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Chemical Composition of the Essential Oil, Total Phenolics, Total Flavonoids and Antioxidant Activity of Methanolic Extracts of *Satureja montana* L.

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Abstract: Aerial parts of *Satureja montana* L. (Lamiaceae) were collected from seven growing wild populations (four populations in Kosovo, two in Albania and one in Montenegro) in 2013 with the aim of assessing the natural variation in the chemical composition of the essential oils, total flavonoids, total phenolics and the antioxidant activity of their methanolic extracts. Essential oils were obtained by steam distillation and analysed using GC-FID and GC-MS, whereas total flavonoids, total phenolics and antioxidant activities were determined using spectrophotometric methods. Sixty-one volatile constituents were identified. The main constituents were myrcene, p-cymene, γ-terpinene, linalool, thymol, carvacrol and viridiflorol. Total phenolics ranged from 68.1 to 102.6 mg/g dry mass, the total flavonoid content ranged from 38.3 to 67.0 mg/g dm, and the antioxidant activity according to the DPPH assay ranged from 253.3 to 342.9 mg TE/g dm and according to the FRAP assay ranged from 8.9 to 11.4 mg TE/g dm.
Hierarchical cluster analysis and principal component analyses were used to assess the geographical variations in the essential oil composition. Statistical analysis revealed that the analysed populations are grouped into four main clusters that appear to reflect the environmental impact on the chemical composition, which is influenced by differences in habitat composition, altitude and microclimatic conditions.

Keywords: Winter savoury; GC MS; volatile oil; polyphenols; natural variability; bioactivity.

1. Introduction

Satureja montana L. (Winter savory) is a perennial species with a stout woody stock (40-70 cm long), linear to oblanceolate leaves and verticillaster inflorescences. It is distributed in southern

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Europe as a native species [1]. In Balkans ethnobotany, it used for different purposes, such as a nutraceutical in Albania [2] and a spasmolytic, anti-diabetic, respiratory tract infection treatment, anti-tussive, and expectorant in Kosovo [3], whereas in the food industry, it is used as a flavouring agent [4]. This species has a rich and diverse composition of secondary metabolites as well as diverse biological activities, including antioxidative properties [5]–[7], antibacterial properties of the essential oil [5], [7]–[10], and antifungal activity [8], [10], [11]. Several studies have reported the chemical composition of the essential oil of *S. montana* originating from different regions of the world [5], [6], [8], [10]–[14] and total phenolics and total flavonoids [15]. *Satureja montana* is characterized by a wide variability in its morphology even in same population, which causes confusion from taxonomic and chorological perspectives. Variations in the content and chemical composition between populations were also previously reported, showing the presence of different chemotypes [16] – [19]. According to the International Standards (ISO 7928-1-1991), the minimum yield of essential oil (on a dry basis) required for springs of *S. montana* is 0.3% and the following main constituents: γ-terpinene, p-cymene, linalool, 1-terpinen-4-ol and carvacrol. The principal aims of our study were to analyse the chemical compositions of the essential oils of the aerial parts of *S. montana* and to assess the natural variation in the essential oils, total phenolics, total flavonoids and antioxidative activity of the methanolic extracts between wild populations growing in Kosovo, Albania and Montenegro.

2. Materials and Methods

2.1. Plant Materials

Aerial parts of *Satureja montana* L. were collected from July to September 2013 in seven locations from naturally growing populations in Kosovo, Albania and Montenegro (Table 1). Four of these populations were from Kosovo, two were from Albania and one was from Montenegro. Three samples were collected in each location (10-20 individual plants were grouped in each sample). Each sample was distilled and analysed separately. Voucher specimens of each population were deposited at the Herbarium of the Department of Biology, University of Prishtina (Table 1).

Table 1. Basic characteristics of the sites from where the plant materials of *S. montana* populations were collected

Location	North	South	Elev. m a.s.l.	Substrate	Climate	Herbarium accession no.	EO yield %v/w
Pashtrik (Kosovo)	42°12'82"	20°31'55"	1417	Limestone	Continental, modified by Mediterranean	LEB/2013/4	0.4-0.7
Koritnik (Kosovo)	42°04'39"	20°36'06"	1307	Limestone	Continental, modified by Mediterranean	LEB/2013/2	0.3-0.5
Morinë (Kosovo)	42°24'08"	20°14'59"	530	Serpentine	Continental, modified by Mediterranean	LEB/2013/7	0.02-0.09
Golesh (Kosovo)	42°34'04"	20°57'06"	713	Serpentine	Continental	LEB/2013/5	0.07-0.14
Valbonë (Albania)	42°26'34"	19°57'40"	776	Limestone	Continental	LEB/2013/3	0.8-1.0
Theth (Albania)	42°23'51"	19°46'11"	769	Limestone	Continental	LEB/2013/6	0.2-0.4
Ulcinj/Ulqin (Montenegro)	41°59'08"	19°15'09"	88	Limestone	Mediterranean	LEB/2013/1	0.8-0.9

2.2. Distillation of Plant Materials

Plant material was air dried in the shade at room temperature and cut in small pieces (>0.5 cm). Essential oil was obtained by hydrodistillation (50 g of cut tissue in 0.5 litres of water contained in a 1 litre flask) at a distillation rate of 3 $mL.min^{-1}$ in a Clevenger apparatus for 3 h. The samples were

stored in the dark at -18°C in a freezer until further analysis. The yield of essential oil is expressed as the volume percentage of the dry mass of the air-dried plant material.

