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Some Quality Criteria of Valerian (*Valeriana dioscoridis* Sm.) Growing in Different Environments

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Abstract: Valerian (*Valeriana dioscoridis* Sm.) is a perennial herb of the Caprifoliaceae family. The genus *Valeriana* L. is represented by 14 species (15 taxa) in Turkey. This plant contains flavone glycosides, iridoids and lignans. Among these components, the medically important active ingredient is valerianic acid. Essential oils from valerian roots and rhizomes are used for the treatment of various diseases, including insomnia, mental illness, anxiety, menstrual cramps and physical stress conditions. In this study, evaluations were made of the root and rhizome of valerian plants grown in a natural environment and in greenhouse condition. The macro and micro nutrient contents of the powdered plant samples, and the antioxidant and antimicrobial activity values of the extracts were reported. Except for some macro and micro elements, it was determined that antioxidant and antimicrobial activities of plants cultivated and grown in nature were not different. The major components of both the natural and cultivated forms were determined to be 9-Borabicyclo [3.3.1] nonane, 9-[3-(dimethylamino) propyl]- (17.55% and 22.65%, respectively). The heavy metales such as Fe (415.21±47.8 mg/kg), Cu (50.9±0.2 mg/kg) and Mn (274.6±9.5 mg/kg), were obtained above limit values in grown plants of natural environment conditions.

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1. INTRODUCTION

Valeriana L. of the Caprifoliaceae family, has approximately two hundred species throughout the world [1, 2]. In Turkey, there are 14 species (15 taxa) [2-5] *Valeriana dioscoridis* Sm. is a perennial herbaceous plant with rhizomes, pink flowers and is commonly known as

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Kediotu or Çobanzurnası in Turkey. The flowering time is February-May. It grows on rocky slopes and scrubland, at up to 1500 meters above sea level [2, 3].

The dried roots and rhizomes are known to be cause soothing and sleep. It is also used in wound treatment [6] and is known to calm the nerves and relieve spasms [7]. In the roots of *V. dioscoridis*, there are lipophilic compounds known as valtrates at the rate of 0.5%. Another species, *Valeriana officinalis*, is reported to have been used as a poisonous plant and in witchcraft at the time of Cervantes [8]. Essential oils obtained from *V. dioscoridis* rhizomes have been found to have an antifungal effect [9] and the aqueous extracts of *V. dioscoridis* have an antioxidant effect [10].

Although the use of this plant is so common, there are limited data on its reliability [11]. Most of the medicinal and aromatic plants are collected from natural areas and are offered to trade. For drugs that are mostly obtained from naturally growing plants, it can be difficult to provide a sufficient amount of medicinal plants or these plants are not of the necessary quantity and quality due to mistakes made during harvesting and drying. When this is the purpose of medicinal and aromatic plants, such factors reduce the utility values of plants and even adversely affect them.

Different relaxation methods are used to eliminate the problems caused by the stressful conditions of modern life. Anxiety, which is caused by stress, has become an important problem affecting the quality of life of current society. Although various drug treatments are available, because of the side-effects of these drugs, many people have turned to alternative medicine and as a result there is an increase in the use of medicinal and aromatic plants. There is a constantly increasing demand for valerian plants, which are medicinal and aromatic plants with potential effects on the significant modern-day disorder of anxiety. In order to meet this demand, the roots and rhizomes of the valerian plant are collected from areas of natural growth. Seed production can be a problem of valerian plants as there is a low percentage of seed production and germination [12]. At the same time, irregular collection of this plant throughout the world is endangering populations of the species [13], resulting in insufficient numbers of plants, and the risk of extinction. Production or cultivation of these plants is one of the factors protecting natural flora. Therefore, cultivation and germplasm studies of this plant are important [13]. Furthermore, sometimes the wrong or various chemical strains of the plant can be collected, and there are specific periods when the effective substances in medicinal plants are highest, so they must be harvested at that time. However, it is not easy to determine when and how plants are collected, since it is not possible to control the collectors. Therefore, often plants are not of the desired quality, with regulated cultivation, the quality and productive varieties of these plants can be improved.

