Research Article

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Comparison of phenolic profiles and antioxidant activity of three *Ornithogalum* L. species

Üç *Ornithogalum* L. türünün fenolik profillerinin ve antioksidan aktivitelerinin karşılaştırılması

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Abstract

Background: Aboveground parts and bulbs of *Ornithogalum* species are consumed as food and used in traditional medicine in worldwide.

Objective: It is aimed to report the antioxidant capacity and phenolic compounds content of *Ornithogalum sigmoideum*, *Ornithogalum orthophyllum* and *Ornithogalum oligophyllum* for the first time.

Materials and methods: Antioxidant activity of the crude methanol extracts of the aerial parts and the bulbs of the species were determined with 1,1-diphenyl-2-picryl-hydrazyl, superoxide radical scavenging, ferrous ion-chelating effect, phosphomolybdenum-reducing antioxidant power

and ferric-reducing antioxidant power assay. The ethyl-acetate, diethylether and water subextracts from leaf and flower were analyzed to quantify selected phenolic compounds by HPLC-UV.

Results: Among the six extracts, the methanol extract of the aerial parts of *O. orthophyllum* contained the highest amount of phenolic compounds (GAE, 11.0 mg/g extract). The aerial parts of *O. orthophyllum* showed higher DPPH and SOD activities than the other extracts with the SC₅₀ values of 0.39 ± 0.05 mg/mL and 0.44 ± 0.08 mg/mL, respectively. Protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid and *p*-coumaric acid were the most abundant compounds at all the subextracts.

Conclusion: The antioxidant activity is found to be in accordance with the levels of phenolic content in the extracts.

Keywords: HPLC; *Ornithogalum oligophyllum*; *Ornithogalum orthophyllum*; *Ornithogalum sigmoideum*; Phenolic acids; Flavonoids.

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Öz

Geçmiş: *Ornithogalum* türlerinin topraküstü kısımları ve soğanları, dünya çapında gıda olarak tüketilmekte ve halk ilacı olarak kullanılmaktadır. *Ornithogalum sigmoideum*, *Ornithogalum orthophyllum* ve *O. oligophyllum* türlerinin daha önce çalışılmamış olan antioksidan kapasite ve fenolik bileşiklerini sunmak amaçlanmıştır.

Yöntem ve Gereçler: Türlerin toprak üstü kısımları ve soğanlarının ham metanol ekstraktlarının antioksidan aktiviteleri 1,1-difenil-2-pikril-hidrazil, süperoksit radikal süpürücü, demir iyon-şelat etkisi, fosfomolibdenyum indirgeyici antioksidan güç, demir indirgeyici antioksidan güç yöntemleriyle araştırılmıştır. Yapraktan ve çiçeklerden elde edilen etil asetat, dietil eter ve su alt ekstraktları seçilen fenolik bileşikleri ölçmek için YBSK-UV ile analiz edildi.

Bulgular: Altı ekstre arasında *O. orthophyllum*'un toprak üstü kısımlarının metanol ekstresi en yüksek miktarda fenolik bileşik (GAE, 11.0 mg/g ekstre) taşımaktadır. *O. orthophyllum*'un toprak üstü kısımlarından elde edilen ekstre diğer ekstrelerle göre sırasıyla 0.38 ± 0.05 mg/mL ve 0.44 ± 0.08 mg/mL SC_{50} değerleriyle yüksek 1,1-difenil-2-pikril-hidrazil ve süperoksit radikal süpürücü aktivitelerine sahiptir. Protokatekuik asit, *p*-hidroksibenzoik asit, vanilik asit ve *p*-kumarik asit tüm alt ekstrelerde en fazla bulunan bileşiklerdir.

Sonuç: Antioksidan aktivitenin, ekstrelerdeki fenolik madde düzeyleriyle uyumlu olduğu bulunmuştur.

Anahtar Kelimeler: Fenolik asitler; Flavonoidler; *Ornithogalum oligophyllum*; *Ornithogalum orthophyllum*; *Ornithogalum sigmoideum*; YBSK.