2.3. GC and GC-MS Analyses

GC/FID analyses were performed using an Agilent 7890A GC system equipped with an FID detector (Agilent Technologies). The separation was conducted on a HP-5MS column (30 m × 0.25 mm with a 0.25 μm film thickness). Helium was used as the carrier gas with an initial flow rate of 0.6 mL/min and then at a constant pressure of 50.0 psi. The front inlet was maintained at 250°C in a split ratio of 50:1. The GC oven temperature was increased from 60°C to 260°C at a rate of 5°C/min, and the FID was operated at 250°C with an air flow of 350 mL/min and a hydrogen flow of 35 mL/min. The injection volume was 1.0 μl.

GC/MS analyses were performed using an Agilent 7890A GC system coupled to a 5975C MSD (Agilent Technologies). The ionisation energy was 70 eV with a mass range of 40-400 *m/z*. The separation was conducted using the same column and temperature program as for the analytical GC.

The identification of each of the components of the essential oil was performed by comparing their Kovats retention indices with those in the literature [20]. The Kovats index was calculated based on a linear interpolation of the retention times of a homologous series of n-alkanes (C9-C28) under the same operating conditions. The components were also identified by comparing the mass spectra of each constituent with those stored in the NIST 08.L and WILEY MS 9th databases and with mass spectra from the literature [20]. Furthermore, some of the main peaks were identified by comparing the retention times and mass spectra with those of authentic constituents. The percentage composition of the oils was computed using the normalization method from the GC peak areas, calculated as the mean of three samples, without correction factors.

2.4. Determination of Total Phenolics and Total Flavonoids

For the analysis of total phenols, total flavonoids and antioxidant activity (DPPH and FRAP), 150 mg of dried leaves and inflorescences was ground and extracted with 25 mL of methanol (50%) in a shaking water bath for 90 minutes at 75°C and stored at -18°C in a freezer until further analyses.

The total flavonoids in the extracts were determined using a photometric method according to [21]. Catechin (0-10 mg/mL) was used as a standard to establish the calibration curve. Absorbance was measured at a wavelength of 510 nm. The total content of flavonoids was expressed as mg catechin equivalent/g plant dry weight.

The total phenolic content in the extracts was determined using the Folin-Ciocalteu method in an alkaline environment [22]. Caffeic acid (0-25 mg/mL) was used as a standard to establish the calibration curve, and absorbance was measured at 725 nm against the blank. The results were expressed as mg caffeic acid equivalent/g plant dry weight.

2.5. Evaluation of Antioxidant Activity

The DPPH (2.2-diphenyl-l-picrylhydrazyl) radical scavenging assay and Trolox (2.5 mM in methanol) were used as reference substances following the protocol of [22]. Trolox (0-50 mg/mL) was used to construct the calibration curve. The absorbance of the decolorizing process was measured at 515 nm against the blank. The results are expressed as the percent scavenging of DPPH free radicals and were measured using the following equation: % DPPH radical scavenging = [(absorbance of control - absorbance of test sample) / (absorbance of control)] x 100.

The ferric reducing antioxidant power (FRAP) assay measures the ability of antioxidants to reduce the ferric 2,4,6-tripyridyl-s-triazine complex $[Fe(III)]^{3+}$ to the intensely blue-coloured ferrous complex $[Fe(II)]^{2+}$ in acidic medium. The FRAP assay was performed as described by [22]. The calibration curve was constructed using calibration standards of Trolox from (0 to 400 mg/mL) in ethanol, and absorbance was measured at a wavelength of 593 nm. The results were estimated as mg Trolox equivalent/g plant dry weight.

All spectrophotometric measurements in the following analyses were performed using a UV-Vis spectrophotometer (Thermo Scientific™ GENESYS 10S UV-Vis spectrophotometer), and the results represent the average of 5 measurements.

2.6. Statistical Analysis

Hierarchical cluster analysis (HCA) and principal component analyses (PCA) were used to evaluate whether the identified essential oil components can be useful for reflecting the chemotaxonomy of *S. montana*. PCA and HCA were performed using the statistical analysis software XLSTAT Version 2014.2.03 (STATCON, Witzenhausen, Germany). The oil components with concentrations higher than 2% (bold in Table 1) of the total oil were subjected to statistical analyses.

