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Variation of Bioactivities and Phytochemical Compositions of Valeriana dioscoridis Sm. Extracts

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ABSTRACT

Valerian (*Valeriana dioscoridis* Sm.) is a perennial herb of the Caprifoliaceae family. The aim of this study was to determine the variation of *V. dioscoridis* plants grown in different regions in respect of the quality criteria traits such as nutrient values, extract contents and in vitro antioxidant and antimicrobial activity values. In this study, roots and rhizomes of *V. dioscoridis* Sm. were collected from eight different locations in Antalya province. The obtained materials were ground in a blender and extracted with 80% ethanol. The macro and micro-nutrient elements contents of the powdered plant samples were evaluated and the antioxidant and antimicrobial activities of the extracts were investigated. Based on the data obtained, there were different values in the antioxidant, antimicrobial activities, nutrient content and chemical composition content of extracts of *V.dioscoridis* Sm. plants grown in different locations.

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Valeriana dioscoridis Sm. Ekstraktlarının Biyoaktivite ve Fitokimyasal Kompozisyon Değişimleri

ÖZET

Kedi otu (*Valeriana dioscoridis* Sm.) Caprifoliaceae familyasının çok yıllık bitkisidir. Bu çalışmanın amacı tüketimi gittikçe artan bitkilerden olan *V. dioscoridis* bitkisinin besin değerleri, ekstrakt içerikleri, antioksidan ve antimikrobiyal aktivite değerleri gibi kalite kriterleri yönünden, farklı bölgelerde yetişen bitkilerin toplandığı bölgelere gore her hangi bir farklılık oluşturup oluşturmadığının belirlenmesi içindir. Bu çalışmada *Valeriana dioscoridis* Sm. kök ve rizomları Antalya ilinin sekiz farklı bölgesinden toplanmıştır. Elde edilen materyaller toz haline getirilmiş ve % 80 etanol ile ekstrakte edilmiştir. Toz halindeki bitki örneklerinin makro ve mikro besin elemet içerikleri değerlendirilmiş ve ekstraktların da antioksidan ve antimikrobiyal aktiviteleri değerlendirilmiştir. Elde edilen verilere gore farklı bölgelerde yetişen *Valeriana dioscoridis* Sm. bitki ekstratlarının antioksidan, antimikrobiyal aktiviteleri ve besin içerikleri ve kimyasal kompozisyonunda farklı değerler elde edilmiştir.

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INTRODUCTION

Valeriana L. contains 14 species and belongs to the Caprifoliaceae family (Richardson, 1972; Davis et al., 1988; Güner at al., 2000; Piccinelli et al., 2004; Güner et al., 2012). *Valeriana dioscoridis* Sm. is a perennial herbaceous plant having rhizomes. The dried roots and rhizomes of this plant have lipophilic compounds (0.5 %) and have sedative, sleep transmitter, wound healing, nerve- calming, and spasm relief effects (Bulut, 2006; Lopez-Munoz et al., 2006; Guarrera et

al., 2008). Previous studies explored that the extracts and essential oils of *V. dioscoridis* have antifungal and antioxidant activity (Tzakou et al., 2004; Karadeniz et al., 2015). Although the use of this plant is very common for medicinal purposes, however there is scarcity of information about reliability (Bogacz et al., 2014).

Valerianic acid present in the plants of genus *Valeriana* has a palliative property, especially for anxiety. Anxiety is caused by stress conditions and can create difficulties in dealing with some problems arising from the intensity of daily and work life. Therefore, it is an important and very common problem affecting the quality of life of people in the modern world. People use different treatment methods, such as drugs or alternative medicine to relieve this anxiety. There is a tendency of higher usage of natural products because of the side-effects resulted by the intake of synthetic drugs. Considering this factors, various plant parts are used for medicinal and aromatic purposes including substances such as cellulose, corn starch, pectin, protein and sugar, in addition to the active substance components with pharmacological effects (Ceylan, 1987). Generally, the essential oils within these plants contain major components, however some other components are also present even in small concentrations. These different components increase the power of major components by facilitating the intake of major components. A better effect may be provided according to the pure substances of the essential oils. The use of medicinal components may be preferred when the pure drugs cannot be used or when it is not economic to do so. These plants gained popularity and more concentration among the medicinal industry and interest is also increasing for the cultivation of these plants. However, where these plants grow naturally and under what conditions they are collected is of importance. Just as plants collected from the roadside could contain heavy metal components and there may be variations in the active substance content according to the conditions around where they are found. Moreover, collection of the plants at the wrong time could also affect the quality of the active substance of the plant. The aim of this study was to determine whether or not there was any difference in *V. dioscoridis* plants grown in different regions in respect of the quality criteria such as nutrient values, extract contents and *in vitro* antioxidant and antimicrobial activity values.

