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Effects of different ecological and phenological factors on antioxidant activity and phenolic content of *Ornithogalum sigmoideum* Freyn & Sint. from Turkey

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The quality and quantity of metabolites in plants responsible for biological activities are influenced by a multitude of factors, chief among them, environmental. Furthermore, part of the plant to be tested and selected extraction solvent affect these kinds of activities. Ethanol and water extracts of above ground and below ground parts of the *Ornithogalum sigmoideum* Freyn & Sint. collected from different altitudes of Ordu province at two different period (begining and end of flowering) were studied in terms of phenolic contents and antioxidative activities. Thus, the effect of four different factors (altitude, flowering period, plant section, extraction solvent) on these parameters were examined. Both the main effects and the interactions of these factors were evaluated separately, and it was concluded that quaternary interaction was statistically significant on the antioxidant activity tested according to the ferric reducing antioxidant power (FRAP) method. Binary interactions such as altitude*solvent and section*solvent were statistically significant on total phenolic content values. On the other hand, section*solvent and altitude*period*section interactions were statistically significant for 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity.

Keywords: Altitude gradient

Therapeutic use of plants is as old as human civilization, and medicinal herbs are an integral part of the health system as herbal remedies to show that 80% of the population trusts the traditional medical system[1]. Since herbal medicinal products do not cause any side effects, the interest in herbal resources is increasing day by day all over the World[2]. In addition, medicinal plants are widely preferred due to their accessibility and affordability features, as well as their cultural beliefs[3]. Alkaloids, flavonoids, phenols, quinones, tannins and terpenoids as secondary metabolites of medicinal plants are used throughout the world for the treatment of various diseases[4-10]. Due to their potential as natural sources of biodymamic compounds, medicinal plants are widely used for drug development. The quality and therapeutic efficacy of medicinal and aromatic plants depend on their secondary metabolites, which vary according to environmental factors[11].

Diversified topography, combined with various environmental conditions, supports the growth of a series of medicinal plants[12]. Height differences of the environment, where plants grow, influence environmental factors such as fast seasonal and daily temperature changes, low atmospheric pressure, low CO_2 concentration, short-term vegetation, and increased UV radiation, keeping plants under serious stress[13]. As the height of the environment where the plants grow increases, the body length, body diameter, biomass production and specific leaf surfaces of plants decrease. On the other hand, leaf thickness increases with this change. In short, the height of the environment, where the plants grow, causes changes in morphological, physiological, and biochemical properties[14]. At high altitudes, high light intensity causes pigment bleaching, lipid peroxidation, protein damage, enzyme inactivation, and ultimately accumulating reactive oxygen species that cause cell death[12]. On the other hand, the environmental temperature, which is one of the most important parameters that changes with height, is effective in the development of plant biochemicals. For example, plants in temperate habitats are exposed to severe environmental stresses, including temperature and radiation excesses. As a result of this, an increase in the production of antioxidative properties and UV-B preservative compounds (Anthocyanins, ascorbic acid, flavonoids and phenolic acid) is observed but a decrease in the production of allelophatic and anti-herbivorous

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substances (alkaloids, iridoid and sesquiterpene lactones)[15].

The *Ornithogalum* L. (Asparagaceae) genus comprises about 160 species worldwide and 54 species are recorded in the flora of Turkey[16]. *Ornithogalum sigmoideum* Freyn & Sint., is a perennial geophyte species widespread in the Central Black Sea Region of Turkey and occurs at low and high elevations under contrasting ecological conditions. Taxon flowers in March- July months and usually occurs under forest canopies, meadow and stony slopes. Fresh bulbs and above gorund parts (flower and leaf) are also fried, used as salad or canned food[16]. Due to antimicrobial[17], antioxidant[18], cytostatic[17] and antitumor[19] activities *Ornithogalum* species are often mentioned for their heart spams relieving and heart regulator and protector effects[20]. In this study, we investigated whether factors such as altitude, plant part, flowering period and extraction solution change the phenolic content and antioxidative activity in the medicinal herb *Ornithogalum sigmoideum*.