The aim of this study was to determine and evaluate some quality criteria of samples taken from the subsoil organs of plants grown in greenhouse conditions and in plants collected from natural areas. There are ongoing studies to eliminate the danger of extinction of the valerian plant. These studies can be considered of value as the cultivation of these plants to specific standards, which are in high demand for drugs, will contribute to the economy of Turkey.

2. MATERIAL and METHODS

The research was carried out between 2016 and 2019 in The Greenhouse of Crop and Animal Production Department, Sivas Vocational School, Cumhuriyet University, Department of Plant and Animal Production and Cumhuriyet University Advanced Technology Research Center laboratories. *Valeriana dioscoridis* Sm. plant were used as materials.

2.1. Supply of Plant Materials

The valerian (*Valeriana dioscoridis* Sm.) plants used in this research were collected from Ahmetler village, in the Manavgat district of Antalya (Turkey. C3 Antalya: Manavgat, Ahmetler village, near a stream, in a red pine forest and damp places, 36 S 0383784-UTM 4076925, 639 m, 19.II.2016, Çinbilgel s.n.).

Later, some of these plant roots were grown in pots in a greenhouse environment. Samples were collected from the root and rhizome of plants grown in both the natural environment and in the greenhouse culture conditions. The obtained plant samples were dried in the shade and grinded to the appropriate size for extraction with a laboratory grinder.

2.2. Obtaining Extracts

The powdered plant materials were macerated with 80% ethanol. After one day of agitation in the shaker, the plant particles were filtered, and dried in an oven to obtain the extracts [14].

2.3. 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 [15]. 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 injection volume using splitless mode was programmed among 80-300 at rate of 5 in minutes. Post run was set at 300 °C for 2 min. Total run time was 60 minutes [16].

2.4. Determination of Macro-Micro Element Contents

First, the samples were ground for further analysis. The N content was determined using the modified Kjeldahl method [17]. For the P, K, Fe, Mn, Zn and Cu contents, 0.200 g plant samples were weighed in a porcelain crucible then dried in the oven at 550 ºC for 5 hours to obtain ash as contents. After removal from the oven, 1/3 HCl and distilled water were added to the extracted samples. Using a P 880 nm UV-spectrophotometer [18], the levels of K, Fe, Mn, Zn and Cu were determined with a Atomic Absorption Spectrophotometer (AAS) [19].

2.5. *In vitro* Antioxidant Activity

2.5.1. DPPH Radical Method

The DPPH radical scavenging activity of the extracts was evaluated according to the Blois method [20]. Briefly, 1mL of 1.5×10^{-4} M DPPH solution in methanol was mixed with 3mL sample solution in ethanol at different concentrations and incubated for 30 min in the dark. Absorbance was measured at 520 nm. Gallic acid was used as a positive control. The percent of DPPH radical scavenging activity was calculated according to the following equation:

$$\% \text{ DPPH radical scavenging activity} = (Ac - As) / Ac) \times 100$$

Where Ac was the absorbance of the control without the sample and As was the absorbance in the presence of the extract.

2.5.2. Linoleic Acid/Thiocyanate Method

To determine the antioxidant activity of the extracts, the ferric thiocyanate method was used. In this method, linoleic acid oxidation is formed in vitro, and during oxidation Fe^{+2} ions are oxidized to Fe^{+3} ions. Specifically, the formation of peroxides is monitored by spectrophotometric measurement of a sample of the mixture in the incubation period. A high absorbance value indicates a high peroxide concentration. The sample solution (10 mL) and standard solution (Vitamine A and BHT) at concentrations of 100-1000 µg/ml were mixed with

10 mL of linoleic acid (2.52 %), 20 mL of phosfate buffer (0.02 M, pH 7.0) and 9.74 mL of distilled water. After vortexing, the mixture was incubated for 53 h at 37 ºC. The negative control was prepared without linoleic acid. Thereafter, at 0, 5, 8, 24, 27, 32, 48, and 53 hours, 9.6 mL of 75% ethanol, 0.1 mL of 30% ammonium thiocyanate were added to the 0.2 mL mixture. After 3 min, 0.1 mL of 20 Mm ferrous chloride in 3.5 % HCI was added to the mixture. The absorbance was calculated at 500 nm after 5 min incubation [21].

