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Altitude Variation in Volatile Composition of Blueberry Leaf Analyzed by SPME GC-MS

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ABSTRACT

Background: The present work was aimed to carry out volatile component analyzes in *Vaccinium arctostaphlyos* L., *V. uliginosum* L., *V. vitis-idaea* L. and *V. myrtillus* L leaf growing at different altitudes of the East Blacksea Region of Turkey. **Methods:** The leaf of Vaccinium species were harvested from twenty-one different altitudes (748-3035 m) from six cities (Artvin-Ardahan-Rize-Trabzon-Gümüşhane-Giresun) of Turkey. The diversity of volatiles in the leaf was investigated by SPME GC-FID/MS. **Results:** The major constituents of the Vaccinium leaf showed variation with changes in altitudes. The identified volatile components of *V. arctostaphlyos* and *V. uliginosum* were represented mainly by aldehydes in all altitudes, whereas monoterpenes were found the major constituent of *V. vitis-idaea* at Posof-Ardahan (2376 m) and Artvin (2553 m) samples. In all altitudes, capronaldehyde (7.23-28.96%) and 2(E)-hexenal (8.90-53.59%) in the leaf of *V. arctostaphlyos*; capronaldehyde (17.04-37.09%) and limonene (16.50-47.51%) in the leaf of *V. vitis-idaea* and capronaldehyde (4.55-39.90%), 2(E)-hexenal (25.08-80.99%) and hexadecane (2.97-11.32%) in the leaf of *V. uliginosum*; and capronaldehyde (14.66-37.26%) and 2(E)-hexenal (18.18-37.59%) at the altitudes of 1912m, 2533 m and 2565 m in the leaf of *V. myrtillus* were the major constituents with the different percentages, respectively. 2-Bornanone (32.86%) at the altitude of 2613 m and 3-penten-2-one (93.60%) at the altitude of 2811m in the leaf of *V. myrtillus* were found to be the major compounds. **Conclusion:** Comparisons of the volatile components of *V. arctostaphlyos*, *V. uliginosum*, *V. vitis-idaea* and *V. myrtillus* in different sites showed significant differences among populations at different altitudes.

Key words: *Vaccinium arctostaphlyos, V. uliginosum, V. vitis-idaea, V. myrtillus*, SPME GC-FID/MS.

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INTRODUCTION

The genus *Vaccinium* L. (Ericaceae) is represented approximately with 450 species of shrubs or small trees worldwide.[1] Four of the species (*V. arctostaphlyos* (*Va*), *V. uliginosum* (*Vu*), *V. vitis-idaea* (*Vvi*) and *V. myrtillus* (*Vm*)) occur in Turkey and these are mostly distributed in North-East and North-West Anatolia.[2] *Vaccinium* is a medicinal plant that leaf consist on deciduous or evergreen and berries are vary from blue, purple, black or red. Berries can be eaten fresh; leaf can be dried and steeped. Also *Vaccinium* leaf, flower, berries and rhizomes are more usually processed.[3] The genus is rich sources of dietary anthocyanins and antioxidants. Leaf and berries are traditionally used in for the treatment of diabetes[4,5] and also including antimicrobial, antiinflammatory and antimutagenic properties.[6,7] *Vaccinium* species are an industrial medicinal plant with various pharmaceutical and nutritional applications. Major constituents of the essential oil of *Vaccinium* species were mentioned to be as; α-terpineol (14.99%) and linalool (13.7%) for *Va;*[8] nerolidol (20.01%), (*Z,Z,Z*)-1,5,9,9-tetramethyl-1,4,7-cycloundecatriene (17.99%) and caryophyllene (9.59%) for *Vaccinium bracteatum* Thumb.[9] α-terpineol (4.7%) and linalool (17.0%) for *Vvi;*[10] α-pinene (15.5%), linalool (11.7%), sandaracopimaradiene (5.9%) and safranal (8.8%) for *V. arctostaphylos;*[11] diisobutyl phthalate for *Vvi* (17.96%);[12] decahydro dimethylnaphthalene (47.04%), linalool (3.19%), 3,7-dimethyl-1,5,7-octatrien-3-ol (1.40%) and benzene acetaldehyde (1.03%) for *Vaccinium dunalianum* var. *dunalianum* (C. B. Clarke) Ridley;[13] α-pinene for *Vaccinium angustifolium* Aiton, (23%) and *Vaccinium arboreum* Marsh., (63%), methyl benzoate for *Vaccinium varingiaefolium* Miq., (18%) and ethyl benzene for *Vaccinium poasanum* Donn. Sm. (21%);[14] and butyl butanoate, *cis*-3-hexen-1-ol, α-terpineol, geraniol and *trans*-2-hexenal for *Vaccinium corymbosum* L.[15] HS SPME GC-MS analysis for fruit juice of *Vm* were mentioned.[16] The complexity of blueberry (*Vm*) aroma was explored by SPME GC-MS and VOCs were reported as aldehydes, alcohols, terpenoids and esters.[17] Volatile compounds, the antifungal activity and the phytotoxic activity of essential oil of *Vm* were also reported.[18,19] In the literature, polar metabolites of leaf of *Vm* and *Vvi* were also studied by HPLC-DAD, HPLC-MS and GC-FID,[20] and the extracts of entire fruits and leaf of *Vm* collected in Finland and Poland were quantitated by GC-FID/MS and the main bilberry constituents were given as α- and β-amyrin, α- and β-amyrenone, campesterol, cholesterol and citrostadienol.[21] However, to the best of our knowledge of literature survey, no data about altitude variation in volatile composition of *Va*, *Vu, Vvi* and *Vm* leaf analyzed by SPME GC-MS have been reported up to date. The present study aim to test whether there is any altitude effect on volatile compounds of *Va*, *Vu, Vvi* and *Vm* leaf growing spontaneously in different altitudes in the East Blacksea region of Turkey.