One-way analysis of variance (ANOVA) was used to determine the differences of total phenolics and total flavonoids and the antioxidant activities (DPPH and FRAP) among the localities. To evaluate the correlation of total phenolics and total flavonoids with both antioxidant test systems, the Pearson correlation coefficients were calculated. Statistical data analyses were performed using SPSS for Windows, version 15.0.

3. Results and Discussion

3.1. Essential Oil Composition

Hydrodistillation of the *S. montana* aerial parts yielded light-yellowish essential oils. The yield of essential oil differed depending on the origin of the populations and ranged from 0.02 to 1.0% based on dry weight (Table 1). The highest content of essential oil was found in the population from Valbonë (0.8-1.0% v/w), whereas the lowest contents were found in the populations from Golesh and Morinë (0.02-0.09% and 0.07-0.14 v/w, respectively). The plant populations collected in Golesh and Morinë were grown in serpentine substrate, which appears to be the major force that determines the low content of the essential oil. Previous studies showed that the yield of *S. montana* growing in Croatia was 1.75% [11] and 0.80–1.46% w/w [12]; in Albania, 0.22 - 1.61% w/w [17]; and in Italy, 0.48% [8]. Samples that originated from Pashtrik, Koritnik, Valbonë and Ulcinj/Ulqin meet the required standards of ISO 7928-1-1991 regarding the yield percentage; the samples that originated from Theth partially meet these requirements, whereas the samples from Golesh and Morinë do not meet these requirements.

The results of the analysis of essential oils extracted from the aerial parts of *Satureja montana* L. collected from seven locations in Kosovo, Albania and Montenegro are presented in Table 2. In total, sixty-four components were separated, which are listed in order of their elution from an HP-5MS column. Among these components, sixty-one components were identified, which accounted for 97.2 to 99.9% of the total composition of the oils. The main components were myrcene, p-cymene, γ-terpinene, linalool, thymol, carvacrol and viridiflorol, and their concentrations differed among the plant populations (Table 1).

Table 2. Composition (%) of the aerial part of *Satureja montana* from different locations

			Kosovo				**Albania**		**Monte negro**	
No.	KI[a]	Compounds[b]	Golesh	Morinë	Pashtrik	Koritnik	Theth	Valbonë	Ulcinj/ Ulqin	Id
1	926	Tricyclene	0.17	0.00	0.00	0.00	0.01	0.00	0.00	1,2
2	930	α-Thujene	0.08	0.00	0.21	0.54	0.25	0.86	0.67	1,2
3	**939**	**α-Pinene**	**2.57**	**1.94**	**0.31**	**0.53**	**4.05**	**0.95**	**0.60**	**1,2,3**
4	948	Camphene	1.90	1.60	0.37	0.85	0.84	0.67	0.70	1,2
5	979	1-Octen-3-ol	0.95	0.72	0.87	1.18	1.79	1.84	1.37	1,2
6	983	Octanone-3	0.37	0.00	0.07	0.14	0.19	0.18	0.17	1,2