MATERIAL and METHOD

The research was carried out in 2019 in the laboratories of Cumhuriyet University Advanced Technology Research Center (CÜTAM). *Valeriana dioscoridis* Sm. plants were collected from eight different locations used as the study materials. The collected materials were dried and have been stored in CÜTAM laboratory.

Supply of plant materials

Valerian (*Valeriana dioscoridis* Sm.) was collected from eight different locations (LOC1: Burdur, C2, Altınyayla District, Ballık Village, Ballık Canyon road, 35S 0715480-UTM 4081603, 1479 m, rocky places on mixed Cedar forest, 22.IV.2016, Çinbilgel 10352, LOC2: Antalya, C3, İbradı District, Başlar Village, 36S 0358486-UTM 4122760, 1200 m, rocky places on mixed Torch pine forest, 15.IV.2016, Çinbilgel 10336, LOC3: Antalya, C3, Manavgat District, Ahmetler Village road, 36S 0383468-UTM 4076318, 605 m, moist places, 11.III.2016, Çinbilgel 10271, LOC4: Antalya, C3, Manavgat District, Ahmetler Village, 36S 0383784-UTM 4076925, 639 m, Red pine forest-moist places, 19.II.2016, Çinbilgel s.n., LOC5: Antalya, C3, Manavgat District, Beşkonak-Sağırin neighborhood, Akpınar plato, 36S 0352351-UTM 4113161, 1436 m, rocky places on mixed Juniper forest, 14.IV.2016, Çinbilgel sn.,) LOC6: Antalya, C3, Manavgat District, Ahmetler Village road, 36S 0383440-UTM 4076188, 569 m, maquis-moist places, 19.II.2016, Çinbilgel s.n., LOC7: Antalya, C3, İbradı District, Ürünlü Village, Altınbeşik Cave National Park, 36S 0377622-UTM 4100098, 646 m, maquis-rocky places, 15.IV.2016, Çinbilgel 10340,) LOC8: Burdur, C2, Yeşilova District, Salda Lake, 35S 0740188-UTM 4158060, 1155 m, rocky places on maquis, 22.IV.2016, Çinbilgel 10355. The collected plant specimens were determined by Dr. Çinbilgel according to the Flora of Turkey and East Agean Islands (Richardson, 1972). The obtained roots and rhizomes were dried and pulverized with a laboratory grinder.

Obtaining the Extracts

The powdered plant materials were macerated with 80% ethanol. After one day of agitation in the shaker, the plant particles were filtered. The filtrates were dried in an oven (40 °C) to obtain the extracts.

Gas Chromatography-Mass Spectrometry (GC / MS) and GC analysis of extracts

Gas Chromatography / Mass Spectrometer was used to identify the components of the extracts and Gas Chromatography was used to determine the relative percentages (Sacchetti et al., 2005). GC–MS analyses were worked with mass spectrometer detector. Helium gas was used as a carrier gas at a constant flow rate of 1.5 mL in minutes, and 1 µl Linjection volume using splitless mode was programmed among 80-300 at rate of 5 per minutes. Post run was set at 300 °C for 2 min. Total run time was 60 minutes (Eruygur and Dural, 2019).

Determination of Macro-Micro element contents

First, the samples were ground for further analysis. The N (nitrogen) content was determined using the modified Kjeldahl method (Bremner, 1965). For the P (phosphorus), K (potassium), Fe (iron), Mn (manganese), Zn (zinc) and Cu (copper) contents, 0.200 g plant samples were weighed in porcelain crucibles and dried in the oven at 550 ºC for 5 hours to obtain ash. After removing the samples from the oven, 1/3 HCl and distilled water were added to the extracted samples. Using a P 880 nm UV-spectrophotometer (Murphy and Riley, 1962), K, Fe, Mn, Zn and Cu were determined with Atomic Absorption Spectrophotometry (AAS) (Güzel et al., 1992).