Materials and Methods

Study area and plant sampling

The sample (*Ornithogalum sigmoideum* Freyn & Sint. plant) selected to work collected from three natural populations, which show an upward trend as sea level (41°01′56″N−37°30′01″E), 600 m a.s.l (40°52′51 ″N− 37°09′37″E) and 1100 m a.s.l (40°52′51 ″N− 37°15′18″E) in Ordu province in Black Sea Region of Turkey from forest clearings, the edge of the hazelnut plantations and under shrubs, respectively (Suppl. Fig S1. *All supplementary data are available only online along with the respective paper at NOPR repository at http://nopr.res.in*). The plant individuals were collected during the begining and end of flowering period (from March to July). The identity of the plant specimen was clarified by Dr. Sevda TÜRKİŞ (Department of Mathematics and Science Education, Ordu University) based on the book 'Flora of Turkey and the East Aegean Island'[21]. All specimens are kept at the Botanical Laboratory of Ordu University. Three 5×5 m plots were selected along the elevational gradient from sea level to 1100 m a.s.l. for plant sampling. At least 15 individuals were collected from each plot at different altitudes at both periods (begining of flowering — 1st and end of flowering- 2nd). To analyse plant sections separately, above ground parts (flower and leaf) and below ground parts (bulbs) of the collected samples were dried separately in the shade at room temperature (25°C). Dried samples were powdered using a mortar. For the preparation of the aqueous extract, a part of the powdered sample was extracted in appropriate amount of distilled water using shaking water bath at 25°C. This process was continued until the extraction was completed. After then extract was filtrated and lyophilized. To prepare ethanol extract, the method followed was as in the preparation of water extract in the initial stage. However, after the filtration of the extract, the extraction solvent was removed under vacuum using a rotary evaporator instead of using lyophilizator. Lyophilized and evaporated dry samples were weighed for quantification of extractable compounds from plants and resolved in water and ethanol, respectively[22]. The resultant extracts were diluted and used throughout the study in all tests.

Determination of total phenolic content

Folin-Ciocalteu method[23] was used to determine content of total phenolics in methanol and distilled water extracts of *O. sigmoideum*. This method is based on the reduction of phosphomolybdic-phosphotungstic acid components of Folin-Ciocalteu reagent by phenolic molecules believed to be present in the extract. At the end of this redox reaction, the phenolic content is calculated by spectrophotometric measurement (at 760 nm) of the blue colour formed. The results were expressed as gallic acid equivalents[24] (mg GAE/g extract) by using the graph drawn using gallic acid as standard (Fig. S2).

DPPH free radical scavenging activity

Free radical scavenging capacity of the extracts were assayed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical purchased by commercially. The method is based on the fact that the extracts with antioxidant content open the colour of purple DPPH solution as a result of their ability to deliver a proton or electron[25]. For this purpose, different concentrations of extracts were added to DPPH solution and the resulting mixtures were kept in the dark at room temperature for 30 min. At the end of this period, absorbance of each mixture was measured at 517 nm against blank containing extraction solvent instead of extract. DPPH free radical scavenging activity was calculated by using following equation.

$$\text{Scavenging activity}(\%) = \frac{(\text{Absblank} - \text{Abssample})}{\text{Absblank}}$$

The extract concentration, which provide 50% of the radicals to be swept up, was calculated as SC_{50} (mg/mL) by plotting the activity values against the extract concentration[26] (Fig. S3).

Ferring reducing antioxidant power (FRAP)

The method used for this purpose is based on reduction of Fe(III)-TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) complex to blue coloured Fe(II)-TPTZ complex in the presence of antioxidants. The intensity of the blue colour measured at 593 nm is directly attributed to the antioxidant power[27]. For this test, the freshly prepared FRAP reagent [300 mM pH 3.6 acetate buffer: 10 mM TPTZ: 20 mM $FeCl_3$ (10:1:1)] is combined with the known amount of extract and the absorbance of the resulting colour after incubation at 37°C for 30 min is recorded. The results were expressed as trolox equivalent (µmol TXE/g extract) using the graph drawn using trolox as standard[28] (Fig S4).

