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ORIGINAL ARTICLE / ÖZGÜN MAKALE

EVALUATION OF HPLC, PHYTOCHEMICAL, ANTICHOLINESTERASE AND ANTIOXIDANT PROFILES OF THE AERIAL PARTS OF *ASPERULA TAURINA* SUBSP. *CAUCASICA*

ASPERULA TAURINA SUBSP. *CAUCASICA*'NIN TOPRAK ÜSTÜ KISIMLARININ YBSK, FİTOKİMYASAL, ANTİKOLİNESTERAZ VE ANTİOKSİDAN PROFİLLERİNİN DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: *In this study, we aimed to evaluate the HPLC, phytochemical, anticholinesterase and antioxidant profiles of the aerial parts of Asperula taurina subsp. caucasica.*

Material and Method: *The fingerprint of the phenolic compounds of the methanolic extract of the plant was obtained using RP-HPLC method. The method was also validated in terms of detection limits, quantification limits, linearity, accuracy, precision and selectivity. The phenolic contents of A. taurina subsp. caucasica were detected as proto-catechuic acid, p-OH benzoic acid and benzoic acid. In the phytochemical studies, quercetin 3-O-β-galactoside was isolated from the ethyl acetate subfraction of A. taurina subsp. caucasica using by several chromatographic methods. The structure of the pure compound was elucidated by means of spectral analysis (^{1}H NMR, ^{13}C NMR, and ESI-MS). Anticholinesterase and antioxidant activity studies were performed on quercetin 3-O-β-galactoside and the methanolic extract of the plant.*

Result and Discussion: *While quercetin 3-O-β-galactoside shown moderate inhibitory activity against butyrylcholinesterase at 200 µg/ml, quercetin3-O-β-galactoside and the metanolic exract of*

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the plant did not show acetylcholinesterase inhibitory activity. Quercetin 3-O-β-galactoside shown DPPH free radical scavenging activity at 50 and 100 µg/ml, moderate lipid peroxidation inhibitory activity at 25, 50 and 100 µg/ml; the methanolic extract of the plant moderate lipid peroxidation inhibitory activity at 25, 50 and 100 µg/ml. In conclusion, A. taurina subsp. caucasica and quercetin 3-O-β-galactoside could be important and valuable sources for protecting our body health, especially Alzheimer's disease.

Keywords: *anticholinesterase activity; antioxidant activity; Asperula taurina subsp. caucasica; fingerprint; HPLC; quercetin 3-O-β-galactoside; Rubiaceae*

ÖZ

Amaç: *Bu çalışmada, Asperula taurina subsp. caucasica'nın toprak üstü kısımlarının YBSK, fitokimyasal, antikolinesteraz ve antioksidan profillerinin değerlendirilmesi amaçlanmıştır.*

Gereç ve Yöntem: *Bitkinin metanolik ekstresinin fenolik bileşiklerinin parmak izi kromatogramı, geliştirilen RP-YBSK yöntemi kullanılarak elde edilmiştir. YBSK yöntemi saptama limitleri, nicelik sınırları, doğrusallık, doğruluk, hassaslık ve seçicilik açısından valide edilmiştir. A. taurina subsp. caucasica fenolik içeriği, protokatekuik asit, p-OH benzoik asit ve benzoik asit olarak tespit edilmiştir. Fitokimyasal çalışmalarda ise, çeşitli kromatografik yöntemler kullanılarak A. taurina subsp. caucasica etil asetat alt fraksiyonundan kersetin 3-O-β-galaktozit izole edilmiştir. Saf bileşiğin yapısı, spektrum analizi (^{1}H NMR, ^{13}C NMR ve ESI-MS) yardımıyla aydınlatılmıştır. Kersetin 3-O-β-galaktozit ve bitkinin metanolik ekstresi üzerinde antikolinesteraz ve antioksidan aktivite çalışmaları yapılmıştır.*