Raw protein value (%) is calculated by the formula below: Raw protein value (%): N value x 6,25

In vitro antioxidant activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the extract was evaluated according to the methodology of Blois method (1958) with slight modification. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity was evaluated by the method of Re et al. (1999) with minor modifications. Total phenolic content was determined with spectrophotometric method (Clarke et al., 2013) and expressed as gallic acid equivalents. Flavonoid contents were determined with the aluminum chloride colorimetric method of Molan and Mahd (2014). Total flavonoids content was expressed as milligrams of catechine equivalent per gram of the dry weight of the extract.

Antimicrobial activities of *V. dioscoridis* extracts

In this study, the microdilution Broth method (Eloff, 1998) was used to determine the Minimum Inhibition Concentration (MIC) of *V. dioscoridis* extracts against micro-organisms of *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853),, *Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC 27853), *Candida albicans* (ATCC10231) and *Candida tropicalis* (DSM11953).

The extract was dissolved in DMSO (50 mg mL^{-1}). 90 µl of media were added to the first row of the microtiter plates and 50 µl of the remaining wells. The 11th wells were used as the reproductive controls and 100 µL broth was added. 10 µL extract was added in the first line of the microtiter plate and serial two-fold dilutions were prepared. The concentration of the extract in the wells was ranging from 2.5 to 0.004 mg mL^{-1}. The bacteria and fungi suspensions (50µL) were added to prepared samples. The final inoculum size was 5×10^5 CFU mL^{-1} in the bacteria wells and $0.5\text{-}2.5\times10^3$ CFU mL^{-1} in the *Candida* sp. wells (CLSI, 2002, CLSI, 2012). The microtiter plates were incubated at 37 ºC and the MICs were recorded after 24 h of incubation. The MIC was defined as the lowest concentration of the extract that produced an 80% reduction in visible growth compared with control.

Statistical Analysis

Range, coefficient of variation and east significant difference (LSD) for various macro and micro elements was investigated using using the statistical software XLSTAT (www.xlstat.com). Similarly, distribution graphs for total phenol contents and antioxidant activity were also devloped using XLSTAT (www.xlstat.com) software.

RESULTS and DISCUSSION

The effect on the quality criteria of the extracts of *Valeriana dioscoridis* plants collected from eight different locations were evaluated.

GC-MS analysis values of the extracts obtained

In the evaluation of the chemical composition of the extracts obtained from the *V. dioscoridis* collected from eight different locations, a total of 43 components were determined, both single and common for each location (Table 1). Maximum components (total 18) were determined in LOC 1 and minimum components (total 8) in LOC 8. Ispiro [2.1.2.4] undecane, 8-methylene-, Tricyclo [4.4.0.0(2,8)] decan-3-ol, Tricyclo [4.4.0.0(2,8)] decane, 5-hydroxy-, acetate, (-)-Caryophyllene oxide, Cyclopentanol, 2-cyclopentylidene-, 2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3.alpha.,5a.alpha.,9.alpha.,9a.alpha.)]-, Limonen-6-ol, pivalate, 1-Propene-1,2,3-tricarboxylic acid, tributyl ester, Decanedioic acid, dibutyl ester (CAS), Tributyl acetylcitrate, Benzyldiethyl-(2,6-xylylcarbamoyl methyl)-ammonium benzoate, Stigmast-5-en-3-ol, (3.beta.)- (CAS) components were found in 4 or more common locations. For LOC 1, LOC 2, LOC 3, LOC 4, LOC 5, LOC 6 and LOC 8, the components of Tributyl acetylcitrate (22.26, 20.58, 14.81, 18.22, 14.71, 19.13 and 15.87, respectively), and for LOC 7, 2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetra methyl-, [3R-(3.alpha.,5a.alpha.,9.alpha.,9a. alpha.)]- (17.46) were determined as major components (Table 1). According to Bogacz et al. (2014) and Dimpfel (2007), the most important chemical component of the genus *Valeriana* plants is valerianic acid.

Macro-Micro Nutrient Content

The evaluations of the macro and micro-nutrient elements content of the *V. dioscoridis* plants collected from eight different locations are shown in Table 2. Various macro elements, such as phosphorus and potassium ranged 0.15-0.50 and 1-6 mg kg^{-1}, respectively. The P content of the *V. dioscoridis* in different locations varied between 0.476% (LOC 5)-0.950% (LOC 6) and was found to be 0.688% on

average. P deficiency in plants results in a decreased growth and low resistance to biotic and abiotic stresses (Plaster, 1992; Aktaş and Ateş, 1998). Maximum potassium content in this plant was 3.813% in LOC 1, at the lowest in LOC 7 (1.290%) and 2.274% on average. In the *V. dioscoridis* collected from different locations, the N content varied between 0.680% (LOC7) and 1.733% (LOC6) and the crude protein content is varied 4.250% (LOC7)-10.60% (LOC6).