Introduction

The *Ornithogalum* L. (Asparagaceae) genus comprises about 160 species worldwide and 54 species are recorded in the flora of Turkey [1–5]. The name of genus is reported to come from antiquity based on the Greek word 'Ornithogalen' which has a meaning of something wonderful [6]. It was reported that the bulbs of *Ornithogalum* species which are economically valuable, are used against abscess and also as emetic since Dioscorides [7]. Aboveground parts and bulbs of *Ornithogalum narbonense* L., *Ornithogalum oligophyllum* E.D. Clarke, *Ornithogalum platyphyllum* Boiss. and particularly *Ornithogalum sigmoideum* Freyn & Sint are used as food and sold at farmers markets at different parts of Turkey [8, 9]. Leaves, stems and bulbs of *O. sigmoideum* are cooked with rice and eaten in Izmit which is a city at Northwest part of Turkey [8]. *Ornithogalum sigmoideum*, *O. oligophyllum*, *O. platyphyllum* and *Ornithogalum umbellatum* L. are also eaten after roasting or boiling, at the Black Sea Region of Turkey [10, 11]. *Ornithogalum cuspidatum* Bertol. has been used as a spice in Iran [12]. *Ornithogalum* species are rich in saponins and cholestane, cardenolide and flavonoid glycosides [13, 14]. Although cardenolide or cholestane glycosides are highly toxic compounds, some plants from this genus have been used to treat various medical conditions, including diabetes, cardiac troubles, hepatitis and even some cancer types by traditional healers [13–16]. The infusion of *O. umbellatum* is used for the treatment of digestive and prostate related diseases [17]. Also, the bulbs are used externally after boiling [18]. Many of the compounds especially flavonoids and

phenolic compounds in these species, have diverse biological effects [19]. *Ornithogalum* species are reported to exhibit antimicrobial [20], antioxidant [21], cytostatic [22] and antitumor [23] activities. In recent studies, saponins which have hepatic protective effect [24] and protective effect against acetaminophen-induced acute liver injury [25], were isolated from *Ornithogalum saundersiae*. Wan et al. reported the antioxidative activity of *O. saundersiae* and mentioned the importance of antioxidative activity which might be involved in the pathological process of *O. saundersiae* against acetaminophen-induced acute liver injury [25].

Antioxidant activities of the bulb, stem and seed extracts of the *O. narbonense* were investigated by using DPPH⁺, ABTS⁺, FRAP, CUPRAC, metal chelating and phosphomolybdenum assays. It was found that the ethyl acetate extract of the bulb demonstrated the most antioxidant activity and had the highest phenolic content [26].

The volatile compounds of *O. sigmoideum*, *O. orthophyllum* and *O. oligophyllum* were analyzed using solid phase microextraction (SPME) and GC-MS previously [27].

Because of the antioxidant properties and extensive distribution in plant species, polyphenols are mentioned to be one of the common bioactive compounds of the human diet. A number of studies have shown that polyphenols play an important role in the prevention of degenerative diseases [19]. However, to our best knowledge, phenolic compositions and antioxidant properties of *O. sigmoideum*, *O. orthophyllum* Ten., and *O. oligophyllum* remained unexplored. Herewith our aim is to report the antioxidant capacity of bulbs and aerial parts of these three species using DPPH, SOD, ferrous ion-chelating, PRAP and FRAP assays and to analyse the correlation between biological activity and phenolic compounds by finding the phenolic compounds occur in the more active part with HPLC-UV analysis, in the light of traditional usages.

Materials and methods

Plant materials

Ornithogalum sigmoideum (OS), *O. orthophyllum* (OO), and *O. oligophyllum* (OL) were collected in flowering season from Geçit Village, Trabzon in March 2013; Ordu in March 2013; Karadağ, Trabzon in May 2013, respectively. The voucher specimens (ISTE 101047, ISTE 101045, ISTE 101048, respectively) were deposited in the Herbarium of İstanbul University Faculty of Pharmacy (ISTE).

Extraction and sample preparation

Twenty gram of pulverized bulbs and aerial parts were separately extracted with 80% methanol (300 mL $\times$ 3) for 15 min under reflux at 40°C, filtered and evaporated to obtain crude extracts. Methanol extracts of the aerial parts were suspended in water and fractionated with EtOAc and diethyl ether to give subfractions. All of extracts were filtered and dried under vacuum with a rotary evaporator (Heidolph Instruments, Schwabach, Germany).