MATERIALS AND METHODS

Plant materials

Va, *Vu, Vvi* and *Vm* leaf (25 g, wet) were harvested from different altitudes in the East Blacksea Region (Artvin-Ardahan-Rize-Trabzon-Gümüşhane-Giresun) of Turkey (Table 1) and were identified.[2,3]

Vouchers were deposited in the Herbarium of Biology (KTUB) at Karadeniz Technical University, Turkey (Table 1).

Solid Phase Micro Extraction (SPME)

The blended leaf (2 g) were place to a sealed SPME vial (10 mL) with a silicone-rubber septum cap then submitted to solid-phase micro extraction device (Supelco, USA). A DVB/Carboxen/PDMS coating fiber was used to obtain volatile components. The SPME fibers were conditioned for 5 min at 250°C in the GC injector. Extraction were achieved with magnetic stirring at 80°C using an incubation time of 5 min and an extraction time of 10 min. Fiber with extract of volatile compounds were subsequently injected into the GC injector. Each sample was analyzed and reported. Conditioning time for subsequent assays was set at 4 min of desorption after each extraction. The temperature, incubation and extraction times were set according to the reported experiment.[22-24]

Gas chromatography-Mass spectrometry (GC-FID/MS)

The gas chromatography-flame ionization detector (GC-FID) analysis was carried out on a Shimadzu QP2010 plus gas chromatography equipped with a flame ionization detector (FID) using a Rtx-5MS capillary column (30 m x 0.25 mm, film thickness, 0.25 µm). Shimadzu QP2010 Plus gas chromatograph was coupled to a Shimadzu QP2010 Ultra mass selective detector. The injection was performed in split mode (1:30) at 230°C. The column held initially at 60°C for 2 min and then increased to 240°C with a 3°C/min heating ramp. The oven program was as follows: the initial temperature was 60°C for 2 min, which was increased to 240°C at 3 min, the final temperature of 250°C was held for 4 min. Helium (99.999 %) was used as carrier gas with a constant flow-rate of 1 mL/min. Detection was implemented in electronic impact mode (EI); ionization voltage was fixed at 70 eV, scan mode (40-450 *m/z*) was used for mass acquisition.[22-25] Each sample was analyzed and mean reported.

Identification of Volatile Constituents

Retention indices of the volatile components of leaf belong to four *Vaccinium* species were determined by Kovats method using *n*-alkanes (C_6-C_{32}) as standards.[26] Volatile compounds were identified by comparing mass spectra and Kovats' Retention Indices with those in literature[22-31] and by computer searching followed by matching the mass spectra data with those held in computer library (NIST, Wiley7NL, FFNSC1.2 and W9N11).