Statistical analysis

The assumptions of data normality and homogeneity of variance, which are prerequisite for ANOVA, were tested with the Kolmogorov Smirnov and the Levene's tests, respectively. Four-way ANOVA[29] variant analyses were used to determine the relationship between variables (elevation, period, plant part and extraction solvent). The means were compared with Tukey's HSD/Dunn post-hoc test and the results were displayed in the form of letters. The alpha level was set at 5%. All calculations were performed with SPSS v25 (IBM Inc., Chicago, IL, USA) statistical software.

Results

The total phenolic contents of the alcohol and water extracts of the *O. sigmoideum* plants collected in two different flowering periods, which were grown at different altitudes and therefore considered to be subject to different ecological conditions, were determined. When the calculated descriptive statistics values and comparison results are examined, it can be seen that the total phenolic content values calculated in the case of water extract are higher. It is also observed that the calculated values for total phenolic contents of the above ground parts are higher than the below ground parts, especially in the case of water extract (Table 1). This apparent difference can be more easily seen from the Table 2 arranged to contain the descriptive statistics values calculated for quadruple interaction (altitude*period*section*solvent). When the aforementioned tables are examined, effective difference observed due to the use of different

Table 2 — Descriptive statictical values according to four-way interaction (Altitude*Period*Section*Solvent) for total phenolic content values (n=3)

Altitude	Period	Section	Solvent	Average	SE	SD
Locality 1	1st	Below ground	Ethanol	15.477	0.464	0.805
			DW	7.140	0.781	1.353
		Above ground	Ethanol	16.860	0.908	1.573
			DW	43.913	12.257	21.229
	2nd	Below ground	Ethanol	24.217	10.069	17.440
			DW	5.530	0.308	0.534
		Above ground	Ethanol	16.290	3.513	6.084
			DW	40.803	3.671	6.358
Locality 2	1st	Below ground	Ethanol	15.623	2.000	3.464
			DW	6.930	3.113	5.391
		Above ground	Ethanol	27.280	1.650	2.857
			DW	33.463	2.084	3.610
	2nd	Below ground	Ethanol	24.770	4.644	8.044
			DW	6.327	0.258	0.447
		Above ground	Ethanol	16.870	2.566	4.445
			DW	44.520	5.254	9.100
Locality 3	1st	Below ground	Ethanol	18.020	1.159	2.007
			DW	10.603	0.676	1.171
		Above ground	Ethanol	15.333	0.628	1.087
			DW	49.503	13.311	23.056
	2nd	Below ground	Ethanol	12.057	0.533	0.924
			DW	6.307	0.368	0.637
		Above ground	Ethanol	17.293	1.946	3.370
			DW	52.340	3.823	6.622

[DW: Distilled water; SE: Standard Error; SD: Standard Deviation; 1st period: Begining flowering period; and 2nd period: end of flowering period]

Table 1 — Descriptive statistics and comparison results for total phenolic content values

Factor	Factor level	n	Extraction Solvent: Ethanol			Extraction Solvent: Distilled Water		
			Average	Std. Error	Std. Deviation	Average	Std. Error	Std. Deviation
Altitude	Locality 1	12	18.211Aa	2.516	8.717	24.347Aa	6.091	21.101
	Locality 2	12	21.136Aa	1.962	6.795	22.810Aa	5.205	18.031
	Locality 3	12	15.676Ab	0.865	2.997	29.688Aa	7.073	24.503
		P-Değeri				0.042*		
Section	Below ground	18	18.361Aa	1.952	8.281	7.139^{Bb}	0.611	2.593
	Above ground	18	18.321Ab	1.220	5.178	44.091^{Aa}	3.125	13.259
		P-Value				0.000***		

[*, statistically significant (P <0.05); ***, statistically significant (P <0.001). The difference between altitude averages not contain common capital letters for same solvent is statistically significiant (P <0.05). The difference between solvent averages not contain common small letter for same altitude is statistically significiant (P <0.05). The difference between section averages not contain common capital letter as superscript for same solvent is statistically significiant (P <0.05). The difference between solvent averages not contain common small letter as superscript for same section is statistically significiant (P <0.05)]

extraction solvent or plant section is not reflected with period difference.

