Correlation between chemical and genetic variation in *Helichrysum leucocephalum* (*Asteraceae*)

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Abstract. The essential oils of five geographical populations of the Iranian *Helichrysum leucocephalum* were analyzed by gas chromatography and mass spectrometry (GC and GC/MS). There were 69 chemical compounds in the studied populations: *trans*-caryophyllene (11.2–21.44 %) and α-humulene (9.37–18.68 %) occurred with the highest percentage in the Abadeh-Tashk population. Some other interesting compounds obtained were: α-copaene (3.23–8.40 %), α-pinene (2.90–7.86 %), β-selinene (2.93–6.37 %), and Δ-cadinene (2.50–4.74 %). Compounds occurred in different quantities in all studied populations. A STRUCTURE analysis of the combined genetic and chemical contents showed the divergence between populations.

Key words: Chemical diversity, *Helichrysum leucocephalum*, STRUCTURE analysis

Introduction

Helichrysum is one of the largest genera of the *Asteraceae* with about 600 species distributed mainly in the African continent, Madagascar and Eurasia (Bayer & al. 2007). The species are highly diverse with respect to their phenotype and metabolite profile (Casinov & al. 1968; Orzalesi & al. 1969). Several species have ornamental and medicinal value. For instance, *H. plicatum* subsp. *plicatum* and *H. italicum* have antioxidant and antibacterial effect (Facino & al. 1988; Aslan & al. 2007).

Natural populations of the same plant species may vary in the content of active phytochemicals. This variation in the concentrations within or between populations may be controlled by genetic, environmental and interaction of genetic and environmental factors (Harrigan & al. 2007; Skogerson & al. 2010). Intraspecific chemical variation has been reported in many genera (Whiffin & Ladiges 1992; Butcher 1996) and some of the species of *Helichrysum*. Some of these forms have been distinguished as chemotypes and qualitative and quantitative variation in oil is genetically determined (Angioni & al. 2003; Ornano & al. 2014).

In Iran, some of the species have been studied for their content of essential oils, for example: *H. oligocephalum* DC., *H. oocephalum* Boiss., *H. leucocephalum* Boiss, *H. artemisioides* Boiss. & Hausskn. and *H. aucheri* Boiss. The main significant components were: α-pinene, rosifoliol, cineole, hexadecanoic acid, thymol, β-caryophyllene, menthone, dodecane, α-humulene, and menthol (Firouznia & al. 2007; Javidnia & al. 2009; Sajadi & al. 2009; Torabbeigi & al. 2011). However, no information was found in literature on the variation of essential oils in *Helichrysum*.

H. leucocephalum is an endemic species to Iran distributed in different geographical localities in central and southern parts of the country. It is able to colonize environments ranging from 300 m to 3000 m a.s.l. (Georgiadou & Rechinger 1980; Azizi & al. 2014). This species is a perennial shrub, 25–60 cm high, with sessile resting buds and a single erect stem with tomentum, linear or oblanceolate-spathulate basal leaf and middle stem leaves, the capitula are terminal to branches; a corymb inflorescence contains many opaque white florets recurved behind the involucral bracts; fruits are brown achenes no longer than 1.2 mm (Georgiadou & Rechinger 1980). It was selected for the present study due to its pharmaceutical importance (Javidnia & al. 2009) and lack of population structure studies.

According to the results in our previous paper, *H. leucocephalum* was proven to exhibit genetic variation among and within populations (Azizi & al. 2014). Our aim in the present study was to verify if this variability is influencing the volatile composition with quail-quantitative differences in essential oils among and within populations coming from different regions. Therefore, we have studied the variation in essential oil components, and the correlation between the chemical and genetic structure of five geographical populations of *H. leucocephalum* from Fars and Yazd provinces.