7	**990**	**Myrcene**	**17.36**	**21.09**	**0.65**	**0.70**	**2.47**	**1.26**	**1.85**	**1,2,3**
8	997	3-Octanol	0.15	0.00	0.02	0.09	0.40	0.18	0.11	1,2
9	1018	α-Terpinene	0.22	0.00	1.01	1.35	0.84	1.33	1.62	1,2
10	**1020**	**p-Cymene**	**1.02**	**0.66**	**12.16**	**29.58**	**5.14**	**13.73**	**26.14**	**1,2,3**
11	**1026**	**β-Phellandrene**	**0.00**	**7.66**	**0.48**	**0.52**	**3.90**	**0.70**	**0.61**	**1,2**
12	**1031**	**1,8-Cineole**	**7.41**	**0.00**	**0.08**	**0.24**	**0.00**	**0.00**	**0.57**	**1,2**
13	**1037**	***cis*- β- Ocimene**	**3.09**	**2.09**	**2.27**	**0.85**	**1.14**	**1.60**	**2.56**	**1,2**
14	1050	*trans*-β- Ocimene	1.19	0.70	0.76	0.33	1.01	0.39	0.42	1,2
15	**1062**	**γ-Terpinene**	**0.61**	**0.00**	**2.52**	**8.65**	**5.72**	**7.13**	**0.00**	**1,2**
16	**1070**	***cis*-Sabinene hydrate**	**0.36**	**0.00**	**5.27**	**3.11**	**3.30**	**1.53**	**1.96**	**1,2**
17	1072	*cis*-Linalool oxide	0.00	0.00	0.99	0.17	0.07	0.00	0.06	1,2
18	1088	Terpinolene	0.28	1.73	1.24	0.54	0.39	0.15	0.25	1,2
19	**1098**	**Linalool**	**4.95**	**1.98**	**50.42**	**11.20**	**21.45**	**0.68**	**5.71**	**1,2,3**
20	1098	*trans*-Sabinene hydrate	0.05	0.00	0.32	0.28	0.04	0.01	0.10	1,2
21	1126	α-Campholenal	0.13	0.00	0.27	0.23	0.16	0.09	0.16	1,2
22	1146	Camphor	1.90	0.00	0.16	0.19	0.74	0.27	0.54	1,2
23	**1169**	**Borneol**	**3.62**	**2.53**	**1.73**	**2.65**	**1.83**	**1.04**	**2.83**	**1,2,3**
24	**1177**	**Terpinene-4-ol**	**0.95**	**0.53**	**4.65**	**3.21**	**1.99**	**0.94**	**1.69**	**1,2,3**
25	1182	p-Cymene-8-ol	0.06	0.00	0.76	0.71	0.12	0.22	0.64	1,2
26	1188	α-Terpineol	0.30	0.00	0.53	0.43	1.97	0.57	0.67	1,2
27	1199	γ-Terpineol	0.00	0.00	0.05	0.63	0.00	0.00	0.00	1,2
28	1232	3Z Hexenyl 2-methyl butanoate	0.00	0.00	0.04	0.39	0.05	0.22	0.37	1,2
29	1235	Thymol methyl ether	0.00	0.00	0.00	0.28	0.07	0.00	0.41	1,2
30	**1244**	**Carvacrol methyl ether**	**0.07**	**0.00**	**0.67**	**2.85**	**0.94**	**7.28**	**0.00**	**1,2**
31	1252	Thymoquinone	0.43	0.00	0.00	0.00	0.00	0.00	0.12	1,2
32	**1255**	***cis*-Geraniol**	**0.00**	**0.00**	**0.42**	**6.82**	**0.11**	**0.84**	**3.56**	**1,2**
33	1267	Geranial	0.08	0.00	0.03	0.59	0.11	0.00	0.19	1,2
34	1285	Bornyl acetate	0.15	0.00	0.01	0.06	1.27	0.00	0.20	1,2
35	**1290**	**Thymol**	**0.15**	**0.52**	**0.79**	**7.12**	**3.89**	**31.08**	**14.97**	**1,2,3**
36	**1298**	**Carvacrol**	**0.00**	**0.00**	**0.00**	**1.73**	**4.82**	**16.20**	**17.92**	**1,2**
37	1354	neoiso- Dihydro carveol acetate	0.28	0.71	0.12	1.53	0.20	0.06	0.20	1,2
38	1355	Thymol acetate	1.60	1.98	0.40	0.37	0.49	0.10	0.17	1,2
39	1375	Linalool isobutanoate	0.12	0.00	0.04	0.04	0.00	0.00	0.00	1,2
40	1376	α-Copaene	0.37	0.78	0.00	0.04	0.18	0.05	0.00	1,2
41	**1419**	**E-Caryophyllene**	**6.04**	**6.76**	**1.61**	**2.41**	**6.65**	**1.61**	**2.27**	**1,2,3**
42	1432	β-Copaene	0.27	0.47	0.03	0.07	0.15	0.07	0.00	1,2
43	1454	α-Caryophyllene	0.56	0.58	0.11	0.12	0.44	0.13	0.19	1,2
44	1460	Allo-Aromadendrene	0.56	0.71	0.11	0.12	0.44	0.13	0.04	1,2
45	1479	γ-Muurolene	0.22	0.00	0.02	0.05	0.34	1.20	0.00	1,2
46	**1450**	***cis*- Muurola-3,5-diene**	**0.00**	**0.00**	**0.00**	**0.00**	**3.23**	**0.00**	**0.48**	**1,2**
47	**1481**	**Germacrene D**	**7.44**	**9.38**	**1.34**	**0.95**	**0.20**	**0.27**	**0.85**	**1,2,3**
48	**1500**	**Bicyclogermacrene**	**3.05**	**2.99**	**1.28**	**0.82**	**2.39**	**0.54**	**0.50**	**1,2**
49	**1507**	**β-Bisabolene**	**0.86**	**0.71**	**0.00**	**0.45**	**2.07**	**1.12**	**0.21**	**1,2**