Determination of phenolic compounds by HPLC-UV

HPLC-UV analyses were performed on a reverse phase C18 column (150 mm $\times$ 4.6 mm id, 5 μ m particle; Fortis, France) using a Thermo Finnigan Surveyor HPLC (Thermo Finnigan, San Jose, CA, USA) and UV detector which is simultaneously operating dual-UV wavelength. Gradient elution which had been developed by Turumtay, was used for HPLC analyses [28, 29]. The mobile phase was (A) 2% acetic acid in water and (B) 70:30 acetonitrile:water. The following gradient was used; 0–3 min 5% B; 3–8 min 5–15% B; 8–10 min 15–20% B; 10–12 min 20–25% B; 12–20 min 25–40% B; 20–30 min 40–80% B. The injection volume was 25 μ L, the column temperature was 30°C and the flow rate was 1.2 mL/min. Benzoic acid derivatives were analysed at 280 nm whereas cinnamic acid derivatives, flavone,

flavonols and the flavonol glycoside were analysed at 315 nm. Calibration solutions were prepared at different concentrations (1, 2, 5, 10, 20, and 30 mg/L). External calibration curves were used for all standards. Linearity (R^2) was given in Table 1. Limit of detection (LOD) and the limit of quantification (LOQ) values as the concentration unit of mg/L were calculated as an S/N level of 3 and S/N level of 10 respectively. The compounds in the standard mixture were used at following concentrations: 0.5 mg/L for gallic acid, *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid and syringic acid, 1 mg/L for chlorogenic acid, caffeic acid, *p*-coumaric acid, fisetin, ferulic acid, rutin, myricetin, quercetin, apigenin, kaempferol and isorhamnetin, and 2 mg/L for catechin and epicatechin. The mixture was injected 7 times to verify the LOD and LOQ of each compound. To validate the reproducibility of the method, the percent relative standard deviation (RSD%) of the peak area responses were calculated (Table 1). Analytical grade methanol, ethanol, all reagents and all standard compounds; gallic acid, *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid, syringic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, fisetin, ferulic acid, rutin, myricetin, quercetin, apigenin, kaempferol and isorhamnetin, catechin, epicatechin, gallic acid and butylated hydroxyanisole were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile, methanol, were from Merck (Darmstadt, Germany). Folin-Ciocalteu's phenol reagent was from Fluka Chemie GmbH (Buchs, Switzerland).

Table 1: Calibration and validation parameters of 18 phenolic standards.

No	RT _{Mean}	Compound	%RSD (RT)	%RSD (area)	R ²	LOD ^a	LOQ ^a
1	2.78	Gallic acid	0.30	2.05	0.998	0.03	0.11
2	5.18	Protocatechuic acid	0.24	2.78	0.999	0.04	0.14
3	8.61	<i>p</i> -OH benzoic acid	0.65	1.19	0.999	0.02	0.06
4	9.66	Catechin	0.58	2.10	0.999	0.12	0.39
5	10.28	Chlorogenic acid	0.29	1.50	0.998	0.04	0.15
6	10.73	Vanillic acid	0.35	0.87	0.999	0.01	0.05
7	11.15	Caffeic acid	0.24	0.83	0.999	0.03	0.09
8	11.87	Syringic acid	0.36	1.49	0.999	0.02	0.07
9	12.46	Epicatechin	0.38	1.33	0.999	0.08	0.26
10	14.17	<i>p</i> -Coumaric acid	0.11	0.56	0.999	0.02	0.06
11	15.41	Ferulic acid	0.23	0.63	0.999	0.02	0.07
12	15.82	Rutin	0.53	1.15	0.999	0.03	0.11
13	18.88	Myricetin	0.26	4.09	0.999	0.08	0.27
14	19.28	Fisetin	0.25	2.56	0.999	0.05	0.18
15	22.55	Quercetin	0.21	1.78	0.999	0.04	0.13
16	25.54	Apigenin	0.15	1.49	0.999	0.05	0.17
17	26.08	Kaempferol	0.14	2.68	0.999	0.07	0.22
18	26.66	Isorhamnetin	0.14	6.34	0.998	0.13	0.45

^amg standard $\cdot$ L⁻¹.