RESULTS

The volatile compounds of leaf belong to four *Vaccinium* species collected from different altitudes in the East Blacksea Region of Turkey were analyzed by SPME GC-FID/MS. A total of 16, 23, 13, 21, 16,

Table 1: Examined species, voucher numbers, collection data and localities of the Vaccinium species in Turkey

Sample	Harvested localities	Altitude (m)	Harvested date	Collection number[a]
Vaccinium arctostaphylos (*Va*)				
*Va*1	A8 Rize: Ardeşen, Işıklı village, path route, forestry region	748	19 viii 2017	C&Y-46
*Va*2	A8 Rize: Ardeşen, Siprona plateau, forestry regions	1032	19 viii 2017	C&Y-47
Va3	A7 Trabzon: Araklı, Köyiçi village, forestry regions	1443	06 vii 2017	C&Y-15
*Va*4	A7 Trabzon: Düzköy, Çayırbağı, Kale river, forestry regions	1516	06 vii 2017	C&Y-21
*Va*5	A7 Giresun: Doğankent, Harmancık plateau, forestry regions	1666	02 vii 2017	C&Y-14
*Va*6	A7 Gümüşhane: Kürtün above plateau, forestry regions	1845	01 vii 2017	C&Y-1
*Va*7	A8 Artvin: Şavşat, Pınarlı plateau, forestry regions	2105	07 ix 2018	C&Y-117
*Va*8	A8 Artvin: Murgul, Tiryol mountain, forestry regions	2234	10 vii 2018	C&Y-91
Vaccinium uliginosum (*Vu*)				
*Vu*1	A8 Artvin: Şavşat, Karagöl National Park, alpine meadows	2530	02 vii 2018	C&Y-95
*Vu*2	A8 Rize: İkizdere, Anzer plateau, alpine meadows	2537	02 ix 2018	C&Y-108
*Vu*3	A9 Ardahan: Hanak, Alabalık plateau, alpine meadows	2588	08 vii 2018	C&Y-101
*Vu*4	A7 Trabzon: Çaykara, Multat plateau, alpine meadows	2811	20 vi 2018	C&Y-93
*Vu*5	A8 Rize: İkizdere, Ovit Lake, alpine meadows	3035	20 viii 2017	C&Y-61
Vaccinium vitis-idaea (*Vvi*)				
*Vvi*1	A9 Ardahan: Hanak, Alabalık plateau, alpine meadows	2362	03 vii 2018	C&Y-103
*Vvi*2	A9 Ardahan: Posof, Sesödile Hill, alpine meadows	2376	07 x 2017	C&Y-75
*Vvi*3	A8 Artvin: Şavşat Karagöl National Park, alpine meadows	2533	06 x 2017	C&Y-69
Vaccinium myrtillus (*Vm*)				
*Vm*1	A7 Gümüşhane: Kürtün, Süme Village, alpine meadows	1917	01 vii 2017	C&Y-5
*Vm*2	A8 Artvin: Şavşat, Kirazlı Village, alpine meadows	2533	02 vii 2018	C&Y-98
*Vm*3	A8 Rize: Ardeşen, Fındıklı Plateau, alpine meadows	2565	19 viii 2017	C&Y-52
*Vm*4	A9 Ardahan: Hanak, Alabalık Plateau, alpine meadows	2613	08 x 2018	C&Y-102
*Vm*5	A7 Trabzon: Çaykara, Multat Plateau, alpine meadows	2811	20 vi 2018	C&Y-94

[a]C&Y: Coşkunçelebi and Yılmaz.

12, 14 and 17 volatile compounds were identified from the leaf of *Va*, which were collected from different altitudes in Rize-Rize-Trabzon-Trabzon-Giresun-Gümüşhane-Artvin-Artvin, respectively. Aldehydes, aliphatic hydrocarbons and aromatics were the largest group of volatiles, accounting for 44.61-77.06%, 8.59-22.01% and 3.42-23.19% of total volatiles, respectively (Table 2). In all altitudes, capronaldehyde (7.23-28.96%), 2(E)-hexenal (8.90-53.59%) and nonanal (4.24-10.03%) were generally major compounds in the leaf of *Va*.

A total of 6 to 8 volatile constituents were characterized from the leaf of *Vu*, which were harvested from different altitudes in Artvin-Rize-Ardahan-Trabzon-Rize, respectively (Table 3). Aldehydes and aliphatic hydrocarbons were the major group of volatiles in the leaf of *Vu*, accounting for 50.92-86.95% and 8.87-30.21% of total volatiles and capronaldehyde (4.55-39.90%), 2(*E*)-hexenal (25.08-80.99%) and hexadecane (2.97-11.32%) were the major constituents, respectively.