Material and methods

Plant material

A total of five natural populations of Iranian *H. leucocephalum* were collected in July 2012 from five geographical locations of Mehriz (Yazd Province), Abadeh-Tashk, Arsanjan, Roniz and Neyriz (all from Fars Province) (Fig. 1; Table 1). The botanical identification according to *Flora Iranica* (Georgiadou & Rechinger 1980) was performed by N. Azizi and voucher specimens from each population have been deposited in the Shahid Beheshti University Herbarium. (SBUH)

Isolation of essential oils

The air-dried aerial parts of the plants (200 g) were subjected to hydro-distillation for 4h on a Clevenger-type apparatus as described in the British Pharmacopoeia of 1988.

Fig. 1. Distribution map of studied *Helichrysum leucocephalum* populations. (Numbers in the parenthesis [1-5] are the population numbers of Mehriz, Abadeh-Tashk, Arsanjan, Roniz, and Neyriz, respectively).

Table 1. ***Helicrysum leucocephalum*** **populations and their localities.**

P.	Province	City	Locality	Voucher number	Longitude	Latitude	Altitude (m)
1	Yazd	Mehriz	Damgahan valley	Azizi, N., 2012227	312800	541800	1900
2	Fars	Abadeh-Tashk	Abadeh-Tashk	Azizi, N., 2012228	295000	534339	1676
3	Fars	Arsanjan	Arsanjan	Azizi, N., 2012229	295500	531100	1793
4	Fars	Roniz	Morghak valley	Azizi, N., 2012231	291560	535908	1649
5	Fars	Neyriz	Palangan	Azizi, N., 2012230	291200	541624	1832

GC-FID analysis

The GC-FID analysis of oils was conducted using a Thermoquest-Finnigan Trace apparatus equipped with a DB-5 fused-silica capillary column (30 m_0.25 mm i.d., film thickness 0.25 mm). The oven temperature was programmed from 60° to 250° at 5°/min and held isothermal at 250° (10 min); injector temperature 250°; detector (FID) temperature 280°; carrier gas N2 (1.1 ml/min); split ratio 1:50. Quantitative data were obtained from GC-FID area percentages without applying correction factors (Kanani & al. 2011).

GC/ MS analysis

The GC/MS analysis was carried out with a Thermoquest-Finnigan Trace apparatus equipped with a DB-5 fused-silica cap. column (60 m_0.25 mm i.d., film thick-

ness 0.25 μm). The oven temperature was programmed from 60° to 250° at 5°/min and held isothermal at 250° for 10 min; injector temperature 250°; transfer-line temperature 250°; carrier gas He (1.1 ml/min); split ratio 1:50. The quadruple mass spectrum was acquired for the mass range of 35–465 amu, with ionizing voltage of 70 eV and ionization current of 150 μA. Identification of the individual compounds was based on the comparison of their mass spectra with those of the internal reference mass spectral library (Adams 2007), or with those of authentic compounds. For quantification purposes, the relative area percentages obtained by FID were used without applying correction factors (Kanani & al. 2011).

Statistical analysis

To classify and group the five populations of *H. leucocephalum* on the basis of their essential-oil components, the obtained data were standardized (mean= 0, variance= 1) and then used to determine the Euclidean distance among the studied populations. UPGMA (Unweighted Paired Group using Average Mean), PCA (Principal Components Analysis) and MDS (Multidimensional Scaling) methods were applied for grouping the populations (Podani 2000). Multivariate statistical analyses were done with PAST program, ver. 2.17 (Humer & al. 2001).

Results and discussion

Chemical diversity of essential oils

Sixty-nine components were obtained in the essential oils analyzed for the five studied locations, which supply sufficient information for chemical diversity analysis (Table 2). The major chemical compound, respectively, was *trans*-caryophyllene with a range of 11.2–21.44 %, This compound acts as a selective agonist of the cannabinoid receptor type 2, antinociceptive, neuroprotective, anxiolytic and antidepressant (Bahi & al. 2014; Paula & al. 2014). Next came *α*-humulene with a range of 9.36–18.68 % and significant anticancer effect (Legault & al. 2003). The highest percentage of these chemical constituents was observed in the Abadeh-Tashk population, while the lowest was in the Arsanjan population. Some other interesting essential oils obtained in all studied populations were: *α*-copaene, *α*-pinene, *β*-selinene, and *Δ*-cadinene.