50	1513	γ-Cadinene	1.08	2.50	0.03	0.13	0.38	0.20	0.22	1,2
51	**1523**	**δ-Cadinene**	**1.23**	**3.22**	**0.15**	**0.10**	**0.74**	**0.32**	**0.08**	**1,2**
52	1563	*trans*-Nerolidol	0.00	0.00	0.00	0.00	1.86	0.07	0.57	1,2
53	**1578**	**Spathulenol**	**1.80**	**3.01**	**1.63**	**1.50**	**2.11**	**0.45**	**2.20**	**1,2**
54	**1583**	**Caryophyllene oxide**	**4.24**	**4.86**	**2.12**	**1.79**	**2.59**	**1.44**	**1.58**	**1,2**
55	**1590**	**Viridiflorol**	**13.66**	**9.56**	**0.06**	**0.00**	**0.33**	**0.00**	**0.37**	**1,2**
56	1601	Humulene epoxide II	1.07	1.67	0.22	0.17	0.40	0.08	0.04	1,2
57	1640	epi-α-Cadinol	0.25	0.00	0.00	0.00	0.07	0.00	0.00	1,2
58	1644	α-Muurolol	0.17	0.00	0.00	0.02	0.60	0.02	0.09	1,2
59	1646	Cubenol	0.46	1.51	0.11	0.26	0.44	0.08	0.08	1,2
60	1663	7-epi-alpha-Eudesmol	1.53	1.73	0.07	0.05	0.46	0.03	0.03	1,2
61	1670	14-Hydroxy-9-epi-(E)-caryophyllene	0.45	0.47	0.04	0.04	0.20	0.00	0.00	1,2
62	1672	Unknown 1	0.22	0.00	0.02	0.00	0.71	0.00	0.07	1,2
63	1677	Unknown 2	2.21	1.98	0.23	0.09	1.34	0.07	0.04	1,2
64	1692	Unknown 3	0.27	0.81	0.19	0.27	0.37	0.15	0.00	1,2
		Total identified	97.40	97.21	99.56	99.72	97.58	99.78	99.89	

Yield % v/w	0.07-0.14	0.02-0.09	0.40-0.70	0.32-0.50	0.20-0.43	0.84-1.02	0.78-0.85
Monoterpenes	18.74	16.38	21.41	43.98	23.29	27.51	34.14
Oxygenated monoterpenes	12.98	5.56	66.39	38.07	40.80	53.47	51.12
Sesquiterpenes	21.68	28.10	4.68	5.26	17.21	5.64	4.84
Oxygenated sesquiterpenes	23.63	22.81	4.25	3.83	9.06	2.17	4.96
Others	23.55	27.29	3.33	8.86	10.09	11.34	4.94

[a]Kovats indices calculated against a mixture of C9- C28n alkanes on the HP-5MS column. [b]Compounds are listed in order of elution from a HP-5MS column. The percentage for each population represents the mean values of n calculated samples (n=3 samples). Compounds marked in boldface (with 163 concentrations higher than 1%) were chosen for HCA and PCA statistical analyses. tr = trace < 0.1%. Id.= Peak identification mode: 1. constituent identified by comparison of mass spectra; 2. constituent identified by retention index matching; and 3. constituent identified by comparing the retention times with those of authentic constituents.

In the plant populations originating from Golesh and Morinë, the primary components were myrcene (17.4% and 21.1%, respectively) followed by viridiflorol (13.7% and 9.6%, respectively), germacrene D (7.4% and 9.4%, respectively), and E-caryophyllene (6.0% and 6.8%, respectively), which represents a special chemotype of *S. montana*. Based on a literature search, we found only one population originating from Albania (P4) that showed a quite similar chemical composition [17], with main constituents of myrcene (7.8%), β-phellandrene (6.1%), E-caryophyllene (10.8%), and germacrene D (10.4%).

The essential oils that originated from populations in Pashtrik and Koritnik were dominated by p-cymene (12.2% and 29.6%, respectively) followed by linalool (50.4% and 11.2%, respectively) and p-cymene (12.6% and 29,6%, respectively). The essential oil originating from Ulqin has a quite similar composition to those populations, in which the main constituents were p-cymene (26.1%) followed by carvacrol (17.9%), thymol (14.9%) and linalool (5.7%). An essential oil in which linalool and p-cymene were the main constituents was previously found in Serbia [24], whereas essential oils dominated by p-cymene carvacrol and thymol were previously reported from Albania [17] and Croatia [6]. Linalool (21.45%), E-caryophyllene (6.7%) and carvacrol (4.8%) were the main constituents

found in the population originating from Theth, and the linalool chemotype of *S. montana* was previously reported from populations from Serbia [19]. In Valbonë, thymol (31.1%), carvacrol (16.2%) and p-cymene (13.7%) were found in the essential oil. Thymol followed by carvacrol were previously reported as the main constituents from populations originating from Albania [16], Bosnia and Hercegovina [5] and Croatia [12]. Oxygenated monoterpenes constituted the highest percentage of all components (5.5-66.4%), followed by monoterpenes (16.4-44.0%), sesquiterpenes (4.7-28.1%), oxygenated sesquiterpenes (2.2-23.6%), other hydrocarbons and an unknown compound (3.3-27.3%). It is very obvious that plants from Valbonë and Ulcinj belong to the monoterpene aromatic polyphenol chemotype (p-cymene, thymol and carvacrol chemotype); plants from Golesh and Morinë belong to the myrcene and viridiflorol chemotype; Pashtrik is unique with its linalool chemotype; and the plants of Koritnik and Theth are intermediate chemotypes (p-cymene/linalool) that link the Pashtrik and Valbonë/Ulcinj populations.