The SPME analysis of *Vvi* leaf led to the identification of 6, 11 and 4 constituents and the major compounds were capronaldehyde (17.04-37.09%), α-terpinene (7.40-9.60%), limonene (16.50-47.51%) and terpinolene (7.45-8.70%), respectively (Table 4). Leaf collected from 2533 m altitude afforded limonene (47.51 %) as a major constituent

Table 2: Identified VOCs from the leaf of V. arctostaphylos (Va) growing at different altitudes in Turkey.

No	Compounds	RI*	RI[a]	*Va*1 748 m	*Va*2 1032 m	*Va*3 1443 m	*Va*4 1516 m	*Va*5 1666 m	*Va*6 1845m	*Va*7 2105 m	*Va*8 2234 m
				(%)[b]							
1	Ethyl vinyl ketone	687	694	-	3.40	6.33	3.31	-	-	8.15	5.50
2	Pentanal	704	701	-	-	-	-	3.68	-	3.19	8.00
3	2-Ethyl furan	720	704	5.16	2.68	-	-	-	-	-	-
4	2(*E*)-Pentenal	756	755	-	3.46	4.78	3.49	-	-	-	-
5	Capronaldehyde	803	802	19.32	20.19	15.19	18.13	17.13	31.18	7.23	28.96
6	2(*E*)-Hexenal	855	854	10.4	8.90	27.50	16.26	11.01	10.70	53.59	9.30
7	Heptanal	906	903	4.17	4.38	-	-	-	-	2.23	3.78
8	Matyl caproate	925	924	-	1.55	-	-	-	-	-	-
9	2(*E*)-Heptenal	954	958	-	-	-	-	-	-	-	2.07
10	6-Methyl-5-hepten-2-on	981	989	9.75	3.89	-	6.66	8.76	13.50	0.96	4.06
11	2-Pentyl furan	994	994	5.30	4.30	-	5.08	7.39	4.88	3.42	5.43
12	Decane	1000	1000	-	8.30	-	-	-	-	-	-
13	Octanal	1002	1005	-	5.40	7.00	6.94	4.59	4.15	3.37	-
14	Limonene	1031	1030	-	3.02	-	0.52	-	-	-	4.46
15	2,2,6-Trimethyl cyclohexanone	1035	1041	-	-	-	-	-	-	-	3.12
16	α-Terpinene	1054	1059	-	-	-	1.96	-	-	-	-
17	(*E*,*E*)-3,5-Octadien-2-one	1068	1074	-	-	-	3.19	-	-	-	-
18	Linalool oxide	1073	1078	-	-	-	-	0.67	-		-
19	Linalool	1095	1102	-	-	4.36	-	2.75	3.56	-	-
20	Nonanal	1100	1106	6.79	7.58	10.03	7.14	8.26	8.53	5.81	4.24
21	2,6-Dimethylcyclohexanol	1110	1116	-	-	-	0.78	0.35	-	-	-
22	2-Allylphenol	1191	1195	6.78	3.92	11.81	5.25	15.8	9.47	-	6.97
23	Dodecane	1200	1196	-	0.92	-	-	-	-	-	-
24	Decanal	1201	1208	3.60	3.14	4.23	-	3.57	3.80	1.64	-
25	β-Cyclocitral	1224	1231	1.04	1.51	-	3.42	-	-	-	2.87
26	Vitispirane	1286	1290	5.57	-	-	-	-	-	-	-
27	Tetradecane	1400	1400	2.98	1.90	-	2.69	2.63	-	2.30	1.84
28	Geranylacetone	1453	1458	-	-	-	1.09	1.74	1.75	-	-
29	β-Ionone	1489	1496	-	-	-	1.31	-	-	-	-
30	Pentadecane	1500	1500	3.19	1.63	1.94	3.08	3.12	-	2.00	4.17
31	Dihydroactinidiolide	1537	1548	-	1.06	0.17	-	-	-	-	-
32	Hexadecane	1600	1600	5.09	3.50	3.99	5.25	5.22	4.82	3.28	2.43
33	Heptadecane	1700	1700	-	-	-	2.81	3.30	-	-	-
34	Pristane	1707	1706	8.00	4.30	2.66	1.48	-	3.65	2.76	2.73
35	Methyl myristate	1726	1728	-	1.03	-	-	-	-	-	-