2.5.3. Thiobarbutric Acid Method

In this method, 2 mL of sample solution as prepared in the FTC method was mixed with 2 mL of 20% trichloroacetic acid (TCA) and 2 mL of 0.67% thiobarbituric acid (TBA), then incubated for 10 min in a water bath. After cooling, it was centrifuged for 10 min at 3000rpm/min. The absorbance of supernatant was measured at 532 nm [22].

2.5.4. Ferric Reducing Antioxidant Power Assay

The ferric reducing power for plant extracts was evaluated according to the Oyaizu method [23]. For the experiment protocol, 1 mL of plant extract (50-1000 µg/mL) and standard was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% $K_3Fe(CN)_6$. The mixture was kept in the dark for 20 min at 50 ºC. Then, 5 mL of 10% trichloro-acetic acid was added and centrifuged at 2500 rpm for 10 min. 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ solution were added to the 2.5 mL of supernatants. The absorbance of the mixture was measured at 700 nm.

2.6. Antimicrobial Activities of *Valeriana* Extracts

The microdilution Broth method [24] was used to determine the Minimum Inhibition Concentration (MIC) of *Valeriana* extracts against microorganisms of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus, Candida albicans* and *Candida tropicalis*. Extracts were dissolved in dimethylsulphoxide (DMSO) to prepare stock solutions (50 mg/mL). The Mueller Hinton Broth (Accumix® AM1072) and Saboraud Dekstroz Broth (Himedia ME033) were used to grow and dilute bacteria and yeast, respectively. 10 µL extract was added to the first line of the microtiter plate, which was diluted with 90 µL broth. Next, 50 µL sample was added to the second line of the microtiter plate, which was serially diluted two-fold with broth. Concentration of the extracts in the wells ranged from 2.5 to 0.004 mg/mL. In the 11th well, 100 µL broth was added to be used as the sterilizitation control. The last well (12th) containing broth and inocula without extracts was used as the growth control. Final inoculum size was 5 $x10^5$ CFU/mL of bacteria and 0.5-2.5 $x10^3$ CFU/mL of Candida in every well [25, 26]. The bacteria and yeast suspension (50 µL) was added to the prepared samples. Samples with added bacteria were incubated at 37 (±1) °C and the samples to which *Candida* was added were incubated at 35 (±1) °C for 16-24 hours. The lowest concentration of extract that was capable of inhibiting visible growth of the microorganism was accepted as the MIC value.

3. RESULTS and DISCUSSION

Plants of the same species, obtained from two different growing environments; the cultivated form (grown in greenhouse conditions) and the natural form (collected from nature) were used in the scope of this experiment. The different growth media the effect of on the essential oil content of plants and the quality criteria of plant extracts were evaluated.

3.1. GC/MS Analysis of the Obtained Extracts

A very small amount of volatile oil could be obtained from the plants and the amount obtained remained at the hydrolysate level. Therefore, the plant extracts were used tto determine the content. Gas Chromatography-Mass Spectrometry (GC/MS) was used for the analysis and the results are shown in Table 1. When the 80% ethanol extracts of the plants collected from

the natural areas and the greenhouses were compared, they were seen to have different components.

