inhibitors—Their antidiabetic, anticholinergic and antibacterial potentials. *Bioorganic Chemistry*, *81*, 119–126. https://doi.org/10.1016/j.bioorg.2018.08.003

Zhang, W., Luo, J.-G., Zhang, C., & Kong, L.-Y. (2013). Different apoptotic effects of triterpenoid saponin-rich *Gypsophila oldhamiana* root extract on human hepatoma SMMC-7721 and normal human hepatic L02 cells. *Biological and Pharmaceutical Bulletin*, *36*(7), 1080–1087. https://doi.org/10.1248/bpb.b12-01069

Zheleva-Dimitrova, D., Zengin, G., Balabanova, V., Voynikov, Y., Lozanov, V., Lazarova, I., & Gevrenova, R. (2018). Chemical characterization with in vitro biological activities of *Gypsophila species*. *Journal of Pharmaceutical and Biomedical Analysis*, *155*, 56–69. https://doi.org/10.1016/j.jpba.2018.03.040

How to cite this article: Altay A, Tohma H, Durmaz L, et al. Preliminary phytochemical analysis and evaluation of in vitro antioxidant, antiproliferative, antidiabetic, and anticholinergics effects of endemic *Gypsophila* taxa from Turkey. *J Food Biochem*. 2019;43:e12908. https://doi.org/10.1111/jfbc.12908