36	Octadecane	1800	1800	2.85	-	-	-	-	-	-	-
			Chemical Class;				**%[b] and NC[c]**				
			Monoterpene:	-	3.02:1	-	2.48:2	-	-	-	7.58:2
			Monoterpenoid:	1.04:1	1.51:1	4.36:1	3.42:1	3.42:2	3.56:1	-	-
			Aldehyde:	44.61:5	53.0:7	68.73:6	51.94:5	48.24:6	58.36:5	77.06:7	56.25:6
			Alcohol:	-	-	-	0.78:1	-	-	-	-
			Hydrocarbon:	22.01:5	20.55:6	8.59:3	16.4:6	16.01:5	10.22:3	10.34:4	11.17:4
			Aromatics:	17.14:3	14.76:3	11.81:1	10.33:2	23.19:2	14.35:2	3.42:1	12.4:2
			Ester:	-	2.58:2	-	-	-	-	-	-
			Ketone:	9.65:1	3.85:3	6.50:2	13.16:3	8.76:1	13.50:1	9.11:2	12.58:3
			Other:	5.47:1	-	-	1.31:1	-	-	-	-
			Total:	99.92:16	99.32:23	99.99:13	99.82:21	99.62:16	99.99:12	99.90:14	99.90:17

*Retention Index of references; [a]Retention Index calculated from retention times relative to that of n-alkane (C_6-C_{32}) series; [b]Percentages obtained by FID peak-area normalization; [c]NC: number of compounds; *V. arctostaphylos* species growing at different altitudes in Turkey: *Va*1: 748 m, Ardeşen-Rize; *Va*2: 1032 m Ardeşen-Rize; *Va*3: 1443 m, Araklı-Trabzon; *Va*4: 1516 m, Düzköy-Trabzon; *Va*5: 1666 m, Doğankent-Giresun; *Va*6: 1845 m Kürtün-Gümüşhane; *Va*7: 2105 m, Şavşat-Artvin; *Va*8: 2234 m, Murgul-Artvin.

Table 3: Identified VOCs and chemical class from the leaf of V. uliginosum(Vu) growing at different altitudes in Turkey.

No	Compounds	RI*	RI[a]	*Vu*1 2530 m	*Vu*2 2537 m	*Vu*3 2588 m	*Vu*4 2811 m	*Vu*5 3035 m
						(%)[b]		
1	Pentanal	704	701	-	0.85	9.24	-	8.06
2	2-Ethyl furan	720	724	-	0.40	-	-	-
3	2(*Z*)-Pentenol	765	768	-	3.34	-	-	-
4	Capronaldehyde	803	802	11.67	4.55	11.18	39.90	23.16
5	4-Hydroxy-4-methyl-2-pentanone	844	845	17.47	-	20.45	-	-
6	2(*E*)-Hexenal	855	854	46.41	80.99	30.55	25.08	42.27
7	6-Methyl-5-hepten-2-on	981	989	-	-	-	4.81	13.27
8	Nonanal	1100	1106	4.51	-	-	-	0.48
9	Tetradecane	1400	1400	-	2.74	10.43	9.01	-
10	Pentadecane	1500	1500	-	2.56	-	-	2.37
11	Hexadecane	1600	1600	11.32	2.97	9.58	10.14	5.59
12	Heptadecane	1700	1700	8.60	-	8.55	11.06	4.78
	Chemical Class;					%[b] and NC[c]		
	Aldehyde:			62.59:3	86.95:3	50.92:3	64.88:2	73.97:4
	Alcohol:			-	3.44:1	28.56:3	-	-
	Aliphatic hydrocarbon:			19.92:2	8.87:3	20.45:1	30.21:3	12.74:3
	Aromatics:			-	0.60:1	-	-	-
	Ketone:			17.45:1	-	-	4.81:1	13.27:1
	Total:			99.96:6	99.86:8	99.93:7	99.90:6	99.98:8

*Retention Index of references; [a]Retention Index calculated from retention times relative to that of n-alkane (C_6-C_{32}) series; [b]Percentages obtained by FID peak-area normalization; [c]NC: number of compounds; *V. uliginosum* growing at different altitudes in Turkey: *Vu*1: 2530 m, Artvin; *Vu*2: 2537 m, Rize-Anzer; *Vu*3: 2588 m, Ardahan; *Vu*4: 2811 m, Trabzon; *Vu*5: 3035 m, Rize-Ovit.