To evaluate the variability in the chemical composition of the essential oil between plant populations originating from different geographical locations, hierarchical cluster analysis (HCA) and principal component analyses (PCA) were used as statistical tools. For statistical analyses, the oil components with concentrations higher than 1% (bold in Table 1) in the total oil were selected. The general structure of the dendrogram generated by HCA indicated the existence of four main clusters, corresponding to the chemical compositions of essential oils originating from different plant populations (Figure 1).

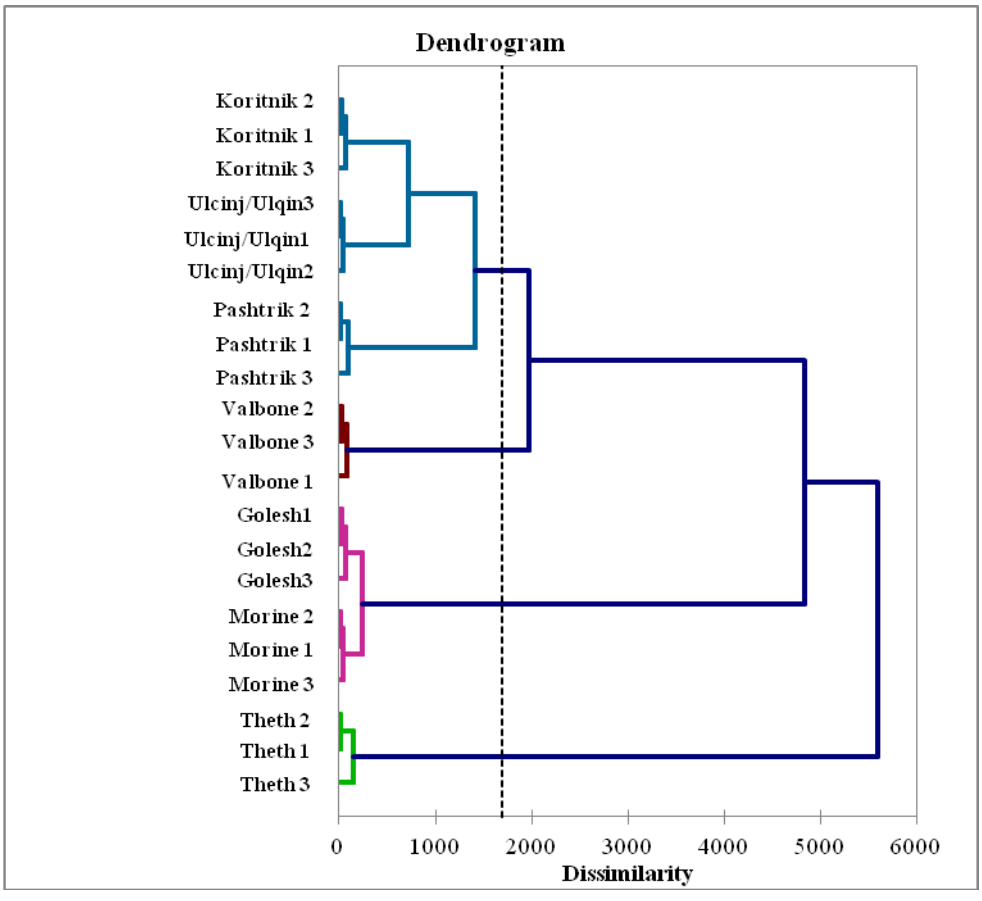

Figure 1. Two-dimensional dendrogram obtained by the cluster analysis of the essential oils of seven populations of *Satureja montana* based on the unweighted pair-group method (square Euclidean distance).

The first cluster includes the oils originating from Koritnik, Pashtrik and Ulcinj/Ulqin; the second cluster groups oils originating from Golesh and Morinë; the third cluster groups samples from Valbonë; and the fourth cluster groups samples originating from Theth. PCA confirmed this clustering