All the amounts of phenolic content including the values for the samples collected from 1100 m.a.s.l. to sea level are arranged in Table 2. Although the effects of the extraction solvent and the selected section of the plant on the total phenolic content are evident, quaternary interaction (altitude*Period*section*solvent) is not statistically significant (Table 3).

When we examine four different variables (altitude, period, solvent, and section) together, it can be concluded that the highest phenolic content values are obtained in case of samples obtained from the 1100 m.a.s.l. region having the highest elevation (Table 2). These values are followed by the values of the samples obtained from the 600 m.a.s.l. In this case, it is possible to say that altitude difference influences phenolic content. These observations were also shown as statistically. A four-way analysis of variance was performed to determine both the main effects and interactions of factors for total phenolics (mg GAE/g extract) and the results are given in Table 2. When Table 3 is examined, it is seen that, binary interactions including Altitude*Solvent and Section*Solvent are statistically significant interactions (P <0.05, P <0.001). Likewise, Duncan multiple comparison test was performed to determine different averages and the results were expressed in letters (Table 1).

The antioxidant activities of the studied samples with significiant phenolic contents were examined by DPPH and FRAP methods. DPPH free radical scavenging activities of the investigated samples vary depending on the concentration. For this reason, activity was determined as SC_{50} (extract concentration that has the power to scavenge 50% of free radicals in reaction medium). The obtained SC_{50} values are compatible with phenolic content values and were lower at extracts prepared from above ground sections of the plant. This difference is more pronounced in the case of water extract. On the other hand, in general, SC_{50} values of alcohol extracts were calculated as lower (Table 4). It can be interpreted that alcohol extracts components that have an antioxidant effect more effectively.

Generally, DPPH free radical scavenging activities of the samples obtained from sea level are higher than the samples obtained from 1100 m.a.s.l. and 600 m. a.s.l. (Tables 4 & 5). Among the activities of the samples collected at different periods there is a noticeable difference especially in the below ground parts (Table 5). There was a high correlation (r^2 = 0.8239) (Fig. S5), between total phenolic content and DPPH free radical scavenging activity in the case of water extracts but there is no such high correlation (r^2 = 0.1321) (Fig. S6) in the case of alcohol extracts.

Table 3 — Analysis of variance optimization of experimental parameters for total phenolic content values

Source of variation	DF[1]	SS[2]	AS[3]	F-Value	P-Value
Altitude	2	23.6	11.82	0.17	0.844
Period	1	6.4	6.44	0.09	0.762
Section	1	6131.1	6131.12	88.54	0.000
Solvent	1	952.4	952.44	13.75	0.001
Altitude*Period	2	40.9	20.44	0.30	0.746
Altitude*Section	2	106.6	53.31	0.77	0.469
Altitude*Solvent	2	468.4	234.18	3.38	0.042*
Period * Section	1	1.7	1.67	0.02	0.877
Period *Solvent	1	0.2	0.23	0.00	0.954
Section*Solvent	1	6157.4	6157.36	88.92	0.000***
Altitude*Period* Section	2	150.6	75.28	1.09	0.345
Altitude*Period* Solvent	2	116.0	57.99	0.84	0.439
Altitude* Section *Solvent	2	97.9	48.96	0.71	0.498
Period* Section * Solvent	1	182.8	182.76	2.64	0.111
Altitude *Period*Section* Solvent	2	205.8	102.89	1.49	0.237
Error	48	3323.7	69.24		
Total	71	17965.5			

[[1]Degrees of freedom for the effect, [2]Sum of squares, [3]Average of square]