Table 1. Components of 80% ethanol extracts of *Valeriana dioscoridis* plants collected from eight different locations
Tablo 1. Sekiz farklı lokasyondan toplanan Valeriana dioscoridis bitkisinin % 80 etanol ekstraktının bileşenleri

No	RT	Components	Relative Percentage (%)	
			Locations of *Valeriana dioscoridis*	% (respectively)
1	18,648	Benzaldehyde, 2-hydroxy-6-methyl-	LOC5	1.96
2	18,683	2-Hydroxy-4-methylbenzaldehyde	LOC7	1.67
3	20,295	Dispiro[2.1.2.4]undecane, 8-methylene-	LOC1, LOC3, LOC5, LOC7	1.07, 1.12, 2.17, 2.12
4	20,759	2-iodo-1-adamantyl acetate	LOC6	2.26
5	21,091	Nerolidol-epoxyacetate	LOC3	1.43
6	21,937	1-Ethyladamantan-2-ol	LOC1	1.24
7	21,956	2,6,6-Trimethyl-1-cyclohexene-1-acetaldehyde	LOC7	2.10
8	22,754	Decanoic acid (CAS)	LOC6	1.87
9	22,852	Quinic acid	LOC5	1.97
10	23,686	3-Buten-2-ol, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-	LOC7	2.07
11	24,085	.beta.-D-Glucopyranoside, methyl	LOC7	1.86
12	24,375	Patchouli alcohol	LOC5	1.47
13	24,745	Dimethyl{bis[(2Z)-pent-2-en-1-yloxy]}silane	LOC1	1.57
14	26,155	Naphth[2,3-b]oxirene, decahydro-	LOC5	14.08
15	26,218	Tricyclo[4.4.0.0(2,8)]decan-3-ol	LOC1, LOC3, LOC4, LOC7	12.59, 6.37, 13.23, 7.83
16	26,344	1,4-Benzenediol (CAS)	LOC6	1.57
17	28,203	Silane, dimethyldi(but-2-enyloxy)-	LOC2	7.23
18	28,206 29,988	p-Mentha-1,8-dien-7-yl acetate	LOC1, LOC5	8.28, 2.93
19	29,316	Tricyclo[4.4.0.0(2,8)]decane, 5-hydroxy-, acetate	LOC1, LOC2, LOC3, LOC4, LOC5, LOC7	5.28, 5.15, 13.30, 14.22, 9.53, 7.72
20	30,015	2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-	LOC1	1.70
21	30,109	(-)-Caryophyllene oxide	LOC1, LOC2, LOC3, LOC4, LOC 5, LOC7, LOC8	1.94, 1.48, 10.94, 10.96, 4.58, 2.06, 4.05
22	30,249	2,2-Dimethyl-1-(3-oxo-but-1-enyl)-cyclopentanecarboxaldehyde	LOC5	2.5
23	30,484	10,12-Docosadiynedioic acid ditms	LOC6	2.80
24	30,262	1a,2,5,5Tetramethyl-trans-1a,4a,5,6,7,8-hexahydro-gamma-chromene	LOC1	1.03
25	31,395	Cyclopentanol, 2-cyclopentylidene-	LOC1, LOC2, LOC7, LOC 8	7.04, 16.61, 9.22, 7.08
26	31,411	trans-5-Hydroxytricyclo[4.4.0.0(3,8)]-4-carboxylic acid	LOC4	8.38
27	31,477	Albuterol	LOC3	6.89
28	32,054	Pentanoic acid 1-methylpropyl ester	LOC3	0.31
29	33,766	1,5-Heptadien-4-ol, 3,3,6-trimethyl-	LOC2	2,32
30	33,955	Andrographolide	LOC6	3.91
31	34,175	Neoplatyphylline	LOC5	1.67
32	34,248	Succinic acid, 3,7-dimethyloct-6-en-1-yl heptadecyl ester	LOC2	1.92
33	34,282	Platyphylline	LOC7	8.05
34	35,521	2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3.alpha.,5a.alpha.,9.alpha.,9a.alpha.)]-	LOC3, LOC4, LOC5, LOC7	6.34, 1.47, 2.65, 17.46