Sonuç ve Tartışma: *Kersetin 3-O-β-galaktozit, butirilkolinesteraz'a karşı 200 µg/ml'de orta düzeyde inhibe edici aktivite gösterirken, kersetin 3-O-β-galaktozit ve bitkinin metanolik ekstresi asetilkolinesteraz inhibitör etki göstermemiştir. Kersetin 3-O-β-galaktozit, 50 ve 100 µg/ml'de DPPH serbest radikal süpürücü aktivite, 25, 50 ve 100 µg/ml'de orta derecede lipit peroksidasyon inhibisyon aktivitesi gösterirken; bitkinin metanolik ekstresi 25, 50 ve 100 µg/ml'de orta düzeyde lipit peroksidasyonu önleyici aktivite göstermiştir. Sonuç olarak, A. taurina subsp. caucasica ve kersetin 3-O-β-galaktozit, özellikle Alzheimer hastalığından korunmada önemli ve değerli bir doğal ürün kaynağı olabilir.*

Anahtar kelimeler: *antikolinesteraz aktivite; antioksidan aktivite; Asperula taurina subsp. caucasica; kersetin 3-O-β-galaktozit; parmak izi; Rubiaceae; YBSK*

INTRODUCTION

The Rubiaceae family is represented by about 500 genera and 6000 species all over the world [1]. Species belong to Rubiaceae contain quinonic compounds [2-4], iridoids [5], coumarins [6], triterpenes [7] and flavonoids [8]. The genus *Asperula* (Rubiaceae) has about 200 known species in the world [1] and has 40 species (52 taxa) in Turkey, and 27 taxa of which are endemic [9]. *Asperula taurina* L. subsp. *caucasica* (Pobed.) Ehrend grows in Northeast Turkey [10].

Some *Asperula* species have been used in folk medicine as a diuretic, tonic and antidiarrheal in Turkey [11]. In our previous studies, we isolated *β*-sitosterol, mollugin, 1-hydroxy-2-methyl-9,10-anthraquinone, 1,3-dihydroxy-2-methoxymethyl-9,10-anthraquinone, 1,3-dihydroxy-2-carboxy-9,10-anthraquinone (munjistin), 2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone 1,4-di-*O-β*-glucoside, and lucidin 3-*O-β*-primeveroside from the underground parts of *A. taurina* subsp. *caucasica* [12]. Other

phytochemical studies have shown that *Asperula* species also contains iridoid glycosides (involucratosides A-C, adoxoside), flavone glycosides (apigenin 7-*O*-*β*-glucopyranoside, luteolin 7-*O*-*β*-glucopyranoside, apigenin 7-*O*-rutinoside, lilacifloroside, quercetin, kaempferol, quercetin 3-*O*-*β*-glucopyranosyl-(1→2)-*β*-galactopyranoside, quercetin 3-*O*-*β*-glucopyranosyl-(1→2)-arabinopyranoside) and phenolic acid derivatives (chlorogenic acid and ferulic acid 4-O-*β*-glucopyranoside) [13, 14]. Some previous studies have shown that some *Asperula* species have antihypoxic and potent sedative, antioxidant activity [15, 16].

Polyphenols, which include phenolic acids and flavonoids, act as free radical scavengers and have shown beneficial health-promoting effects in chronic and degenerative diseases such as Alzheimer [17]. Because of this reason, RP-HPLC method were generated and validated to detect phenolic contents.

A survey of the literature revealed that there have been no phytochemical, anticholinesterase activity and antioxidant activity studies dealing with aerial parts of *A. taurina* subsp. *caucasica*. In the present study, the phytochemical studies have comprised the isolation and structure elucidation of the major compound, and RP-HPLC studies with regard to phenolic contents. Also, anticholinesterase activity and antioxidant activity studies were performed on methanolic extracts of the aerial parts of *A. taurina* subsp. *caucasica*, and quercetin 3-*O*-*β*-galactoside isolated from the plant.

MATERIAL AND METHOD

Plant Material, Extraction and Isolation Procedure

Plant material

The aerial parts of *A. taurina* L. subsp. *caucasica* (Pobed.) Ehrend. (Syn.: *A. caucasica* Pobed.) were collected from Ormanüstü village; from forests and scrub, dry open places (Maçka district, 625 m, August 2000, Trabzon province, Turkey). Voucher specimen of *A. taurina* subsp. *caucasica* was deposited at the Herbarium of Ankara University Faculty of Pharmacy (AEF 19791). *A. taurina* subsp. *caucasica* was identified by Dr. Ufuk Özgen.