[*, significantly (P<0.05); ***, significantly (P<0.001)]

Table 4 — Descriptive statictical values according to four-way interaction (Altitude*Period*Section*Solvent) for DPPH values (n=3)

Altitude	Period	Section	Solvent	Average	SE	SD
Locality 1	1st	Below ground	Ethanol	0.967	0.099	0.172
			DW	2.407	0.436	0.755
		Above ground	Ethanol	0.327	0.013	0.023
			DW	0.503	0.214	0.370
	2nd	Below ground	Ethanol	0.910	0.084	0,145
			DW	3.713	0.736	1,275
		Above ground	Ethanol	0.293	0.053	0.092
			DW	0.367	0.023	0.040
Locality 2	1st	Below ground	Ethanol	0.773	0.090	0.155
			DW	5.130	1.635	2.832
		Above ground	Ethanol	0.157	0.009	0.015
			DW	0.510	0.130	0.225
	2nd	Below ground	Ethanol	0.580	0.089	0.154
			DW	3.403	0.881	1.525
		Above ground	Ethanol	0.293	0.045	0.078
			DW	0.357	0.060	0.104
Locality 3	1st	Below ground	Ethanol	0.757	0.099	0.171
			DW	2.857	0.359	0.622
		Above ground	Ethanol	0.387	0.026	0.045
			DW	0.477	0.134	0.232
	2nd	Below ground	Ethanol	1.193	0.124	0.215
			DW	5.853	1.024	1.773
		Above ground	Ethanol	0.387	0.055	0.096
			DW	0.650	0.345	0.598

[DW: Distilled water; SE: Standard Error; SD: Standard Deviation; 1st period: Begining flowering period; and 2nd period: end of flowering period]

Table 5 — Descriptive statictical values and comparison results according to three-way interaction (Altitude*Period*Section) for DPPH values

Altitude	Period	n	Section: Below ground Average	SE	SD	Above ground Average	SE	SD
Locality 1	1st	6	1.687Ab^{B}	0.379	0.928	0.415Ba^{A}	0.104	0.254
	2nd	6	2.312Aa^{B}	0.709	1.737	0.330Ba^{A}	0.031	0.075
Locality 2	1st	6	2.952Aa^{A}	1.219	2.985	0.333Ba^{A}	0.098	0.240
	2nd	6	1.992Ab^{B}	0.745	1.825	0.325Ba^{A}	0.036	0.089
Locality 3	1st	6	1.807Ab^{B}	0.498	1.220	0.432Ba^{A}	0.064	0.157
	2nd	6	3.523Aa^{A}	1.140	2.791	0.518Ba^{A}	0.167	0.409
P-Value						0.033*		

[SE: Standard Error; SD: Standard Deviation. *, statistically significant (P <0.05). The difference between section averages not contain common capital letters for same altitude and same period is statistically significiant (P <0.05). The difference between period averages not contain common small letters for same altitude and same section is statistically significiant (P <0.05). The difference between altitude averages not contain common capital letters as superscript for same period and same section is statistically significiant (P <0.05)]

Table 6 — Descriptive statistics and comparison results for FRAP values (n=3)