35	37,186	Limonen-6-ol, pivalate	LOC1, LOC3, LOC5, LOC 8	1.20, 1.10, 1.17, 1.09
36	38,629	1-Propene-1,2,3-tricarboxylic acid, tributyl ester	LOC1, LOC2, LOC4, LOC5, LOC6, LOC8	2.08, 2.31, 1.80, 2.11, 1.73, 1.97
37	38,753	Decanedioic acid, dibutyl ester (CAS)	LOC1, LOC2, LOC3, LOC4, LOC5, LOC6, LOC8	7.31, 6.82, 7.59, 6.17, 5.49, 5.44, 6.18
38	39,244	Butyl citrate	LOC1, LOC2, LOC8	1.04,1.90, 1.80
39	40,847	Tributyl acetylcitrate	LOC1, LOC2, LOC3, LOC4, LOC5, LOC6, LOC8	22.26, 20.58, 14.81, 18.22, 14.71, 19.13, 15.87
40	41,205	1-Docosanol (CAS)	LOC7	1.74
41	45,796	Benzyldiethyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoate	LOC1, LOC2, LOC4, LOC5, LOC6, LOC8	2.58, 2.23, 2.06, 1.57, 2.31, 2.9
42	57,434	.gamma.-Sitosterol	LOC1, LOC7	2.47, 5.92
43	57,413	Stigmast-5-en-3-ol, (3.beta.)- (CAS)	LOC1, LOC2, LOC3, LOC4, LOC5	0.84, 1.21, 1.78, 1.10, 2.16

RT: Retention Time

Table 2. Macro and micro element contents of *Valeriana dioscoridis* plant collected from eight different locations
Tablo 2. Sekiz farklı lokasyondan toplanan Valeriana dioscoridis bitkisinin makro ve mikro element içerikleri

LOCATIONS	N(%)	P (%)	K (%)	Ca (%)	Mg (%)
Range values		0.15-0.50	1-6	1.5-2.5	0.3-1
LOC1	0.720±0.01 f	0.720±0.02 b-d	3.813±006 a	8.803±0.28 e	0.493±0.01 b
LOC2	0.876±0.02 d	0.560±0.01 d-e	2.130±0.01 c-d	3.420±0.02 c	0.880±0.01 a
LOC3	1.453±0.01 b	0.630±0.02c-e	1.993±0.02 d	1.500±0.00 d	0.500±0.00 b
LOC4	0.800±0.01 e	0.513±0.01 e	2.010±0.01 d	1.500±0.03 c	0.600±0.00 b
LOC5	1.230±0.01 c	0.476±0.00 e	2.197±0.01 c	0.830±0.01 e	0.590±0.01 b
LOC6	1.733±0.04 a	0.950±0.00 a	1.330±0.01 e	0.220±0.00 a	0.510±0.00 b
LOC7	0.680±0.02 f	0.780±0.03 b-c	1.290±0.01 e	0.117±0.02 e	0.543±0.00 b
LOC8	1.287±0.00 c	0.880±0.01 a-b	3.433±0.02 b	0.083±0.01 b	0.527±0.00 b
Average	1.097	0.688	2.274	2.059	0.580
F	**	**	**	**	**
LSD	0.07554	0.1602	0.1602	0.1602	0.1602
CV (%)	3.99	1.25	0.61	4.58	0.79
LOCATIONS	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Raw Protein (%)
Range values	30-100	100-250	6-20	20-50	
LOC1	408.3±0.00 b	4186±3.53 c	28.26±0.15 f	52.63±0.17 d	4.443±0.06 f
LOC2	186.1±0.69 d	3668±6.23 e	24.31±0.15 g	42.37±0.23 f	5.533±0.10 e
LOC3	86.55±5.03 g	4116±2.35 d	44.70±0.18 d	59.44±0.37 c	9.060±0.06 b
LOC4	63.97±0.47 h	3073±1.27 g	29.42±0.48 f	51.91±0.33 e	5.333±0.10 e
LOC5	144.6±0.16 e	4662±14.47 a	47.74±0.31 c	70.79±0.26 a	7.620±0.06 d
LOC6	111.6±0.96 f	3126±12.48 f	36.75±0.63 e	40.75±0.22 g	10.60±0.00 a
LOC7	1200±4.08 a	4279±10.49 b	63.09±1.26 a	63.86±0.30 b	4.250±0.13 f
LOC8	284.7±0.82 c	4123±7.78 d	51.61±0.18 b	35.87±0.41 h	8.060±0.00 c
Average	310.73	3904.1	40.73	52.20	6.862
F	**	**	**	**	**
LSD	3.535	13.95	1.523	0.5123	0.3462
CV (%)	0.67	0.21	2.21	0.58	3.00

CV (%): Coefficient of Variation, Standart sapmanın ortalamaya göre % değişimi [CV= 100•(s/x)], LSD: Least Significant Difference.