Table 4: Identified VOCs and chemical class from leaf of V. vitis-ideae (Vvi) growing at different altitudes in Turkey.

No	Compounds	RI*	RI[a]	*Vvi*1 2362 m	*Vvi*2 2376 m	*Vvi*3 2533 m
					(%)[b]	
1	Ethyl vinyl carbinol	684	689	18.13	-	-
2	Pentanal	704	701	14.60	3.90	-
3	Acetoin	711	710	-	3.05	-
4	Capronaldehyde	803	802	37.09	17.04	34.18
5	2(*E*)-Hexenal	855	854	9.64	-	-
6	**α**-Terpinene	1014	1021	-	7.40	9.60
7	Limonene	1031	1030	16.50	30.75	47.51
8	**γ**-Terpinene	1054	1059	-	4.60	-
9	Terpinolene	1086	1094	-	7.45	8.70
10	Linalool	1095	1102	-	6.23	-
11	Nonanal	1100	1106	4.01	1.94	-
12	**α**-Terpineol	1186	1192	-	14.75	-
13	Pentadecane	1500	1500	-	2.90	-
	Chemical Class;				%[b] and NC[c]	
	Monoterpene:			16.50:1	50.15:4	65.81:3
	Monoterpenoid:			-	20.98:2	-
	Aldehyde:			65.34:4	22.88:3	34.18:1
	Aliphatic hydrocarbon:			-	2.9:1	-
	Ketone:			18.13:1	-	-
	Others:			-	3.05:1	-
	Total:			99.97:6	99.96:11	99.99:4

*Retention Index of references; [a]Retention Index calculated from retention times relative to that of n-alkane (C_6-C_{32}) series; [b]Percentages obtained by FID peak-area normalization; [c]NC: number of compounds; *V. vitis-ideae* growing at different altitudes in Turkey: *Vvi*1: 2362 m Ardahan-Hanak; *Vvi*2: 2376 m Ardahan-Posof; *Vvi*3: 2533 m Artvin.

whereas a less percentage of limonene (16.50%) was found from other altitude (2362 m). The capronaldehyde was obtained in a high amount form the leaf collected at 2362m and 2533 m altitude. In all three sites, the monoterpene of the *Vvi* leaf collected from the high altitude was higher compared with those collected from the low altitude.

Volatile component analyzes of *Vm* leaf collected from different elevations (1917, 2533, 2565, 2613 and 2811 m) of the Black Sea region were performed by SPME GC-MS. A total of 5, 7, 12, 6 and 6 compounds were identified, respectively (Table 5). The most volatile compounds were illuminated in the sample collected from the 2565 m altitude. The main volatile constituents of *Vm* at 1917 m, 2533 m and 2565 m altitudes were capronaldehyde and 2(*E*)-hexenal. Aldehydes were the main class compounds, except samples harvested at 2613 m and 2811m altitude, monoterpene (39.4%) and ketone (95.27%) was the major class of compounds, respectively. The ratio of capronaldehyde and 2(*E*)-hexenal were decreased up to high altitudes.