Table 2. Chemical compounds identified in the studied *Helicrysum leucocephalum* populations. (All values are in percentage).

	Chemical compound (%)	Pop 1	Pop 2	Pop 3	Pop 4	Pop 5
1	*α*-Pinene	7.86	1.97	3.40	2.96	7.62
2	champhene	0.44	0.14	0.72	0.30	0.98
3	*n*-Octanal	0.17	0.20	0.47	0.22	0.25
4	*p*-Cymene	0.39	0.24	0.83	0.60	0.63
5	Limonene	1.45	0.70	2.27	2.11	1.89
6	1,8-Cineole	2.39	1.04	3.54	2.76	3.53
7	Acetophenone	0.27	0.31	0.68	0.55	0.38
8	Terpinolene	0.08	0.05	0.11	0.14	0.09
9	Linalool	0.23	0.13	0.17	0.15	0.18
10	*n*-Nonanal	0.75	0.43	1.54	0.61	0.42
11	*α*-Campholenal	0.30	0.23	0.48	0.38	0.36
12	Camphore	0.21	0.21	0.17	0.11	0.14
13	endo- Borneol	0.70	0.55	1.17	0.79	0.89
14	*α*-Terpineol	0.20	0.16	0.89	0.65	0.51
15	*n*-Decanal	0.02	0.02	0.07	0.37	0.05
16	trans-Carveol	0.35	0.23	0.27	0.11	0.97
17	Bornyl acetate	0.82	0.42	2.25	1.93	1.38
18	*α*-Longipinene	0.26	0.16	0.09	0.03	0.09
19	Eugenol	0.06	0.39	0.02	0.02	0.03
20	Cycloisosativene	0.16	0.34	0.34	0.21	0.11
21	*α*-Ylangene	0.39	0.49	0.98	0.08	1.97
22	*α*-Copaene	3.23	3.80	5.34	8.57	8.30
23	sativen	0.15	0.15	0.46	0.41	0.42
24	*Z*-Jasmone	0.02	0.08	0.07	0.06	0.12
25	Methyl eugenol	0.05	0.23	0.11	0.24	0.22
26	Longifolene	0.59	0.44	0.59	0.49	0.65
27	*α*-Gurjunene	0.05	0.14	0.4	0.23	0.06
28	trans-Caryophyllene	20.88	21.7	11.16	15.76	15.81
29	*α*-Guaiene	1.61	1.15	3.03	1.13	1.77
30	*α*-Humulene	13.78	18.91	9.33	14.31	10.75
31	9-epi-(E)-Caryophyllene	0.28	0.11	0.34	0.12	0.33
32	*γ*-Muurolene	0.91	1.81	2.49	1.99	1.78
33	*α*-Amorphene	0.10	0.21	0.31	0.24	0.24
34	Valencene	0.90	2.96	1.97	1.55	1.29
35	*β*-selinene	5.87	6.45	3.61	2.99	3.14
36	*α*-selinene	1.45	2.67	1.91	1.71	1.64
37	*α*-Muurolene	0.19	0.65	0.33	0.82	1.04
38	*β*-Bisabolene	0.06	0.06	0.04	0.05	0.05
39	*γ*-Cadinene	1.12	2.80	3.71	2.91	2.49
40	*Δ*- Cadinene	2.50	4.80	4.63	4.27	4.49
41	Cadina-1,4-diene	0.55	1.23	1.91	1.24	1.23
42	*α*-Cadinene	0.06	0.15	0.10	0.16	0.12
43	*α*-Calacorene	0.19	0.41	0.60	0.49	0.41
44	Data ms	0.50	0.81	0.50	1.15	0.91
45	Dodecanoic acid	0.02	0.48	0.40	0.04	0.02
46	Caryophyllenyl alcohol	1.23	1.43	1.20	1.31	1.18
47	Caryophyllene oxide	1.55	1.22	1.97	1.79	1.99
48	Rosifoliol	4.12	3.02	3.06	3.26	3.15
49	1-epi-Cubenol	1.06	1.36	1.47	0.99	1.10