The major components of both the natural and cultivated forms were determined to be 9-Borabicyclo[3.3.1]nonane,9-[3-(dimethylamino)propyl]-(17.55% and 22.65%, respectively). A high level of valerianic acid (pentanoic acid) is desired as this gives the plants medical properties. However, this component remained at the rate of 0.30% in the plants grown in the natural environment, and could not be determined in the plants cultivated in greenhouses. As the environmental temperature sometimes reaches very high levels during GC-MS analysis, this could cause a rupture of the bonds between chemical components of some plants and may have prevented the determination of valerianic acid (pentanoic acid). In addition, as the plants were grown in the controlled conditions of a greenhouse, this may have caused secondary metabolites to be expressed at a low rate as they are usually expressed by the plant to protect itself. As varying environmental conditions are usually produced to protect the plant, there may be differences in metabolite amounts and content. Lopes et al. [27] reported that *Valeriana* roots have highly isovaleric acid. Bogacz et al. [11] and Dimpfel [28] stated that the roots of valerian carry chemical components, especially valerianic acid, and the part of valerian used as herbal medicine is the roots.

Table 1. Main compounds identified in the methanolic extract of *Valeriana dioscoridis* Sm. by GC-MS

No	RT (Retention Time)	Components	Relative Percentage (%)	
			Culture form	Natural form
1	7.550	Pentanoic acid	-	0.30
2	9.055	1,2-Cyclopentanedione	-	1.29
3	28.275	1-Dodecanol	-	1.51
4	28.281	1-Tetradecanol (CAS)	1.54	-
5	29.133	o-Diethyl benzene	1.53	3.29
6	29.391	Phenol, 2,4-bis(1,1-dimethylethyl)- (CAS)	0.73	0.88
7	30.856	4,5-dimethyl-11-methylenetricyclo [7.2.1.0 (4.9)]dodecane	3.20	-
8	33.247	Valeranone, (+)-	0.20	-
9	33.471	Acrylic acid dodecanyl ester	1.47	1.46
10	34.752	1,3-Dimethylthieno[3,4-d]thiepin	6.12	-
11	36.572	1,3-Butadienylidene)cyclohexane	12.34	7.89
12	36.910	1-(3'-Hydroxypropyl)-2,5-dimethoxy -3,4,6-trimethylbenzene	9.50	1.89
13	37.739	Hexadecanoic acid, methyl ester	1.87	4.24
14	38.397	(+-)-3-(3,4-Dimethoxyphenyl)pyrrolidine	2.37	-
15	38.414	1,3-Dimethyl-3-hydroxy-5-methoxyox indole	4.06	-
16	38.861	Hexadecanoic acid, ethyl ester (CAS)	-	2.36
17	39.782	9-Borabicyclo[3.3.1]nonane, 9-[3-(dimethylamino)propyl]-	22.65	17.55
18	40.457	Methyl linoleate	-	1.88
19	41.544	9-Octadecenoic acid, ethyl ester	-	2.71
20	43.261	n-Nonadecanol-1	-	1.31
21	44.388	Oleic acid amide	3.31	8.22
22	49.549	Isophthalic acid, 2,6-dimethoxyphenyl ethyl ester	1.68	-
Total			72.57	56.78

3.2. Macro-Micro Nutrient Element Concentrations

The macro and micro nutrient content of *Valeriana dioscoridis* plants grown in different growth media are presented in Table 2.

Table 2. The macro and micro nutrient content values of *Valeriana dioscoridis* plants collected as the natural form and cultivated form grown in a greenhouse.

Growing Area	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Ca (%)	Mg (%)	N (%)	P (%)	K (%)
Natural Form	274.6±9.5	50.9±0.2	35.9±0.4	4152.1±47.8	3.4±0.02	0.5±0.0	1.29±0.006	0.88±0.01	3.22±0.2
Cultivated Form	133.4±3.7	11.85±0.3	31.99±0.0	3761.1±5.4	1.8±0.01	0.8±0.01	1.86±0.005	1.02±0.01	3.17±0.1