Different micronutrients, such as Mg, Ca, Fe, Zn, Mn and Cu were found in the range of 0.3-1, 1.5-2.5, 100-250, 20-50, 30-100 and 6-20 mg kg^{-1}, respectively. The limit value of the micronutrients such as Zn, Mn and Cu are in the range of 23.2-39.4, 55-104.3 and 4.8-13.5 µg g^{-1}, respectively (Petenatti et al., 2011). It was determined that the obtained data, for Zn and Cu exceeded the limit values in plants grown in different environments. The maximum Cu content of *V. dioscoridis* was in LOC 7 (63.09 mg kg^{-1}), the highest Zn content of the *V. dioscoridis* was in LOC 5 (70.79 mg kg^{-1}). Furthermore, the highest Mn and Fe contents of the *V. dioscoridis* were determined as 1200 mg kg^{-1} in LOC 7 and 4662 mg kg^{-1} in LOC 5, respectively. According to Ekbiç et al. (2017) determined that the Fe (1384-2092 ppm) content in the root of the cucumber plant was very high.

Antioxidant activity

DPPH and ABTS radical scavenging activity

Chemical components which have a reactive structure because of an imbalance in the structures and which are found in an unmatched electron in the orbit of an atom or electron, are known as free radicals. These may interact with all the components forming the cell and may cause a deterioration in the functions of the biological molecules. Free radicals are formed in the body with the natural metabolism and are eliminated with antioxidants taken in externally or with the organism's own antioxidant system. However, when the metabolic system cannot combat the free radicals, cytotoxicity develops with the formation of oxidative stress. Consequently, free radicals may cause several severe diseases such as cardiovascular diseases and cancer (Halliwell, 1997; Yen and Wu, 1999; Halvorsen et al., 2002; Inglet et al., 2011; İçli 2017).

During this study, high absorbance values and low antioxidant activity was observed during the radical scavenging activity analysis. Data obtained with the microplate reader were evaluated in the light of the information, and the DPPH and ABTS radical scavenging capability percentage of extracts of the *V. dioscoridis* plants collected from eight different locations are illustrated in Fig 1. The scavenging effect of extract on DPPH radical increased with increasing concentration from 0.1 to 2.0 mg ml^{-1}, although this was lower than the standard gallic acid.

As the passage of both polar and nonpolar substances could be provided, generally 80 % ethanol or methanol extract showed more antioxidant activity for different in vitro systems. This may have been because 80% ethanol/methanol is the best extraction solvent for phytochemical compounds.

The radical scavenging activities of 80 % ethanol extracts are presented in Figure 1.