Antioxidant methods

Determination of total phenolic contents in the extracts

Folin–Ciocalteu reagent was used to determine the total phenolic content according to the method of Kähkönen [30]. Fifty microliter of sample extract, 250 μ L of Folin–Ciocalteu reagent and 750 μ L of 20% (w/v) sodium carbonate were mixed. After incubating for 120 min in the dark, the absorbance was measured at 750 nm to calculate the phenolic content which was given as gram of gallic acid equivalent (GAE).

DPPH radical scavenging assay

Free radical scavenging effect was tested with a DPPH free radical scavenging assay method [31]. The methanolic DPPH solution (0.1 mM) and different concentrations of samples were mixed and incubated in dark at room temperature for 30 min. The absorbances of the samples (A_{sample}) were measured at 517 nm. Mixture without sample was used as control (control absorbance, A_{control}). Gallic acid (GA) and butylated hydroxyanisole (BHA) were used as positive controls and scavenging effect was calculated with Equation 1.

$$\text{Scavenging effects (\%)} = \left[\frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100 \quad (1)$$

Superoxide radical scavenging assay

A non-enzymatic superoxide radical ($\text{O}_2^{\cdot-}$) generation assay with modified spectrophotometrical nitro blue tetrazolium (NBT) photoreduction method was used to test the superoxide radical scavenging activities of the samples [32]. The absorbances of the mixtures which contained riboflavin (2 μ M), methionine (13 mM), NBT (75 mM), EDTA (0.1 mM), with the test samples (50 mM phosphate buffer, pH 7.8) were measured at 560 nm, after illuminating at 30°C for 10 min by a fluorescent lamp. Assay mixture without the samples was used as a control (control absorbance, A_{control}). All experiments were in triplicates and results were expressed as the mean $\pm$ standard deviation (S.D.). Free $\text{O}_2^{\cdot-}$ radical scavenging effect was calculated using the Equation 1.

Ferrous ion-chelating effect

Ferrous ion-chelating effect of the extracts and reference was estimated by the method of Chua [33]. The total 740 μ L mixture of methanol and the samples (200 μ L) were incubated with 2 mM FeCl_2 solution. The reaction was started by adding 5 mM ferrozine to mixture. The absorbance of the mixture of reaction was measured at 562 nm after incubating for 10 min. The capacity was calculated using Equation 1 and the ferrous ion chelating results were expressed as the mean $\pm$ standard deviation (SD).

Phosphomolybdenum-reducing antioxidant power (PRAP) assay

Each dilution of the extracts was mixed with 10% phosphomolybdic acid solution in ethanol (w/v). The absorbances of the mixtures were read (600 nm) expressed as quercetin equivalent (QE, mg/g extract), after incubating 30 min at 80°C [34].

Ferric-reducing antioxidant power (FRAP) assay

The ferric-reducing power was tested according to the method of Oyaizu [35]. Different concentrations of the extracts and butylated hydroxyanisole (BHA) which was used as reference, were mixed. 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide were added to the mixture which was then incubated at 50°C. At the end of 20 min, 10% trichloroacetic acid was added. After vigorous shaking, the solution was mixed with distilled water and FeCl_3 (0.15%). The absorbance was read at 700 nm and expressed as BHA equivalent (BHA-E, mg/g extract).

Statistical analysis

Results were expressed as means $\pm$ standard deviations (SD) of three independent and parallel measurements. SC_{50} values were determined by linear regression analysis (Microsoft Excel program for Windows and GraphPad Prism 5.0, Albuquerque, NM, USA). The differences among the compounds were investigated by one-way analysis of variance (ANOVA) followed by Tukey tests. $p < 0.0001$ was considered to be significant.

Table 2: Antioxidant activities and total phenol contents of methanol extracts.