Extraction and isolation studies on the aerial parts of A. taurina subsp. caucasica

The air-dried and powdered aerial parts (220 g) of *A. taurina* subsp. *caucasica* were extracted with methanol (2000 ml x 3) under reflux for 3 h for each extraction at 40 °C. The combined methanol extracts were evaporated to dryness (30 g, yield 10.4%) under reduced pressure at 40 °C. The methanol extract was suspended with 200 ml of H_2O:MeOH (9:1). It was partitioned against chloroform (200 ml x 3) and ethyl acetate (EtOAc) (200 ml x 3), respectively. The chloroform and EtOAc subfractions were evaporated at reduced pressure at 40 °C, and were 15.3 g and 1.2 g, respectively. The aqueous phase was evaporated to give a residue (12.9 g).

The EtOAc extract (1.2 g) was subjected to Sephadex LH-20 column chromatography with MeOH. Fractions 2-3 (225 mg) gave compound **1** (16 mg).

Chemicals and Instruments

Electric eel AChE, horse serum BChE, acetylthiocholine iodide, butyrylthiocholine chloride, DTNB [5,50-dithio-bis(2-nitrobenzoic) acid], 100 mM sodium phosphate buffer (pH 8.0), galanthamine, Sephadex LH-20 (Sigma-Aldrich) and silica gel 60 (0.063-0.2 mm Merck 7734, 0.040-0.063 mm Merck 9385 and LiChroprep RP-18 25-40 µm Merck 9303) for column chromatography; silica gel 60 F254 (Merck 5554) for TLC were used. TLC spots were detected with a UV lamp and spraying 1% Vanillin/H_2SO_4. DPPH, BHA, BHT, α-tocopherol, β-carotene and linoleic acid were used for antioxidant activity studies.

^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Mercury plus spectrometer at 400 (100) MHz. 96-well microplate reader (SpectraMax PC340, Molecular Devices, USA) was used for antioxidant and anticholinesterase activity. Softmax PRO v5.2 software was used for anticholinesterase activity studies.

HPLC analyse was practiced using a Shimadzu liquid chromatograph (Shimadzu Corporation, LC 20 AT, Kyoto, Japan) and C18 column (Zorbax, 4,6 mm x 150 mm, 5 µm particle size) for 10 phenolic compounds (gallic acid, protocatechuic acid, protocatecualdehyde, *p*-hydroxy benzoic acid, chlorogenic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid and benzoic acid) (Sigma-Aldrich).

Anticholinesterase Activity Assay

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities were measured by slightly modifying the spectrophotometric method developed by Ellman et al. [18]. The measurements and calculations were evaluated by using Softmax PRO v5.2 software. Percentage of inhibition of AChE or BChE was determined by comparison of reaction rates of samples relative to blank sample (ethanol in phosphate buffer, pH 8) using the formula (E-S) / E × 100, where E is the activity of enzyme without test sample, and S is the activity of enzyme with test sample. The experiments were carried out in triplicate. Galanthamine was used as reference compound.

DPPH free radical-scavenging assay

The free radical-scavenging activity of the methanol extract of *A. taurina* subsp. *caucasica* was determined by the DPPH˙ assay described by Blois (1958) with slight modification [19, 20, 21]. BHA, BHT and α-tocopherol were used as standard compounds.

The ability to scavenge the DPPH radical was calculated by using the following equation:

$$\text{DPPH˙ Scavenging Effect (\%)} = A_{control} - A_{sample} / A_{control} \times 100$$

Determination of the antioxidant activity by the β-carotene bleaching method

The antioxidant activity of the samples was evaluated, using the *β*-carotene-linoleic acid test system by Miller (1971) with slight modifications [16]. BHT and BHA were used as standard compounds. The bleaching rate (R) of *β*-carotene was calculated according to the following equation: R= ln a/b/t where, ln = natural log, a = absorbance at time zero, b = absorbance at time t (120 min).

The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control, using the following equation:

$$AA = R_{control} - R_{sample}/R_{control} \times 100$$

HPLC Analysis

Preparing of Standard Solutions

In this study, 10 phenolic compounds, gallic acid, protocatechuic acid, protocatecualdehyde, *p*-hydroxy benzoic acid, chlorogenic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid and benzoic acid were used as standards. Previously, a stock solution including each standard (100 ppm) was prepared and filtered through 0.45 µm membranes. To make calibration curve, the stock solutions of mixed standards were diluted in the concentrations range of 5-100 ppm.

Preparing of Sample Solutions

The aerial parts of the plant were extracted in methanol for 12 h at room temperature and the solvent was removed under vacuum. The extract was redissolved in HPLC grade methanol (10 mg/ml) and filtered through 0.45 µm membranes.