Altitude	Period	Section	Solvent	Average	SE	SD
Locality 1	1st	Below ground	Ethanol	52.027Ab^{Aa}	4.487	7.772
			DW	23.477Ab^{Aa}	4.178	7.236
		Above ground	Ethanol	120.510Ba^{Ab}	4.588	7.946
			DW	197.090Aa^{Aa}	50.911	88.181
	2nd	Below ground	Ethanol	55.073Ab^{Aa}	7.442	12.891
			DW	15.893Ab^{Aa}	1.174	2.034
		Above ground	Ethanol	136.443Aa^{Aa}	16.743	29.000
			DW	176.920Aa^{Aa}	2.202	3.814
Locality 2	1st	Below ground	Ethanol	59.573Ab^{Aa}	7.293	12.632
			DW	16.873Ab^{Aa}	7.513	13.012
		Above ground	Ethanol	199.697Aa^{Aa}	16.509	28.595
			DW	130.810Ba^{Bb}	29.582	51.238
	2nd	Below ground	Ethanol	80.377Aa^{Aa}	13.330	23.088
			DW	21.583Ab^{Aa}	3.071	5.319
		Above ground	Ethanol	127.887Ba^{Ba}	21.860	37.862
			DW	233.877Aa^{Aa}	49.266	85.331
Locality 3	1st	Below ground	Ethanol	53.977Aa^{Aa}	6.039	10.460
			DW	21.040Ab^{Aa}	0.988	1.711
		Above ground	Ethanol	101.303Ba^{Ab}	2.931	5.076
			DW	168.670Aa^{Aab}	52.328	90.634
	2nd	Below ground	Ethanol	35.923Ab^{Aa}	2.399	4.156
			DW	21.767Ab^{Aa}	7.213	12.493
		Above ground	Ethanol	103.650Ba^{Aa}	10.248	17.750
			DW	182.813Aa^{Aa}	31.577	54.694

P-Value Altitude*Period*Section*Solvent Interaction: 0.029*

[DW: Distilled water; SE: Standard Error; SD: Standard Deviation. 1st period: Begining flowering period, 2nd period: end of flowering period. *, statistically significant (P <0.05). The difference between solvent averages not having common capital letters for same altitude, same period and same section is statistically significiant (P <0.05). The difference between ssection averages not having common small letters for same altitude, same period and same solvent is statistically significiant (P <0.05). The difference between period averages not having common capital letters as superscript for same altitude, same section and same solvent is statistically significiant (P <0.05). The difference between altitude averages not having common small letters as superscript for same period, same section and same solvent is statistically significiant (P <0.05)]

Table 7 — Analysis of variance optimization of experimental parameters for FRAP values

Source of variation	DF[1]	SS[2]	AS[3]	F-Value	P-Value
Altitude	2	6180	3090	2.19	0.123
Period	1	278	278	0.20	0.659
Section	1	252791	252791	179.19	0.000
Solvent	1	890	890	0.63	0.431
Altitude*Period	2	960	480	0.34	0.713
Altitude*Section	2	1585	792	0.56	0.574
Altitude*Solvent	2	5285	2643	1.87	0.165
Period*Section	1	199	199	0.14	0.709
Period*Solvent	1	2543	2543	1.80	0.186
Section*Solvent	1	33412	33412	23.68	0.000
Altitude*Period*Section	2	243	121	0.09	0.918
Altitude*Period*Solvent	2	8081	4041	2.86	0.067
Altitude*Section*Solvent	2	655	327	0.23	0.794
Period*Section*Solvent	1	3141	3141	2.23	0.142
Altitude*Period*Section*Solvent	2	10797	5398	3.83	0.029*
Error	48	67716	1411		
Total	71	394756			

[[1]Degrees of freedom for the effect; [2]Sum of squares; [3]Average of square. *, statistically significant (P <0.05)]

The FRAP values of samples also varied similarly to the DPPH free radical scavenging activity. Numerically, it is possible to reveal this. The correlation coefficient between the calculated values for FRAP and DPPH activities was calculated as 0.8947 (Fig. S7) for the water extracts and 0.915 (Fig. S8) for the alcohol extract. FRAP potential of the extracts prepared from the above ground sections is much higher in case of both water and alcohol extract. This difference is more pronounced, especially in the case of water extract. The FRAP values of the alcohol extracts prepared from the bulb section of the plant are higher than the water extracts. But, there are no significant differences between the activities of the extracts collected from plant samples at different periods (Table 6). For the calculated FRAP values a four-way analysis of variance was conducted to determine both the main effects and the interactions of

Table 8 — Analysis of variance optimization of experimental parameters for DPPH values