Table 3. Continuation.

	Chemical compound (%)	Pop 1	Pop 2	Pop 3	Pop 4	Pop 5
50	Data ms	2.84	1.38	0.81	1.27	0.79
51	epi-α-Cadinol	3.7	0.29	0.19	4.63	1.68
52	β-Eudesmol	1.36	1.58	2.21	1.62	1.59
53	α-Cadinol	0.55	0.61	0.61	0.47	0.53
54	Δ- ms	1.39	1.06	2.05	1.06	1.59
55	Δ- ms	1.00	0.69	1.60	0.80	1.02
56	Cadalene	3.61	2.98	2.70	3.25	2.06
57	Heptadecane	0.16	0.28	0.84	0.50	0.38
58	Tetradecanoic acid	0.09	0.15	0.13	0.22	0.15
59	Δ-ms	1.80	0.82	2.23	0.10	1.01
60	Octadecane	0.13	0.25	0.18	0.14	0.19
61	6,10,14-Trimethyl-2-pentadecanone	0.41	0.70	1.14	0.5	0.47
62	Δ-ms	0.03	0.25	0.20	0.15	0.14
63	Nonadecane	0.04	0.13	0.17	0.09	0.10
64	Dibutyl phthalate	1.85	0.78	1.83	0.74	0.98
65	Sandaracopimara-8(14),15-diene	0.18	0.27	0.25	0.21	0.28
66	Eicosane	0.09	0.23	0.35	0.15	0.20
67	Heneicosane	0.03	0.09	0.18	0.07	0.13
68	Tricosane	0.07	0.08	0.21	0.06	0.18
69	Pentacosane	0.11	0.11	0.23	0.09	0.19
	Total (%)	100	100	100	100	100

Populations' abbreviation: Pop 1–5: Mehriz, Abadeh-Tashk, Arsanjan, Roniz and Neyriz respectively.

In a similar study, Javidnia & al. (2009) reported the occurrence of 92 components in plant specimens of *H. leucocephalum* collected during the flowering stage in the Bastak Mountains in South Iran. Their study showed that rosifoliol (22.3 %), β-caryophyllene (10.1 %) and α-humulene (9 %) were the main components in that locality. These differences could be due to diversity of varieties, polymorphism, stage of plant growth, and environmental factors (Ghahreman 1992; Roussis & al. 2000; Bianchini & al. 2009; Sajadi & al. 2009).

The UPGMA dendrogram (Fig. 2) obtained on the basis of essential oil extracts gives two divisions: the Abadeh-Tashk population was placed in a separate cluster, far from the other studied populations. The Arsanjan and Neyriz populations were placed close to each other and form a separate subcluster. The Roniz and Mehriz populations also showed some degree of chemical similarity and formed a second subcluster.