HPLC Conditions

Chromatographic analysis was performed using a Shimadzu liquid chromatograph. A C18 column (4,6 mm x 150 mm, 5 µm) was used with a gradient elution of 100% HPLC-grade methanol (Solvent A) and 2% (v/v, adjust to pH 2,85) acetic acid in HPLC-grade water (Solvent B) as mobile phase at a flow rate of 1.5 ml/min, injection volume 20 µl for the method. The method was studied with diode array detector at wavelengths between 240 and 320 nm. The method was run with the following gradient elution program: 0,01 min 20% A, 80% B; 4 min 30% A, 70% B; 7 min 40% A, 60% B; 10 min 45% A, 55% B; 12 min 50% A, 50% B; 16 min 60% A, 40% B; 17 min 80% A, 20% B. Mixed standards diluted in the concentrations range of 5-100 ppm were performed five repetitive. The method was run 17 minutes to identify the concentrations of 10 phenolic compounds in the plant.

Method Validation

The validation of the method was evaluated for detection limits, quantification limits, linearity, accuracy, precision and selectivity. LOD and LOQ were calculated to assess the detection limits and

quantification limits of the method using signal-to-noise ratios. Linearity was determined by means of calibration curves including five concentrations of standards and five repetitive data. Accuracy was verified adding known amounts of the phenolic standards to a preparation of the plant extract. Precision was evaluated by measurement of intra-day and inter-day precision. The selectivity of the method was appraised by comparing the chromatograms of the phenolic standards.

RESULT AND DISCUSSION

Compound isolated from *A. taurina* subsp. *caucasica*

Yellow powder. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.84 (1H, d, H-2′, *J* = 2.0 Hz), 7.58 (1H, dd, H-6′, *J* = 8.4 Hz, *J* = 2.2 Hz), 6.86 (1H, d, H-5′, *J* = 8.4 Hz), 6.40 (1H, d, H-8, *J* = 1.8 Hz), 6.20 (1H, d, H-6, *J* = 1.8 Hz), 5.20 (1H, d, H-1″, *J* = 7.7 Hz), 3.85-3.30 (5H, sugar protons). ^{13}C NMR (100 MHz, CD_3OD) δ: 178.3 (C-4), 164.9 (C-7), 161.8 (C-5), 157.6 (C-2), 157.3 (C-9), 148.8 (C-4′), 144.6 (C-3′), 134.6 (C-3), 121.8 (C-6′), 121.7 (C-1′), 116.6 (C-2′), 114.9 (C-5′), 104.4 (C-10), 104.2 (C-1″), 98.7 (C-6), 93.5 (C-8), 76.0 (C-5″), 73.9 (C-3″), 72.0 (C-2″), 68.8 (C-4″), 60.8 (C-6″). ^{1}H NMR and ^{13}C NMR data are in agreement with data given in the literature for quercetin 3-*O*-*β*-galactoside (Figure 1) [22].

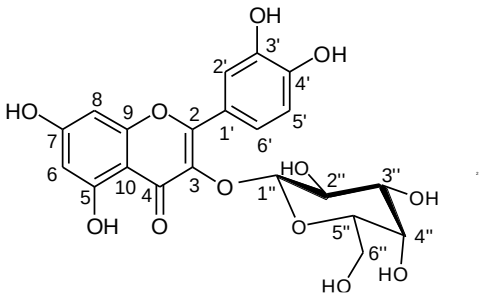

Figure 1. Quercetin 3-*O*-*β*-galactoside

The results of antioxidant and anticholinesterase activity studies

The results of antioxidant and anticholinesterase activity studies of quercetin 3-*O*-*β*-galactoside and the methanolic extract of the aerial parts of *A. taurina* subsp. *caucasica* have been shown in Table 1-4.