Source of variation	DF[1]	SS[2]	AS[3]	F-Value	P-Value
Altitude	2	1.779	0.8896	1.26	0.292
Period	1	0.945	0.9453	1.34	0.252
Section	1	71.023	71.0233	100.95	0.000
Solvent	1	46.096	46.0960	65.52	0.000
Altitude*Period	2	5.777	2.8883	4.11	0.023
Altitude*Section	2	1.171	0.5857	0.83	0.441
Altitude*Solvent	2	2.091	1.0455	1.49	0.236
Period*Section	1	0.964	0.9637	1.37	0.248
Period*Solvent	1	0.589	0.5886	0.84	0.365
Section*Solvent	1	36.823	36.8225	52.34	0.000***
Altitude*Period*Section	2	5.136	2.5681	3.65	0.033*
Altitude*Period*Solvent	2	4.055	2.0276	2.88	0.066
Altitude*Section*Solvent	2	1.703	0.8515	1.21	0.307
Period*Section*Solvent	1	0.852	0.8515	1.21	0.277
Altitude*Period*Section *Solvent	2	2.671	1.3355	1.90	0.161
Error	48	33.770	0.7035		
Total	71	215.444			

[[1]Degrees of freedom for the effect; [2]Sum of squares; [3]Average of square. [*, statistically significant (P <0.05); ***, statistically significant (P <0.001)]

the factors (Table 7). It is seen that from the table, quartet interaction is statistically significant (P <0.05). Accordingly, Duncan multiple comparison test was performed to determine different averages and the results were expressed in letters. Descriptive statistics and comparison results for the FRAP values are given in Table 6. From Table 8 and Fig. 1, it can be concluded that plant section type, the period in which the plant is collected and the geographic location where the plant grows contribute antioxidant activity.

Discussion

Phenolic compounds are among the key components of phytochemicals[30]. Phenolic compounds are believed to be antioxidants that are effective as radical scavenging and metal chelating agents. Phenolics accumulate in different plant organs according to the role of plant life[31]. According to the results of previous studies, it was reported that different anatomical parts of various plant species contain different levels of phenolic[32]. The impact of developmental, genetic, and environmental factors such as climatic factors, soil composition (mineral and organic nutrients), weather (irrigation) and density of sulfur fertilization contributes significantly to phenolic compound content through the effects of phenolic genes, enzymes and metabolites[33].

Although a great deal of research has been done on the morphological, physiological and biochemical properties of tree plants, there is insufficient information about the growth, lipid peroxidation, activities of

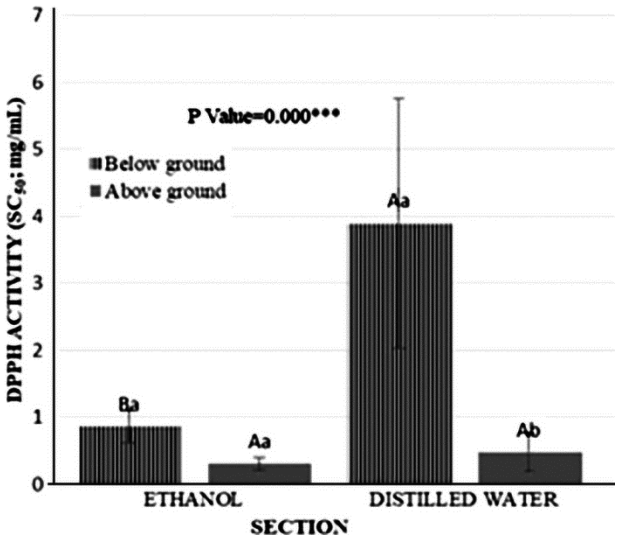

Fig. 1 — Descriptive statictical values and comparison results according to two-way interaction (Extraction Solvent*Section) for DPPH values

antioxidant enzymes and accumulation of secondary metabolites of medicinal plants which are grown under stressful conditions at various altitudes[12]. In the light of this information, the total phenolic contents of the alcohol and water extracts of the *O. sigmoideum* plants were determined. The average total phenolic content was calculated as 50.92 mg GAE/g extract for the water extracts of above ground sections of the samples collected from the 1100 m.a.s.l, which has the highest altitude. But the calculated average value was 8.46 mg GAE/g extract for the below ground sections of the same samples. Thus, it can be said that below ground and above ground differ significantly in terms of phenolic content.