The MDS plot supported the results of clustering (Fig. 3). This plot separated the plant specimens collected from the studied populations in different groups. For example, the Abadeh-Tashk population differed from the other studied populations; the

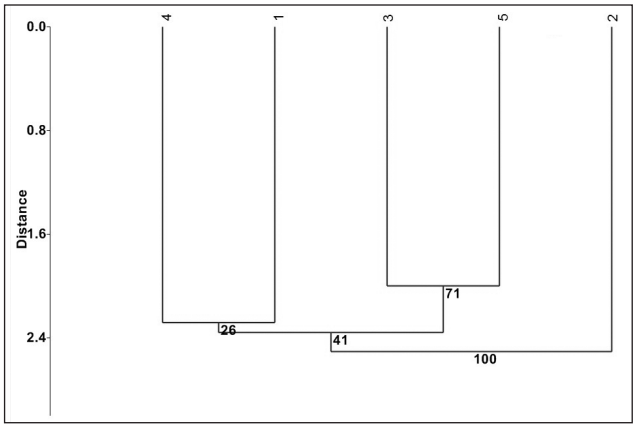

Fig. 2. UPGMA-graph based on chemical data of the studied *H. leucocephalum* populations.

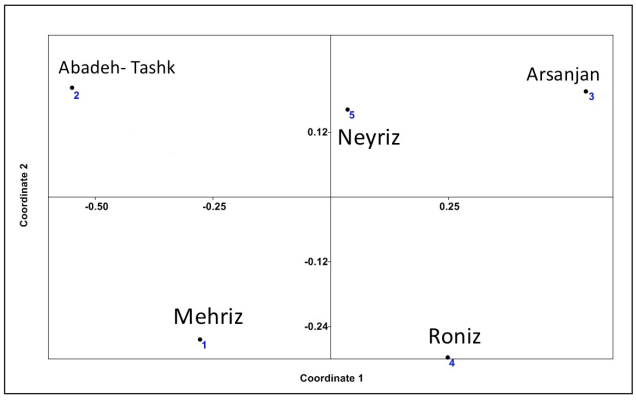

Fig. 3. MDS plot based on chemical data of the studied *H. leucocephalum* populations.

Mehriz population also occupied a distinct position below the center of the MDS plot.

Clustering of all chemical data clearly showed that the populations differ in these compounds. This was further supported by the PCA analysis of chemical data: the first three PCA components accounted for about 83 % of the total variation. Further analysis showed that most of the studied chemical compounds had high correlation values with the first PCA component and varied greatly among the studied populations ($r > 0.08$ and $r < -0.80$) (Table 3).

Comparing the grouping obtained by genetic and chemical analyses

According to the results for *H.leucocephalum* in our previous paper (Azizi & al. 2014), a significant genetic difference ($p < 0.01$) was revealed among the studied populations.

A comparison of the grouping obtained by genetic and chemical analyses indicates some degree of agreement between them. For example, the Mehriz

Table 3. Chemical compounds with highest correlation coefficient with PCA axes.

	Chemicals	Axis 1	Axis 2	Axis 3
1	α-Pinene			−0.8532
2	champhene	0.8332		
3	*n*-Octanal	0.8035		
4	*p*-Cymene	0.9725		
5	Limonene	0.8828		
6	1,8-Cineole	0.9485		
11	α-Campholenal	0.9301		
13	endo- Borneol	0.9659		
14	α-Terpineol	0.886		
17	Bornyl acetate	0.8544		
23	sativen	0.9012		
28	trans-Caryophyllene	−0.9446		
30	α-Humulene	−0.954		
35	β-selinene	−0.8105		
38	β-Bisabolene	−0.9288		
42	α-Cadinene			0.8922
45	Dodecanoic acid		0.9058	
46	Caryophyllenyl alcohol	−0.8069		
47	Caryophyllene oxide	0.9519		
49	1-epi-Cubenol		0.9326	
51	epi-α-Cadinol		−0.9752	
53	α-Cadinol		0.9338	
54	Data ms	0.8416		
55	Data ms	0.8227		
58	Tetradecanoic acid			0.8972
60	Octadecane		0.849	
68	Tricosane	0.8588		
69	Pentacosane	0.8505		

and Abadeh-Tashk populations were placed close to each other, as compared to the other studied populations in both analyses. Similarly, three other populations showed affinity in both analyses. However, the chemical and genetic distances of these populations differ somewhat in the chemical versus the genetic data.