DISCUSSION

In this study, the volatile component analysis of *Vaccinium* species depending on different altitudes, aldehyde compounds were found to be the main component in different proportions. In the literature, the essential oil constituents of the flowering aerial parts of *Va* were analyzed by GC-MS and twenty-six compounds, constituting 80.43% of the oil, were identified. The major compounds were *α*-terpineol (14.99%) and linalool (13.7%).[8] The volatile oil from *V. bracteatum* leaf was analyzed by GC-MS and 49 constituents were reported, which represented about 91.94% of the total content. The main chemical compounds were terpenes (54.75%), open chain alkanes (6.93%), cycloparaffins (20.1%), olefins (0.60%), aromatics (1.60%), phenol, ether and alcohol (3.39%), aldehyde and ketone (2.14%), ester (1.15%), heterocyclic compounds (0.47%) and amines (0.78%). Nerolidol (20.01%), (*Z,Z,Z*)-1,5,9,9-tetramethyl-1,4,7-cycloundecatriene (17.99%) and caryophyllene (9.59%) were the main components in the identified terpenes.[9] The GC/MS analyses of the essential oils from dry leaf *Vv i* were predominant to be terpenoids, fatty acids, fatty acid- and carotenoid-derived compounds and *α*-terpineol (4.7%) and linalool (17.0%).[10] The chemical constituents of blueberry seed oil was analyzed by GC-MS and a total of 34 constituents were reported. The contents in the volatiles were menthyl isovalerate (30.06%), 9,12-octadecadien-1-ol (11.82%), Z-7-tetradecenal (9.77%), *γ*-sitosterol (6.04%), *β*-sitosterol (4.74%), phenylethyl alcohol (4.72%), Z-(13,14-epoxy)tetradec-11-en-1-ol acetate (4.30%), retinal (4.07%), 1-(+)-ascorbic acid, 6-dihexadecanoate (3.93%), 9,12-octadecadienoic acid, ethyl ester (3.10%) and 1,6,10,14,18,22-tetracosahexaen-3-ol (3.00%).[32] The chemical compounds of the essential oils obtained from by hydrodistillation from the flowering aerial parts of *Va* was assessed by GC/MS analysis. *α*-Pinene (15.5%), linalool (11.7%), sandaracopimaradiene (5.9%) and safranal (8.8%) were mentioned to be major constituents.[11] The volatile components of *Vvi* juice were analyzed by HS SPME GC-MS and 41 volatile components were detected. The major volatile compound in *Vvi* juice was reported as diisobutyl phthalate, accounting for 17.96% of total aromatic

Table 5: Identified VOCs and chemical class from leaf of *V. myrtillus* (Vm) growing at different altitudes in Turkey.

No	Compounds	RI*	RI[a]	*Vm*1 1912 m	*Vm*2 2533 m	*Vm*3 2565 m	*Vm*4 2613 m	*Vm*5 2811 m
						%[b]		
1	Ethyl vinyl carbinol	684	689	-	-	-	-	4.22
2	3-Penten-2-one	733	739	9.44	-	14.52	93.60	-
3	Capronaldehyde	803	802	20.26	11.50	37.26	1.01	14.66
4	4-Hydroxy-4-methyl-2-pentanone	844	845	19.94	-	-	1.67	-
5	2(*E*)-Hexenal	855	854	37.59	15.00	18.18	1.31	34.13
6	α-Pinene	932	938	-	12.55	-	-	
7	1-Octen-3-ol	976	979	-	-	-	-	2.70
8	6-Methyl-5-hepten-2-on	981	989	3.86	-	13.82	-	9.36
9	2-Pentyl furan	994	994	-	-	-	-	1.65
10	*p*-Cymene	1020	1029	-	7.12	-	-	-
11	Limonene	1031	1030	-	19.99	-	-	-
12	2,2,6-trimethyl Cyclohexanone	1035	1041	-	-	-	-	4.94
13	Nonanal	1100	1106	8.88	-	2.87	-	2.58
14	2-Bornanone	1161	1154	-	32.86	-	-	-
15	β-Cyclocitral	1224	1231	-	-	-	0.71	2.76
16	Vitispirane	1286	1290	-	-	-	1.30	-
17	Tridecane	1300	1299	-	-	-	-	6.60
18	Tetradecane	1400	1400	-	-	-	-	8.03
19	Hexadecane	1600	1600	-	-	8.70	-	4.57
20	Heptadecane	1700	1700	-	-	4.61	-	3.75
	Chemical Class;					%[b] and NC[c]		
	Monoterpene:			-	39.4:3	-	-	-
	Monoterpenoid:			-	32.9:1	-	0.71:1	2.76:1
	Aldehyde:			66.73:3	27.6:2	58.31:3	2.32:2	51.37:3
	Alcohol			-	-	-	-	2.70:1
	Aliphatic hydrocarbon:			-	-	13.31:2	-	22.95:4
	Ketone:			33.24:3	-	28.34:2	95.27:2	18.52:3
	Others:			-	-	3.05:1	1.3:1	-
	Total:			99.97:6	99.99:6	99.96:7	99.96:6	99.95:12

*Retention Index of references; [a]Retention Index calculated from retention times relative to that of n-alkane (C_6-C_{32}) series; [b]Percentages obtained by FID peak-area normalization; [c]NC: number of compounds; *V. myrtillus* species growing at different altitudes in Turkey: *Vm*1: 1917m, Gümüşhane-Kürtün,; *Vm*2: 2533m, Artvin-Şavşat,; *Vm*3: 2565m, Rize-Ardeşen,; *Vm*4: 2613m, Ardahan-Hanak,; *Vm*5: 2811m,Trabzon-Çaykara.