Extract code	DPPH $\pm$ S.D. (SC ₅₀ , g/mL)	SOD $\pm$ S.D. (SC ₅₀ , mg/mL)	Ferrous ion-chelating capacity $\pm$ S.D. (SC, mg/mL)	PRAP $\pm$ S.D. (QE, mg/g extract)	FRAP $\pm$ S.D. (BHA, mg/g extract)	Total phenol content $\pm$ S.D. (GAE, mg/g extract)
OOA	0.39 $\pm$ 0.05	0.44 $\pm$ 0.08 ^a	3.25 $\pm$ 0.09 ^c	128.00 $\pm$ 0.08	27.39 $\pm$ 0.07	11.00 $\pm$ 0.18
OSA	0.91 $\pm$ 0.08 ^c	0.58 $\pm$ 0.10 ^b	1.85 $\pm$ 0.07 ^c	100.72 $\pm$ 0.11	15.33 $\pm$ 0.09	7.12 $\pm$ 0.21
OLA	3.82 $\pm$ 0.11 ^c	1.19 $\pm$ 0.09 ^c	6.29 $\pm$ 0.12 ^c	50.83 $\pm$ 0.08	1.65 $\pm$ 0.12	0.19 $\pm$ 0.02
OOB	2.36 $\pm$ 0.09 ^c	0.77 $\pm$ 0.08 ^c	1.30 $\pm$ 0.08 ^c	60.80 $\pm$ 0.12	4.80 $\pm$ 0.08	2.04 $\pm$ 0.22
OSB	1.06 $\pm$ 0.07 ^c	0.62 $\pm$ 0.11 ^b	5.01 $\pm$ 0.09 ^c	84.52 $\pm$ 0.10	9.75 $\pm$ 0.10	3.90 $\pm$ 0.08
OLB	0.55 $\pm$ 0.06 ^b	0.49 $\pm$ 0.05 ^a	2.18 $\pm$ 0.10 ^c	113.00 $\pm$ 0.09	22.62 $\pm$ 0.11	7.27 $\pm$ 0.19
Gallic acid	0.065 $\pm$ 0.004	—	—	—	—	—
BHA	0.027 $\pm$ 0.005	—	—	—	—	—
Catechin	—	0.023 $\pm$ 0.009	—	—	—	—
EDTA	—	—	0.014 $\pm$ 0.005	—	—	—

^ap < 0.05; ^bp < 0.001; ^cp < 0.0001. OOA, *O. orthophyllum* aerial parts; OSA, *O. sigmoideum* aerial parts; OLA, *O. oligophyllum* aerial parts; OOB, *O. orthophyllum* bulbs; OSB, *O. sigmoideum* bulbs; OLB, *O. oligophyllum* bulbs.

Results

In vitro antioxidant activity of the crude methanol extracts of the aerial parts (OOA, OSA and OLA) and the bulbs (OOB, OSB and OLB) of three *Ornithogalum* species were determined and tabulated in Table 2. DPPH and SOD of OOA showed higher radical scavenging activities with the SC₅₀ values of 0.39 $\pm$ 0.05 mg/mL and 0.44 $\pm$ 0.08 mg/mL, respectively, among other extracts. The radical scavenging activities of DPPH and SOD of the extracts were sorted as OOA > OLB > OSA > OSB > OOB > OLA. The weakest DPPH and SOD were obtained in OLA extract with SC₅₀ values of 3.82 $\pm$ 0.11 and 1.19 $\pm$ 0.09 mg/mL, respectively. There are previous studies regarding antioxidant properties of different species of *Ornithogalum*. Ebrahimzadeh reported that methanol extracts obtained from bulbs and aerial parts of *Ornithogalum sintenisii* scavenged DPPH radical with SC₅₀ values of 669 $\pm$ 25 μ g/mL and 368.00 $\pm$ 15 μ g/mL, respectively [21]. Methanol extract of bulbs of *O. alpigenum* is reported to show 90.38% inhibition with DPPH dependent antioxidant activity assay [20].