The results of this study showed that the amount of potassium was obtained as 3.22 K % and 3.17 K %, respectively in natural form and cultivated form. Both values were found close to each other. While the N concentration of the natural form was determined as 1.29 N %, the nitrogen concentration of the plant grown in cultivated form was determined as 1.86 N %. At the same time, phosphor concentration was obtained in the nearly same proportions in plants grown in both environments (natural and cultivated form contain 0.88 P % and 1.02 P % , 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, respectively [29]. The Zn contents and range were similar in plants obtained from both growth environments. The Mn content exceeded the limit values in plants grown in both environments, with higher Mn content determined in plants grown in natural conditions. Similarly, Petenatti et al. [29] determined high Mn value in Valeriana officinalis. The Cu content was within the limit values under greenhouse conditions and exceeded the limits in plants grown in natural conditions. The Ca content was remained proportionally low in plants grown under greenhouse conditions. The amount of Fe was found to be higher in the plants grown in the natural environment than other condition. Petenatti et al. [29] reported that the content of Fe obtained as 0.97 mg g^{-1} in *Valeriana officinalis*. The high values of micro elements, some of which are heavy metals, suggested that these plants collected from fields may have been obtained from high traffic areas. When the plant is evaluated in terms of nutrients, it is necessary to cultivate the plants avoiding heavy metal pollution, so cultivation should be in areas with low traffic density.

3.3. Antioxidant Activity

3.3.1. DPPH Radical Scavenging Activity

The percentage DPPH radical scavenging capability of cultured and natural *V. dioscoridis* extracts are illustrated in Figure 1. The scavenging effect of the extract on DPPH radical increased in a linear manner with increasing concentration from 0.1 to 2.0 mg/mL, although at a lower level than the standard gallic acid. Duaheh et al. [30] reported that *V. officinalis* species showed high DPPH radical scavenging activity (IC_{50} = 38 mg/mL). Sudati et al. [31] and Malva et al. [32] reported that *V. officinalis* had an antioxidant effect and this property, made medically valuable to the *Valeriana* plant.

3.3.2. Linoleic Acid/Thiocyanate Method

This method is based on the measurement of the amount of lipid peroxide formed by incubation of an unsaturated fatty acid linoleic acid with oxygen at 40 ℃ in an emulsion medium formed by phosphate buffer. A higher absorbance value shows lower antioxidant activity. The extracts obtained from the valerian plants grown in nature and collected in the

culture medium and the results of total antioxidant activity of Vitamin E are given in the graph below (Figure 2). According to the linoleic acid/ferric thiocyanate (FTC) method, the results are quite low compared to the reference. When evaluated in terms of both cultivation conditions, there were no significant differences in antioxidant capacity.

3.3.3. Thiobarbutric Acid Method (TBA)

The TBA method represents the inhibition of degradation of peroxides in the final stage in the production of carbonyl compounds. The TBA test is used to measure secondary peroxide oxidation products such as aldehyde and ketone. The antioxidant activity values of extracts obtained from valerian plants grown in the two different growing environments, the natural habitat and culture conditions, according to the thiobarbituric acid method, are shown in Figure 3. When the data were examined, it was observed that the results of the antioxidant activity of the extracts were quite low compared to the reference. No significant differences were determined between the two growth environments in respect of antioxidant capacity.

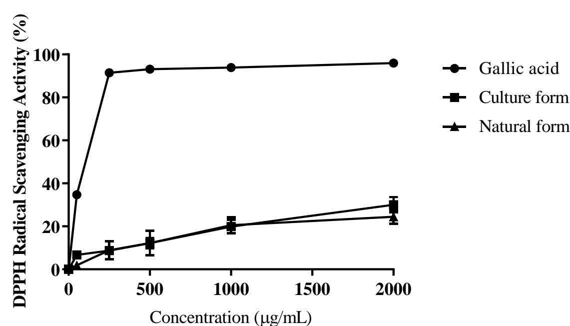

Figure 1. DPPH radical scavenging activity of 80% ethanol extracts of cultured and natural *Valeriana dioscoridis*

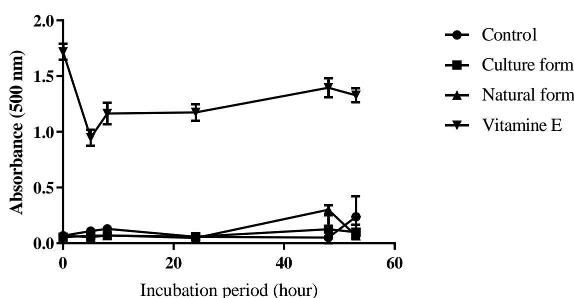

Figure 2. Antioxidant capacity of 80% ethanol extracts of cultured and natural *Valeriana dioscoridis* using the FTC method