Table 1. The results of the DPPH free radical scavenging activity

Sample	DPPH Free Radical Scavenging Activity Inhibition (%)			
	10	25	50	100
	(µg/ml)			
A1	28,51±1,32	52,88±2,62	77,12±1,69	80,51±0,48
Asp	4,71±1,81	19,37±1,37	25,81±0,97	39,79±1,97
α-TOC	32,92±0,26	77,35±0,20	80,38±0,46	81,18±0,87
BHT	38,80±1,01	58,68±1,31	76,78±1,08	81,10±0,43
BHA	57,05±0,48	77,01±0,30	80,79±0,83	81,20±0,54

A1 = Quercetin 3-*O*-*β*-galactoside, **Asp** = The methanol extract of *A. taurina* subsp. *caucasica*, **α-TOC** = α-Tocopherol, **BHT** = Butylatedhydroxytoluene, **BHA** = Butylatedhydroxyanisole

Table 2. The results of the lipid peroxidation inhibitory activity

Sample	**Lipid Peroxidation Inhibitory Activity Inhibition (%)**			
	10	25	50	100
	(µg/ml)			
A1	-	34,02±3,81	54,61±0,02	63,28±5,65
Asp	11,38±2,39	33,27±1,56	55,45±5,20	68,22±3,04
α-TOC	77,66±0,36	79,27±2,38	84,63±0,04	87,99±0,12
BHT	58,16±2,19	71,87±0,39	75,73±0,36	82,65±0,36
BHA	79,22±3,13	82,34±2,07	85,45±0,08	74,59±0,36

A1 = Quercetin 3-*O*-*β*-galactoside, **Asp** = The methanol extract of *A. taurina* subsp. *caucasica*, **α-TOC** = α-Tocopherol, **BHT** = Butylatedhydroxytoluene, **BHA** = Butylatedhydroxyanisole

Quercetin 3-*O*-*β*-galactoside showed important DPPH free radical scavenging activity at 50 and 100 µg/ml; moderate lipid peroxidation inhibitory activity at 25, 50 and 100 µg/ml, and moderate inhibitory activity against butyrylcholinesterase at 200 µg/ ml. The methanol extract of *A. taurina* subsp. *caucasica* have shown moderate lipid peroxidation inhibitory activity at 25, 50 and 100 µg/ ml. None of the samples has shown acetylcholinesterase inhibitory activity.

Table 3. The results of the anticholinesterase activity (AChE) assays

Samples	**AChE Inhibition (%)**			
	25	50	100	200
	(µg/ml)			
A1	-	-	-	-
Asp	-	-	-	-
Galanthamine	77,62±0,39	78,85±0,08	79,52±0,76	79,65±0,60

A1 = Quercetin 3-*O*-*β*-galactoside, **Asp** = The methanol extract of *A. taurina* subsp. *caucasica*,

Table 4. The results of the anticholinesterase activity (BChE) assays

Samples	**BChE Inhibition (%)**			
	25	50	100	200
	(µg/ml)			
A1	6,87±1,69	14,64±1,37	19,97±0,46	25,21±0,92
Asp	-	-	-	
Galanthamine	59,62±0,35	66,65±0,60	69,15±0,42	69,58±0,81

A1 = Quercetin 3-*O*-*β*-galactoside, **Asp** = The methanol extract of *A. taurina* subsp. *caucasica*,

The results of HPLC Studies

Method Development

Solvent type, solvent ratio in the mobil phases, flow rate and detection wavelenght were changed to specify the most useful and quickly separation. The appropiate HPLC conditions were found out 100% HPLC-grade methanol and 2% (v/v, adjust to pH 2,85) acetic acid in HPLC-grade water for mobile phases, 1,5 ml/min for flow rate and 270 nm for detection wavelenght. The chromatogram of the phenolic standards was obtained by using these HPLC conditions (Figure 2).