by HCA; the two-dimensional axis system of the PCA identified four groups of essential oils (Figure 2). Cis-muurola-3,5-diene, α-pinene, β-phellandrene, and E- caryophyllene were the principal components that contributed to population clustering of the plants from Theth. Spathulenol, bicyclogermacrene, γ-cadinene, caryophyllene oxide, myrcene, germacrene D and viridiflorol were the primary components that contributed to the clustering of the populations from Morinë and Golesh. The populations from Pashtrik, Koritnik and Ulcinj/Ulqin were dominated by linalool, terpinene-4-ol, cis-geraniol, p-cymene and cis-sabinene hydrate, whereas the essential oil from Valbonë was dominated by δ-terpinene, carvacrol methyl ether, thymol and carvacrol (Figure 2). Thus, the PCA results showed that the first two principal axes represented 56.3% of the total variance. The first axis (41.6% of the total variance) accounted for positive contributions of the unknown compound, caryophyllene oxide, myrcene, germacrene D, viridiflorol, 1,8-cineol, borneol and Z-β-ocimene and negative contributions of linalool, terpinene-4-ol, cis-geraniol, p-cymene and cis-sabinene hydrate. The second axis (14.7% of the total variance) was due to positive contributions of cis-muurola-3,5-diene, α-pinene, β-phellandrene, E- caryophyllene, spathulenol, bicyclogermacrene, and γ-cadinene and negative contributions of δ-terpinene, carvacrol methyl ether, thymol and carvacrol (Figure 2).

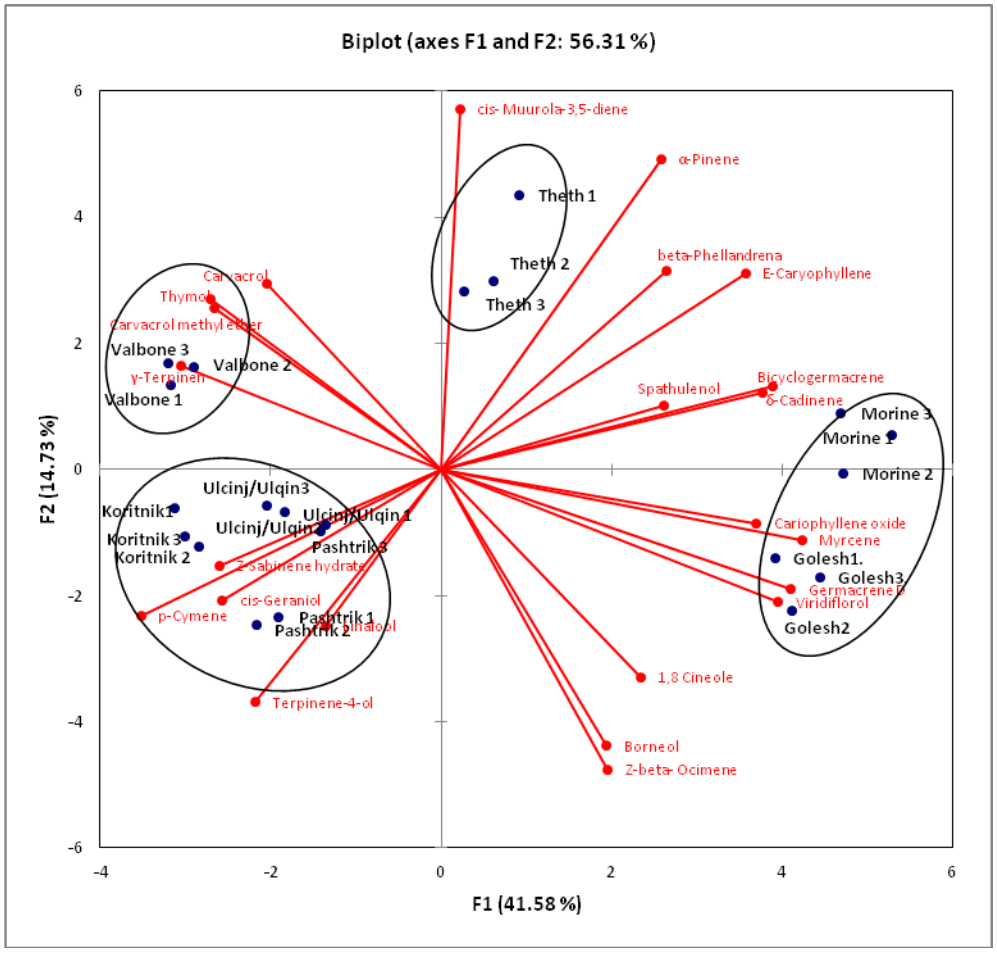

Figure 2. Principal component analysis of the oil constituents obtained from seven populations of *Satureja montana*.