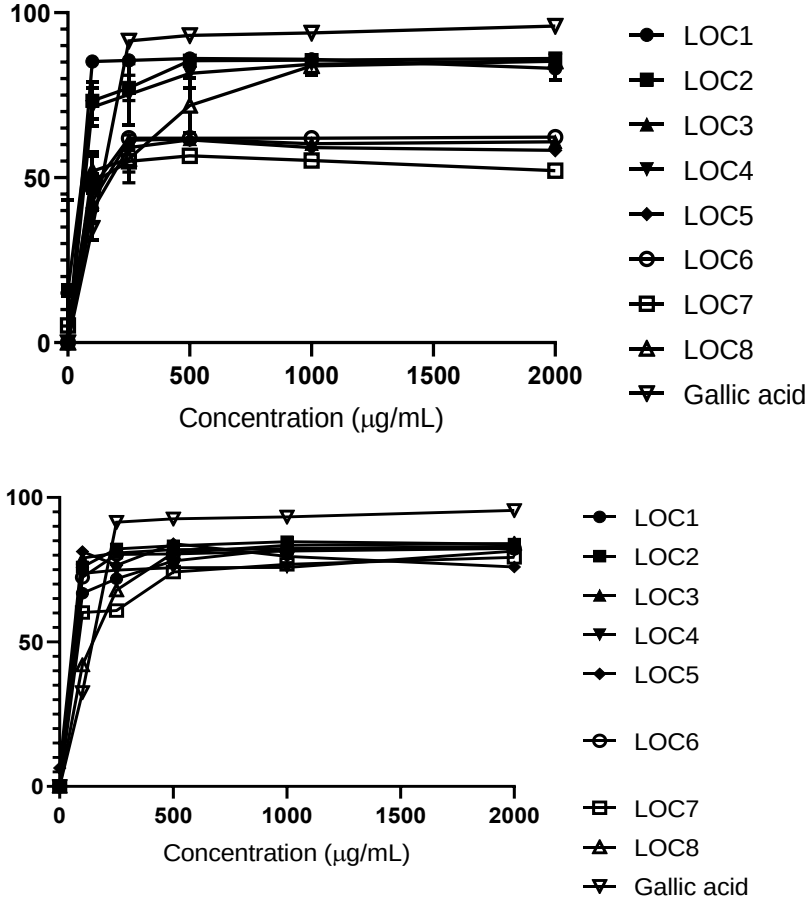

Figure 1. (1) DPPH radical scavenging activity of 80% ethanol extracts of *Valeriana dioscoridis* plants collected from eight different locations, (2) ABTS radical scavenging activity of 80% ethanol extracts of *Valeriana dioscoridis* plants collected from eight different locations

Şekil 1. (1) Sekiz farklı lokasyondan toplanan Valeriana dioscoridis bitkisinin % 80 etanol ekstraktının DPPH radikal süpürücü etkisi (2) Sekiz farklı lokasyondan toplanan Valeriana dioscoridis bitkisinin % 80 etanol ekstraktının ABTS radikal süpürücü etkisi

When the results of the analysis made according to DPPH method were evaluated, the highest antioxidant effect was present in the samples of LOC 1, where the most components were determined in the chemical

composition structure and the lowest antioxidant effect was seen in the samples of *V. dioscoridis* collected from LOC 7. When the results of the analysis were compared with ABTS radical scavenging activity method, the antioxidant activity results, even if lower than the reference substance, were determined to be at a level that could be said to be an antioxidant effect. According to Karadeniz et al (2015), the aqueous extracts of *V. dioscoridis* have an antioxidant effect.

Total Flavonoid Content (TFC) and Total Phenol Content (TPC)

Phenolic components are defined as the two sub-classes of flavonoids and phenolic acids, which are found in many hydroxyl groups in at least one aromatic ring. Flavonoids are polyphenolic antioxidants found in the natural structures of fruit, vegetables and herbal teas (Naczk and Shahidi, 2004).

Generally, biological activity such as antioxidant activity is mostly related to components such as flavonoids and phenolic acids (Saddiqe et al., 2010). In this study, evaluation was made to explore the differences in the total phenol and flavonoid values of the *V. dioscoridis* collected from different locations. Results revealed that total flavonoid value could not be determined in any of the plants collected from any of the locations, while the highest phenol content was determined in LOC 8, followed by LOC 3 (Figure 2).

Antimicrobial Activities

The antimicrobial activity results of all the *V. dioscoridis* extracts are shown in Table 3. According to the results, there was not much difference between the extracts in terms of antimicrobial activity. The antimicrobial activity of plant extracts are accepted significant if the MIC value is ≤0.1 mg ml^{-1}, moderate if MIC is 0.1 - 0.625 mg ml^{-1} and weak if MIC > 0.625 mg ml^{-1} (Kuete, 2010; Awouafack et al., 2013). Among the tested micro-organisms, *S. aureus* was more susceptible to some extracts, with MIC values ranging between 0.312 and >2.5 mg mL^{-1}. The MIC values of ethanol extract from LOC2, LOC5, LOC6, LOC7 and LOC8 were found to be moderate. The results of the microdilution broth assay for *B. cereus* showed that the best result was observed in the samples from LOC8 (0.625mg mL^{-1}) against all the tested micro-organisms.