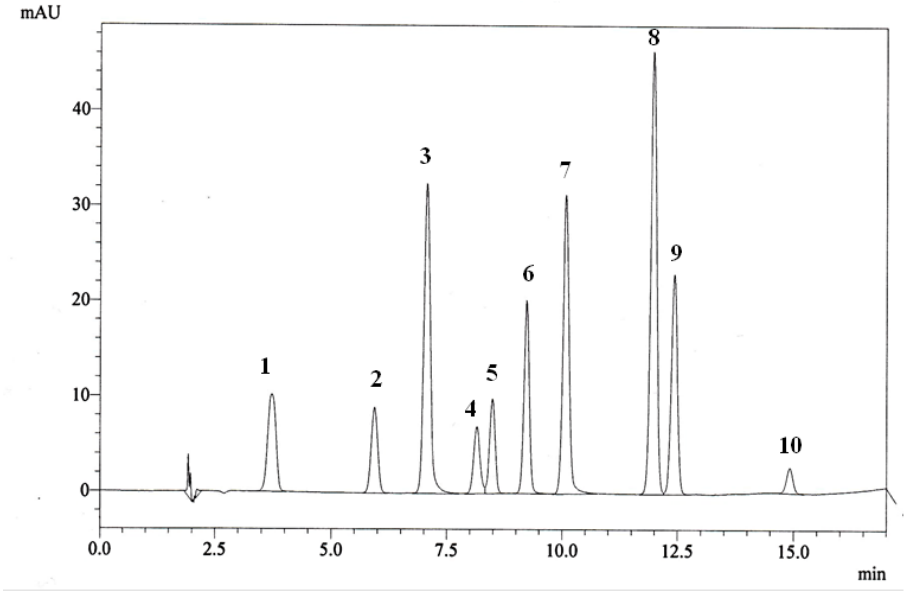

Figure 2. The HPLC chromatogram of the mixture of 10 phenolic standards (1* Gallic acid, 2* Protocatechuic acid, 3* Protocatecualdehyde, 4* p-OH Benzoic acid, 5* Chlorogenic Acid, 6* Vanillic Acid, 7* Caffeic Acid, 8* p-Coumaric Acid, 9* Ferulic Acid, 10* Benzoic Acid)

Validation of the Method

The validation of the method were evaluated in terms of detection limits, quantification limits, linearity, accuracy, precision and selectivity pursuant to ICH guidelines [23].

Determination of limits of detection and quantification

The limits of detection and quantification were determined as signal-to-noise ratios by use of the values of 3:1 and 10:1, respectively (Table 5).

Determination of Linearity

The mixture solution of the phenolic compounds in the range of 5-100 ppm were analyzed in five repetitive and at least five concentrations. The peak areas were plotted against each concentration of the mixture solutions to establish a linear regression equation and to identify value of correlation coefficient (Table 5).

Table 5. Validation data from calibration curves of phenolic compounds

Compound No	Retention time			Regression equation	Correlation coefficient (R)	LOD	LOQ
	Mean (min)	% RSD	Std			(mg/ml)	
1	3,73	0,39	0,015	y = 29361x - 17284	0,9996	0,006	0,020
2	5,91	0,42	0,025	y = 19697x - 2766,8	0,9998	0,009	0,030
3	7,09	0,37	0,026	y = 70768x - 15508	0,9997	0,003	0,008
4	8,18	0,36	0,030	y = 17043x - 16244	0,9997	0,011	0,034
5	8,51	0,33	0,028	y = 17549x + 10845	0,9998	0,011	0,033
6	9,25	0,30	0,028	y = 37378x - 13137	0,9997	0,005	0,016
7	10,11	0,27	0,027	y = 64091x - 9006,5	0,9997	0,003	0,009
8	12,02	0,30	0,036	y = 91761x + 8933,8	0,9998	0,002	0,006
9	12,47	0,26	0,032	y = 46118x - 5106,4	0,9998	0,004	0,012
10	14,96	0,22	0,033	y = 5639x - 679,56	0,9998	0,034	0,105

Determination of Accuracy

The accuracy of the method was verified by addition of standard solutions to sample solution at three different levels 80, 100 and 120% by triplicate analysis. The recovery tests of all compounds were detected range of 97-102%.

Determination of Precision

The intra-day and inter-day precision were identified for retention times. Peak areas were determined for 10 phenolic standards (5 ppm) with repetitive analysis (n= 6). The precision data were predicated as the relative standard deviation (R.S.D) (Table 6).

Table 6. Precision data of phenolic compounds

Compound No	Intra-day R.S.D for RT (%)	Intra-day R.S.D for Peak Area (%)	Inter-day R.S.D for RT (%)	Inter-day R.S.D for Peak Area (%)
1	0,13	0,35	0,44	0,55
2	0,13	0,53	0,17	0,62
3	0,11	0,33	0,16	0,32
4	0,10	0,38	0,16	0,58
5	0,10	0,43	0,06	0,42
6	0,10	0,44	0,16	0,35
7	0,08	0,43	0,11	0,17
8	0,08	0,39	0,17	0,24
9	0,07	0,31	0,15	0,25
10	0,05	1,00	0,09	0,69

Determination of Selectivity

The method selectivity was appraise by the resolution study among standard peaks. Through the HPLC conditions, all standard peaks were completely separated.