The populations originating from Pashtrik, Koritnik and Ulcinj/Ulqin (linalool, p-cymene/linalool and p-cymene/carvacrol/thymol chemotypes) grouped in the first cluster include the oils of populations that are growing in locations dominated by the Mediterranean climate or impacted

by this climate. Thus, the climate condition appears to be the major factor that is responsible for the chemical composition of the essential oil. The second cluster groups samples from Golesh and Morinë (myrcene and viridiflorol chemotypes); these populations grow in a serpentine substrate, which appears to indirectly impact the content and chemical composition of the essential oil. The third cluster groups oils originating from the population collected in a limestone area of Valbona (p-cymene/carvacrol/thymol chemotype) characterized by a Continental climate, whereas the fourth cluster groups samples originating from Theth (p-cymene/linalool chemotype) characterized by a Continental climate and that also continue to grow in a limestone area. The populations originating from Valbonë and Theth have a short geographic distance between them, and although this short distance is close, they were grouped into separate groups in the PCA and HCA graphs (Figure 1 and Figure 2) because these populations are separated by high mountains.

The variability in the chemical compositions of essential oils among populations of *S. montana* appears to reflect the environmental impact on the composition, which is influenced by differences in habitat composition, altitude and microclimatic conditions.

3.2. Total Flavonoid and Total Phenolic Contents and Antioxidant Activity

The results (mean value and standard deviation) of total flavonoids, total phenolics and antioxidant activity (DPPH and FRAP) are presented in Table 2. The concentration of total phenolics ranged from 68.1 to 102.6 mg caffeic acid equivalent/g of plant dry mass. The highest concentration was found in Theth, whereas the lowest was found in Valbonë; significant differences were found between most of the analysed populations (Table 2). Compared with other plant species of the Lamiaceae family from Kosovo, *S. montana* generally has a higher content of total phenolics than leaves of *Stachys sylvatica* (44.3-77.8 mg CAE /g dm) [26] and leaves of *Betonica officinalis* (74.9-80.8 CAE mg/g dm) [27].

The total flavonoid concentration ranged from 38.3 to 67.0 mg catechin equivalent/g of plant dry mass. The highest concentration was found in Theth, whereas the lowest was found in Valbonë; significant differences were found between most of the analysed populations (Table 2). In general, methanolic extracts of *S. montana* have a more or less similar content of total flavonoids compared with other species of the Lamiaceae family, such as leaves of *Stachys sylvatica* (38.8-70.6 mg CAE /g dm) [26] and leaves of *Betonica officinalis* (74.9-80.8 CA mg/g dm) [27].

The methanolic extracts of *S. montana* exhibited different degrees of DPPH radical scavenging capacity (37.6-51.8% inhibition). In particular, the plant material originating from Theth exhibited the highest antioxidant activity compared with the antioxidant activity of the population; no significant differences were found between the analysed populations (Table 2). The methanolic extracts of *S. montana* show a more or less similar DPPH radical scavenging capacity compared with other species of the Lamiaceae family, such as the leaves of *Stachys sylvatica* (37.2-57.2%) [26] and leaves of *Betonica officinalis* (38.4-53.5%) [27].

Table 3. Mean Values and Standard Deviations of Total Flavonoids, Total Phenolics, DPPH and FRAP Antioxidant Activity in Leaves and Inflorescence of *S. montana*.

Localities	Total flavonoid mg CE/g dm	Total phenolics mg CAE /g dm	FRAP mg TE/g dm	DPPH mg TE/g dm	DPPH % inhibition
Theth	67.0±12.7^{a}	102.6±26.9^{a}	11.4±1.2^{a}	342.9±92.4^{a}	51.8±14.6^{a}
Morinë	54.6±3.0^{ab}	77.4±4.4^{ab}	10.2±0.7^{ab}	320.2±28.1^{a}	48.2±4.4^{a}
Golesh	51.1±3.7^{bc}	88.9±6.1^{ab}	9.4±0.3^{b}	316.0±30.3^{a}	47.5±4.8^{a}
Pashtrik	50.5±4.1^{bc}	75.3±10.0^{a}	9.5±0.6^{b}	302.5±20.4^{a}	45.4±3.2^{a}
Koritnik	41.7±6.4^{bc}	70.5±4.1^{b}	8.9±0.7^{b}	253.3±16.4^{a}	37.6±2.5^{a}
Ulcin	39.9±2.8^{bc}	69.9±13.7^{b}	9.7±0.5^{ab}	268.2±40.8^{a}	39.9±6.4^{a}
Valbonë	38.3±8.1^{c}	68.1±5.4^{b}	10.0±1.2^{ab}	332.6±16.6^{a}	50.1±2.6^{a}

Different subscript letters within the same column indicate significant differences ($P< 0.05$ according to Tukey's HDS test) of means between localities.

As shown in Table 2, the highest FRAP antioxidant capacity was found in the population collected in Theth, whereas the lowest was found in the population collected in Koritnik (8.9-11.4 mg TE/g dm). Significant differences were found between most of the analysed populations (Table 2). Compared with the FRAP antioxidant capacity of the leaves of *Betonica officinalis* (40.1-59.5 mg TE/g dm) [27] and *S. sylvatica* (95.2-103.4 mg TE/g dm) collected in Kosovo [26], *S. montana* exhibits lower antioxidant activity.

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