compounds.[12] The volatile components from the fresh leaf buds of *V. dunalianum* var. *dunalianum* were mentioned. 27 Compounds has been identified and decahydro dimethylnaphthalene (47.04%), linalool (3.19%), 3,7-dimethyl-1,5,7-octatrien-3-ol (1.40%) and benzene acetaldehyde (1.03%) were the main compounds.[13] The floral volatile compounds of *V. angustifolium, V. varingiaefolium, V. arboreum* and *V. poasanum* has been provided. A total of 40 from *V. angustifolium*, 34 for *V. varingiaefolium*, 37 for *V. arboreum* and 17 for *V. poasanum* volatile compounds were detected in the headspace surrounding flowers. The most abundant volatile compounds for the each species was reported to be *α*-pinene for *V. angustifolium* (23%) and *V. arboreum* (63%), methyl benzoate for *V. varingiaefolium* (18%) and ethyl benzene for *V. poasanum* (21%).[14] Five volatile compounds consisting of butyl butanoate, *cis*-3-hexen-1-ol, *α*-terpineol, geraniol and 2(*E*)-hexenal has been found from *V. corymbosum* using GC-MS.[15] *Vm* juice has been analyzed by HS SPME GC-MS and altogether, 56 volatile compounds were identified.[16] The complexity of *Vm* aroma has been explored by SPME GC-MS. This comprehensive characterization of aroma allowed the identification of volatile organic compounds, for the most aldehydes, alcohols, terpenoids,and esters.[17] A study has been conducted to determine the antifungal and the phytotoxic activity of *Vm* plant essential oil.[18] In another work, volatile compounds and the antifungal activity of Bilberry essential oil of *Vm* were mentioned and 22 components were identified as mainly 1,8-cineole (41.07%), *β*-linalool (12.72%), *α*-pinene (12.17%) and myrtenol (6.48%).[19] Polar metabolites of leaf of *Vm* and *Vvi* has been studied by HPLC-DAD, HPLC-MS and GC-FID. Chlorogenic acid was the major phenolic compounds in bilberry leaf and arbutin in lingonberry leaf. Flavonol glycosides were another major group of phenolic in bilberry [5-28 mg/g DM (dry mass)] and lingonberry (15-20 mg/g DM) leaf.[20] The extracts of entire fruits and leaf of *Vm* collected in Finland and Poland has been quantitated by GC-FID/MS and the

main bilberry constituents were found to be α- and β-amyrin, α-and β-amyrenone, campesterol, cholesterol and citrostadienol.[21]

CONCLUSION

Vaccinium species is one of the industrial medicinal plant that is traditionally used for the treatment of diabetes throughout the world. VOCs composition of the *Va*, *Vu, Vvi* and *Vm* leaf has been analyzed. Comparisons based on the mean relative amount of the volatile components in different sites showed significant differences among populations at different altitudes. In all three and five sites, the monoterpene and aldehydes ratio of the *Vvi, Vm* and *Vu* collected from the low altitude was higher compared with those collected from the high altitude, respectively. The aldehydes ratio of the *Va* harvested from the all eight altitudes was more or less the same. Principal components analyzed based on the mean relative amounts of the volatile components have led to the identification of three chemotypes (capronaldehyde, 2(*E*)-hexenal and nonanal). Since, the amount of plant constituents affected by altitude and time collected would vary accordingly. These results suggest that altitude is a factor influencing the volatile components of these plants.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

***Va*:** *V. arctostaphlyos;* ***Vu*:** *V. uliginosum;* ***Vvi*:** *V. vitis-idaea*; ***Vm*:** *V. myrtillus*; **SPME:** Solid phase micro extraction; **GC-MS:** Gas Chromatography-Mass Spectrometry; **HS:** Headspace; **VOC:** Volatile organic compound; **HPLC:** High-Performance Liquid Cromatography; **DVB:** Diviniylbenzene; **PDMS:** Polydimethylsiloxane; **FID:** Flame ionization detector; **mL:** Millilitre; **KTUB:** Karadeniz Technical University, Herbarium of Biology (Turkey); **C&Y:** Coşkunçelebi and Yılmaz; **RI:** Retention Index; **NC:** Number of compounds.

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