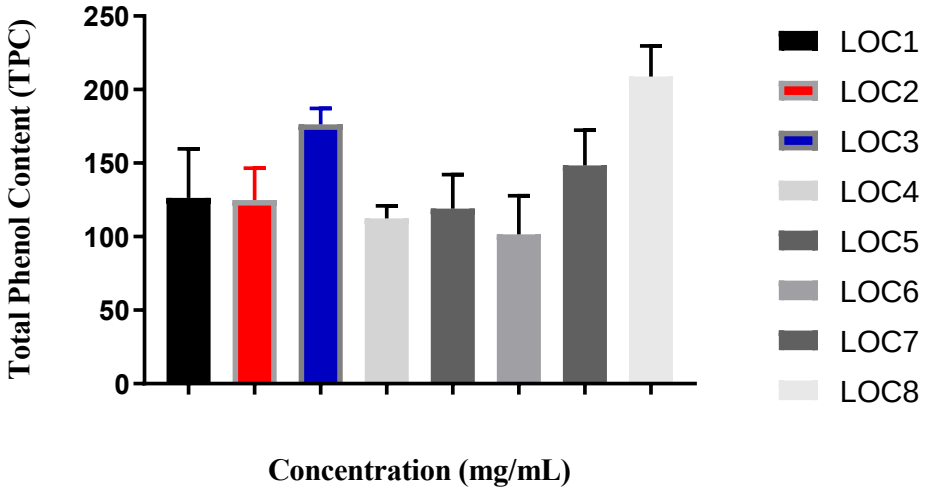

Figure 2. Total phenol content of the 80% ethanol extracts of *Valeriana dioscoridis* plants collected from eight different locations
Şekil 2. Sekiz farklı lokasyondan toplanan Valeriana dioscoridis bitkisinin % 80 etanol ekstraktının Toplan fenol içeriği

Table 3. Antimicrobial capacity (*MIC (minimum inhibitor concentration)*) of the 80% ethanol extracts of *Valeriana dioscoridis* plants collected from eight different locations
Tablo 3. Sekiz farklı lokasyondan *toplanan Valeriana dioscoridis* bitkisinin % 80 etanol ekstraktının antimikrobiyal aktivite (*MIC (minimum inhibitor konsantrasyon)*) kapasitesi

Growing area	*E. coli ATCC 25922* (µg/ml)	*S. aureus ATCC 29213* (µg/ml)	*P. aeruginosa ATCC 27853* (µg/ml)	*B. cereus ATCC11778* (µg/ml)	*C. albicans ATCC10231* (µg/ml)	*C. tropicalis* DSM11953 (µg/ml)
LOC1	>2.5	>2.5	>2.5	1.25	>2.5	>2.5
LOC2	>2.5	0.312	>2.5	1.25	>2.5	>2.5
LOC3	>2.5	>2.5	>2.5	1.25	>2.5	>2.5
LOC4	>2.5	>2.5	>2.5	1.25	>2.5	>2.5
LOC5	>2.5	0.625	>2.5	1.25	>2.5	>2.5
LOC6	>2.5	0.312	>2.5	1.25	>2.5	>2.5
LOC7	>2.5	0.312	>2.5	1.25	>2.5	>2.5
LOC8	>2.5	0.312	>2.5	0.625	>2.5	>2.5

CONCLUSION

In this study, the antioxidant, antimicrobial and nutrient contents of *Valeriana dioscoridis* plants grown in different locations were investigated. Maximum chemical components were present in LOC 1 and minimum in LOC 8. Generally, the amount of plant macro and micro nutrient elements were moderate in all growing conditions but wasn't obtained in the same proportions in plants grown in all environments. The highest antioxidant effect was observed in LOC 1, but there were no significant different value for antioxidant activity between the locations. Maximum phenol contents were observed in LOC 8, followed by LOC 3. The results of the microdilution broth assay for *B. cereus* showed that the best result was in the samples from LOC 8 against all the tested micro-organisms. Oxidative stress is associated with many chronic disease. Therefore, herbal products can play an important role in the management of these diseases due to the rich antioxidant compound content. However, the synthesis of the secondary metabolites, which are responsible for the main effect in the plant, varies according to the regions where the plant grows, so the biological effect of the plant also changes. In this regard, the *V. dioscoridis* plants of in different locations were evaluated and it was determined that the different locations were created differences in the content values of the plant.

Author contributions

Concept – E.U., M.A., Y.Ç., İ.Ç., N.E., T.K; Design – E.U., M.A., Y.Ç., İ.Ç., Supervision – E.U., N.E.; Resources – E.U., M.A., Y.Ç., İ.Ç., N.E.; Materials – İ.Ç.; Data Collection and/or Processing – E.U., M.A., Y.Ç., N.E.; Analysis and/or Interpretation – E.U., M.A., Y.Ç., İ.Ç., N.E., T.K.; Literature Search – E.U., M.A., Y.Ç., İ.Ç., N.E.; Writing – E.U., M.A., Y.Ç., İ.Ç., N.E., T.K.; Critical Reviews – – E.U., M.A., Y.Ç., İ.Ç., N.E., T.K.

Conflict of interest statement

The authors declared no conflict of interest.

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