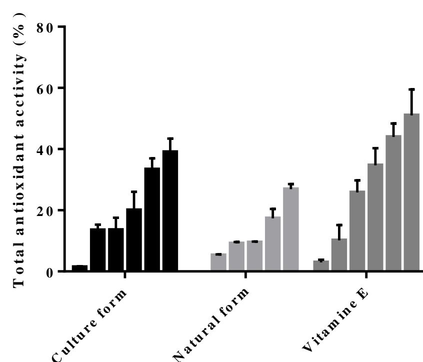

Figure 3. Antioxidant capacity of 80% ethanol extracts of cultivated and natural *Valeriana dioscoridis* using the TBA method

3.3.3. Thiobarbutric Acid Method (TBA)

The reduction power method is based on the principle that potassium ferrocyanide (Fe^{2+}) is formed by reacting the substances with the potential for reduction with potassium ferricyanide (Fe^{3+}), then reacting with ferric chloride to give maximum absorbance at 700 nm. Figure 4 below shows the comparative results of plant extracts collected from nature and grown in culture conditions and vitamin E used as reference for reducing power. According to the obtained data, the antioxidant activity values of the extracts did not show good results compared to the reference compound and there were no significant differences between the two growth conditions.

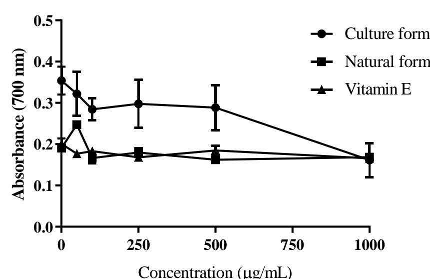

Figure 4. Ferric reducing power of 80% ethanol extracts obtained from cultivated and natural *Valeriana dioscoridis*

3.4. Antimicrobial Activities

The antimicrobial activity results of the *Valeriana* extracts are shown in Table 3. It has been reported that antimicrobial activity of plant extracts to be significant if the MIC value is 0.1 mg/mL or less, moderate if the MIC value is in the range of $0.1 < MIC \leq 0.625$ mg/mL and weak if the MIC value is bigger than 0.625 mg/mL [33, 34]. There is little difference in terms of antimicrobial activity in the comparisons of the extracts. Among the tested microorganisms, *Bacillus cereus* was more susceptible to some extracts, with MIC values ranging between 0.312 and 2.5 mg/mL. According to Düzgüner and Erbil [35] reported that *Valeriana* plant extracts have low antibacterial effect.

Table 3. Antimicrobial capacity of 80% ethanol extracts of cultivated and natural *Valeriana dioscoridis*

Growing area	*E. coli* ATCC 25922	*S. aureus* ATCC 29213	*P. aeruginosa* ATCC 27853	*B. cereus* ATCC11778	*C. albicans* ATCC10231	*C. tropicalis* DSM11953
Culture form	>2.5	>2.5	>2.5	1.25	>2.5	>2.5
Natural form	>2.5	>2.5	>2.5	1.25	>2.5	>2.5

4. CONCLUSION

Phenolic compounds that one of the most important substances with antioxidant activity, can prevent oxidative cell damage in living organism. The generally, medicinal and aromatical plants have antioxidant compounds. In this context, these plants are in high demand and consumed. If plants are harvested only from nature, their generation may face the danger of extinction. Some application mistakes are made during the collection from nature. This can also affect the plant's quality criteria. In this study, it was evaluated whether there is a decrease or increase in nutrient content, antioxidant and antimicrobial activity values of plants grown in nature and cultivated. It was observed that there was no difference in the quality criteria among plants grown in nature and cultivated. In fact, it is thought that quality criteria can be increased as a result of some plant cultivation processes.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author(s).

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