A combined dataset was used of genetic and chemical, along with geographical data such as longitude, latitude and altitude of the studied populations and they were analyzed by CCA (Canonical Correspondence Analysis). The results (Fig. 4) clearly separated the Mehriz population of the Yazd Province from the other studied populations of Fars Province. Also, we found a clear genetic and chemical difference among the populations of the Fars Province, irrespective of almost similar ecological conditions. Moreover, the STRUCTURE plot (Fig. 5), obtained from combined genetic and chemical characteristics of the studied populations, has revealed that these populations differ too much in their genetic and chemical contents (difference in the colored segments).

According to the significant Mantel test between the chemical distance and geographical distance (r = 0.50. p <0.01), these populations also differ more chemically across the studied geographical area.

The significant Mantel test between the chemical distance and genetic distance has indicated that genetic diversity of the studied populations increases and their chemical difference enhances (r = 0.54, p <0.01).

Intraspecific variation in the essential oil composition could be attributed to presence of chemotypes in a single species (Whiffin & al. 1992). Oil yield can be substantially modified by environmental and genetic factors (Scora & al. 1966; Butcher & al. 1996). Melito & al. (2013) studied the intraspecific variation in the oil composition of *H. italicum* and suggested correspondence between the gene pool and chemical diversity and recognized two chemotypes.

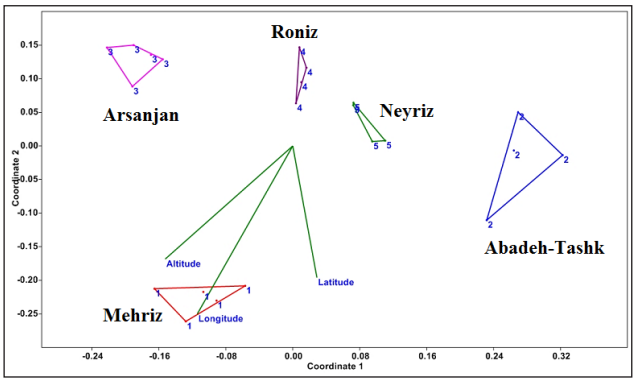

Fig. 4. CCA plot of combined data.

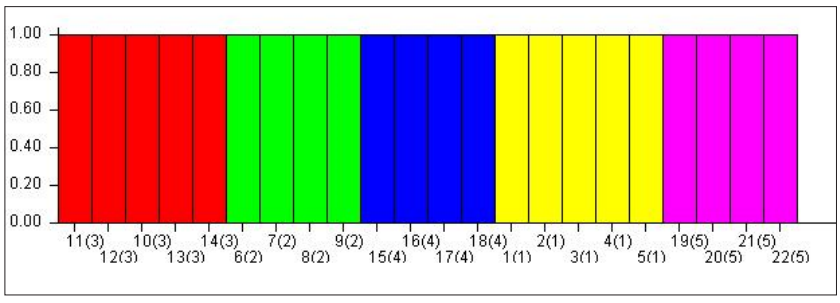

Fig. 5. STRUCTURE plot of genetic and chemical data. (Numbers in the parenthesis [1-5] are the population numbers of Mehriz, Abadeh-Tashk, Arsanjan, Roniz, and Neyriz, respectively).

Plummer & al. (1999), who studied the intra- and interspecific variation in the oil composition of *Boronia megastigma* Nees. (*Rutaceae*), reported great intrapopulation and interpopulation variation, but the qualitative and quantitative variation was continuous and not partitioned into chemotypes. Lack of chemotypes was supported by PCA analysis which consistently failed to separate populations.

In the present study, the populations differed both in chemical contents and in genetic characteristics and the PCA and MDS plots clearly separated the populations into different groups. Moreover, the STRUCTURE plot revealed genetic and chemical distinctness of the populations. Therefore, we may have clear chemotypes in *H. leucocephalum*.

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