OOB extract with SC₅₀ value of 1.30 $\pm$ 0.08 mg/mL chelated more iron than the other extracts (Table 2), but consequently all extracts were less efficient than commercial chelator EDTA. Similarly, Fe²⁺ chelating activity of the aerial parts of *O. sintenisii* has been reported to be greater than bulbs of the same species with the values of IC₅₀ 340.00 $\pm$ 14 μ g/mL for aerial parts and 684.00 $\pm$ 27 μ g/mL for bulbs [21]. The absorbances measured for the samples were converted to BHA equivalent antioxidant capacity (BHA) values obtained from the absorbance – [BHA]

calibration graph, and the mg/g extract BHA values are given in Table 2.

The results showed that all extracts have a strong ferric reducing power. OOA extract showed the highest reducing capacity (BHA, 27.39 $\pm$ 0.07 mg/g extract). The weakest capacity to reduce iron(III) (BHA, 1.65 $\pm$ 0.12 mg/g extract) was obtained in the OLA extract. According to our results, especially OOA, OLB, OSA extracts showed high antioxidant activity in PRAP assay; 128.00 $\pm$ 0.08, 113.00 $\pm$ 0.09 and 100.72 $\pm$ 0.11 (QE, mg/g extract), respectively (Table 2). The reducing capacity of aqueous ethanolic extract of *O. umbellatum* stems was determined according to the same method and reported to be smaller than 0.2 μ g/mL [11].

Among the five extracts, OOA extract was found to contain the highest (GAE, 11.0 $\pm$ 0.18 mg/g extract) amount of phenolic compounds followed by OLB (GAE, 7.27 $\pm$ 0.19 mg/g extract), OSA (GAE, 7.12 $\pm$ 0.21 mg/g extract), OSB (GAE, 3.90 $\pm$ 0.08 mg/g extract), OOB (GAE, 2.04 $\pm$ 0.22 mg/g extract) and OLA (GAE, 0.19 $\pm$ 0.02 mg/g extract) (Table 2).

The method that used for HPLC-UV analyses of phenolic compounds was revalidated, and all values were within acceptable criteria (Table 1) [28]. LOD and LOQ values were lower than 0.12 and 0.39 mg standard $\cdot$ L⁻¹ respectively. Over the selected range, peak areas linearly depended on concentrations for all phenolics with high correlation coefficients (>0.998). The injection repeatability of the system was evaluated by seven consecutive injections of the standard solution of phenolics. The RSD values of retention times of phenolics were less than 0.60% (Table 1).

Discussion

Antioxidant activities of several *Ornithogalum* species has been described in previous studies and correlated with the phenolic contents of the extracts [11, 19, 20, 26, 36]. In connection with our studies on *Ornithogalum* species [27], we tested three *Ornithogalum* species growing in five types of antioxidant activity assays and the OOA extract exerted the highest activity in three of the assays except ferrous ion-chelating capacity and PRAP assay. The bulb extracts showed comparatively weak activity overall the studied extracts (Table 2). It is very important to point out that; there was a positive relationship between antioxidant activity potential and amount of phenolic compounds of the crude extracts. Phenolic compounds have been associated with the overall antioxidant activities [35–38]. From this point of view, we decided to investigate the more active aerial parts with HPLC-UV because of their rich phenolic composition.

Phenolics in diethylether, ethyl acetate and water subextracts were analyzed through the revalidated HPLC method. According to the determinations; protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid and *p*-coumaric acid were found to be the most abundant compounds

among the identified compounds in the extracts (Table 3). The distribution of phenolics varied by plant part; leaves were found to be rich in amount of phenolic compounds compared to the flowers. Diethyl ether subextract of the leaves of OO has the highest phenolic content according to HPLC-UV analysis whose total phenolic content and antioxidant potential were the highest in the extracts as well. According to our findings, radical scavenging activities of the aerial parts of OO and OS were higher than the bulbs of same species. These findings are in a line with our HPLC experiment results. OS and OO are richer in terms of polyphenol source than *O. oligophyllum*. While this is the first report on the phenolics of *O. sigmoideum*, *O. orthophyllum* and *O. oligophyllum*, the phenolics of *O. narbonense* were investigated before and major phenolic compounds were found as benzoic acid, *p*-hydroxybenzoic acid, chlorogenic acid, rutin and caffeic acid [26]. The comparison of our data with those reported in literature proved that the extracts of the *Ornithogalum* species which were rich in phenolic contents had high antioxidant property. Especially methanol extract of aerial parts of *O. orthophyllum* was found to show higher radical scavenging activities, FRAP and PRAP reducing capacities and highest amount

Table 3: Phenolic constituents of three *Ornithogalum* species determined by HPLC-UV.