RP-HPLC Analysis of the Methanolic Extract of the Plant

The determination of phenolic compounds found in the plant was carried out using the same RP-HPLC conditions. Sample peaks were detected by comparing retention time of known phenolic standards. As a result, three phenolic compounds (proto-catechuic acid, *p*-OH benzoic acid and benzoic acid) were identified (Figure 3, Tablo 7).

Table 7. The phenolic contents of *A. taurina* subsp. *caucasica*

	Compounds	Retention Time (Mean)	Peak area (Mean)	Concentration (mg/100 g)
2	Protocatechuic acid	5,91	301536	135,67
4	*p*-OH Benzoic acid	8,18	4779259	2568,84
10	Benzoic Acid	14,96	181560	623,41

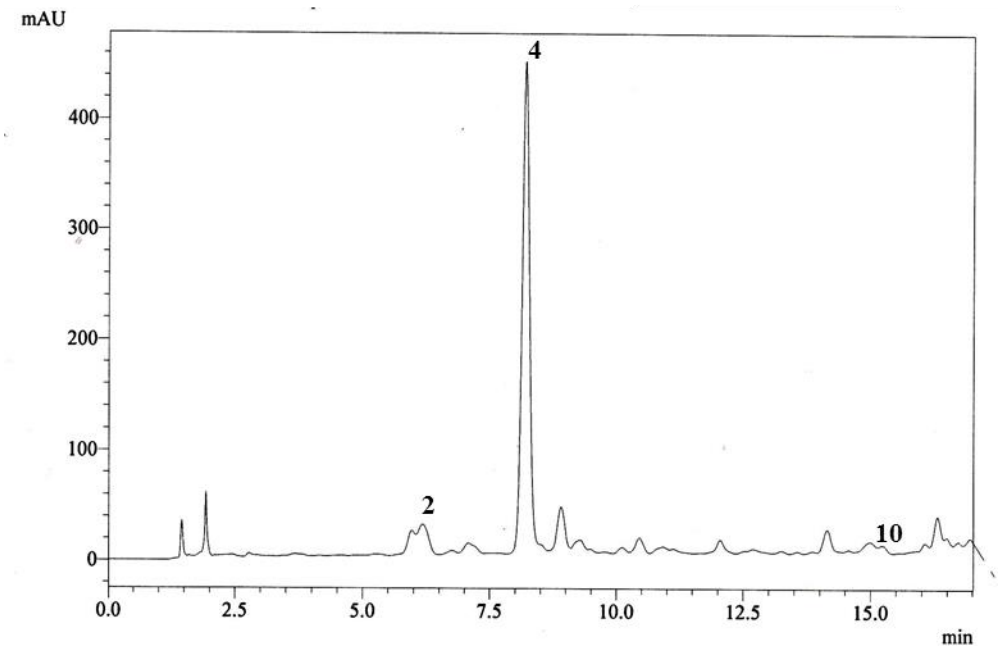

Figure 3. The HPLC chromatogram of the plant extract

CONCLUSION

This study is the first one to evaluate the antioxidant and anticholinesterase activity of quercetin 3-*O*-*β*-galactoside, and the methanolic extract of the aerial parts of *A. taurina* subsp. *taurina*. Anticholinesterase activity of quercetin 3-*O*-*β*-galactoside has been reported for the first time herein.

The substances with cholinesterase inhibitory activity have been used for treating of some diseases such as mystenia gravis, Alzheimer's disease. Galanthamine, well known as a medicine used for the treatment of vascular dementia and Alzheimer's disease, is used as positive control in anticholinesterase studies. Quercetin 3-*O*-*β*-galactoside having similar effect with galanthamine may be suggested to reduce the progression of Alzheimer's disease (AD) and neuronal degeneration. Initial studies have indicated that phenolic compounds may have preventive effects on the development of dementia or AD. According to approach, we investigated the phenolic contents with RP-HPLC profiles. While the methanolic extract of the plant include rich phenolic contents, the cholinesterase inhibitory activity of the extract was not observed. Quercetin 3-*O*-*β*-galactoside isolated methanolic extract of the plant has shown moderate butyrylcholinesterase inhibitory activity. As is seen, pure compounds may show more strong activity in comparison with total extract. In conclusion, quercetin 3-*O*-*β*-galactoside is an important natural compound for protecting our body and brain health.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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