According to results of the study on ethyl acetate, methanol, and water extracts of *Ornithogalum narbonense* L. collected from Bingöl village of Sivas (at an altitude of about 1380 m) at the end of the flowering season, it was concluded that the total phenolic content changes significantly compared to the solvent used, as well as the plant section and the higher phenolic content value had been calculated for ethyl acetate extracts of bulb section of the *O. narbonense* L. They reported the highest phenolic content as 21.05 mg GAE/g extract in the case of extracts prepared with ethyl acetate from bulb sections of the plant[31].

In addition to this altitude difference has also an effect on phenolic content[33]. There are results in literature that support this finding. As a result of the study performed to evaluate the antioxidant activity of

Thalictrum foliolosum against height change, it was revealed that the phenolic content was clearly influenced from height and the phenolic contents of samples collected from higher altitudes were higher. Furthermore, there is also information that it is important to study the effect of elevation on the production of secondary metabolites and the production of relevant medicinal properties in order to obtain effective chemotypes[34]. Jaakola & Hohtola[35] and many researchers have shown that plants grown in higher areas are exposed to higher levels of UV-B radiation with a pleiotropic effect on plant growth, morphology and physiology, and the most effective protection mechanism induced by this light is the biosynthesis of flavonoids and phenols[36]. These findings also explain the high rate of phenolic and flavonoid content in plants growing in high altitudes. The opposite situation has also been reported in the literature. It was reported by Zhang *et al*.[37] that black tea obtained from low plantation height contained 22-28% more polyphenols than obtained from high altitude.

Based on the results of current study and other studies from literature, we can say that detection of phenolic content at remerkable level in different sections of plant is an indicator of protective role of it against oxidative damage results from biotic and/or abiotic sources[31].

There has been an increasing interest in nutraceutical and pharmaceutical use of substances with antioxidant properties since a decade ago. It is known that plant extracts have antioxidant activity[38]. Therefore, the antioxidant activities of the studied samples with significiant phenolic contents were examined by DPPH and FRAP methods. Obtained results of antioxidant activity depend on selected solvent are consistent with the reported studies from literature[39]. They showed that the choice of solvent leads to difference in antioxidant activity.

Strong free radical activity can be attributed to a high level of phenolic content. In other words, free radical activity has a positive correlation with the phytochemical content of the extracts. Similarly, previous studies have shown that phenolic compounds contribute significantly to the antioxidant activity of medicinal plants. There are studies showing that increasing the amount of dietary phenolics reduces several diseases disease risks[31,40,41]. The FRAP values of samples also varied similarly to the DPPH free radical scavenging activity. Briefly, it can be said that the samples have the potential to have FRAP potential, i.e., they can serve as the electron server that can stop radical chain reactions.

Conclusion

The quality and quantity of metabolites in plants responsible for biological activities are influenced by a multitude of factors, chief among them, environmental. Although other *Ornithogalum* species have been studied quite frequently, *Ornithogalum sigmoideum* has been rarely studied in terms of biological activities. Particularly, there are no reports in the literature showing that antioxidative capacity and phenolic content of the *O. sigmoideum* are examined according to mentioned factors such as geographical locations, growth elevation and harvest period of the plant. On the other hand, part of the plant to be tested and selected extraction solvent affect these kinds of activities. When all the data obtained are evaluated, it will be possible to safely meet the increasing demand for therapeutically important secondary metabolites by mapping the differences determined as a result of the investigated parameters. It was revealed that the altitude variable at which the plant is grown causes significant differences in biochemical values as well as in plant physiology. Therefore, it is noteworthy to examine the variation in plant secondary metabolites caused by ecological factors, thanks to the altitude variable. In this context, the present study makes an important contribution to the nutritive values of wild edible plants.

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Conflict of Interest

Authors declare no competing interests.

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