	Gallic acid	Caffeic acid	Protocatechuic acid	<i>p</i> -OH benzoic acid	Vanillic acid	Syringic acid	Epicatechin	Ferulic acid	<i>p</i> -Coumaric acid	TIP
OO flower										
EtOAc	ND	ND	347.6	1843.5	1366.2	28.2	232.4	102.9	478.5	4399.4
DEE	ND	ND	368.6	1060.0	619.3	ND	ND	ND	399.3	2447.1
H ₂ O	ND	ND	90.3	120.9	45.7	ND	ND	ND	441.4	698.3
OO leaf										
EtOAc	149.2	ND	568.1	260.6	201.7	68.9	ND	ND	1308.4	2557.1
DEE	6796.0	ND	14768.0	6157.6	4423.2	ND	ND	2200	2710.0	37054.8
H ₂ O	277.0	ND	381.2	55.3	52.0	ND	ND	ND	ND	765.6
OL flower										
EtOAc	ND	ND	350.6	1788.8	1240.6	ND	ND	312.5	3280.0	6972.5
DEE	ND	ND	121.2	588.8	326.5	ND	ND	ND	2391.8	3428.2
H ₂ O	ND	ND	21.3	157.0	71.3	ND	ND	ND	2056.5	2306.2
OL leaf										
EtOAc	ND	ND	3.6	266.0	2377.6	65.9	ND	ND	162.7	2875.7
DEE	ND	ND	149.2	594.2	4065.8	173.8	ND	ND	ND	4983.1
H ₂ O	ND	ND	28.9	42.5	191.0	3.4	ND	ND	99.0	364.8
OS flower										
EtOAc	130.7	ND	765.7	4290.5	1069.8	ND	ND	993.3	5507.1	12757.1
DEE	168.7	ND	711	3589.6	814.8	ND	ND	975.0	11385.4	17644.6
H ₂ O	ND	ND	160.9	131.5	7.4	ND	ND	ND	ND	299.8
OS leaf										
EtOAc	229.5	189.5	469.5	477.4	366.3	ND	ND	114.7	1019.5	2866.3
DEE	ND	ND	902	680.0	283.0	ND	ND	ND	1230.0	3095.0
H ₂ O	19.2	ND	222.9	145.0	ND	ND	ND	ND	1945.0	2332.1

Results are expressed in µg phenolic compound per g dry samples. ND, Non-detected; EtOAc, ethyl acetate; DEE, diethyl ether; TIP, total identified phenolics; OO, *O. Orthophyllum*; OS, *O. Sigmoideum*; OL, *O. Oligophyllum*.

of phenolic compounds among other extracts. Among the samples studied, the epicatechin was found only in the ethyl acetate extract of the OO flowers which was found to show highest activity. Also, gallic acid is found to be in high amount in OO leaf extract and absent in the extracts of OL (Table 3). Also the antioxidant activity of protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid and *p*-coumaric acid which were the most abundant compounds, were previously reported [39–41]. Considered in terms of structure activity relationships, antioxidant activities of the compounds are in correlation with the number of hydroxyl groups that are bonded to the aromatic ring. Besides, epicatechin was reported to scavenge hydroxyl, peroxy, superoxide, and DPPH radicals while peroxy radical and DPPH radical scavenging effects of gallic acid was showed. (+)-catechin and (–)-epicatechins' peroxy radical scavenging activity was reported to be 10 times higher than those of L-ascorbate and β -carotene [42]. It is specified in the studies that the antioxidant activities of flavonoids and phenolic acids are directly related to the number and position of aromatic hydroxyl groups [43, 44].

All these results verified the traditional usage of *Ornithogalum* species. Considering the connection between plant-derived antioxidants in food and human health, *Ornithogalum* species consumed as food, could be evaluated as rich sources of bioactive supplements in human nutrition due to their rich polyphenol content.

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