Progress in Biological Sciences
Vol. 5, Number 1, Winter / Spring 2015/85-96

Essential oil variations among the natural populations of *Francoeuria undulata*

Received: January 1, 2015; Accepted: March 1, 2015

Marjan Bastan [1], Hamid Sadeghi* [2]

1. Department of Horticulture, Jahrom Branch, Islamic Azad University, Jahrom, Iran
2. Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, Iran

ABSTRACT

Francoeuria undulata (L.) LACK is often synonymous with two species of the genus Pulicaria, i.e. *Pulicaria undulata* (L.) C.A. Mey and Pulicaria crispa (Forssk.) olive, which are often included under the genus Francoeuria. The essential oil yield and compositions in the five natural populations of *Francoeuria undulata* from Fars Province in southern Iran were determined by GC and GC/MS analysis. The essential oil yields ranged between 0.34 and 0.52% (w/w) with a mean of 0.42%, in the five populations studied. Fifty-six compounds were identified in the oil samples, representing 92.9% of the total oil. Eighteen major compounds detected in the oil samples at an average concentration of about 1% of the total oil in the five populations have been considered for statistical analyses. The main components included 1,8-cineol (21.1%), alloaromadendrene epoxide (16.9%), eudesma-4(15),7-dien-1-ß-ol (15.7%), α-terpineol (8.1%), α-pinene (5.0%) and terpinen-4-ol (5.0%). By evaluating the changes in the essential oil components, we have concluded the existence of a high intraspecific genetic variation among the *Francoeuria undulata* populations. Based on the canonical discriminant functions the analysis enabled the identification of four chemotypes. Moreover, the presence of *trans*-methyl dihydrojasmonate and high levels of oxygenated mono- and sesquiterpenes in the essential oils of the plant, indicate the high chemical defensive ability of *Francoeuria undulata*.

Keywords: chemotypes, essential oils, *Francoeuria undulata*, oxygenated monoterpenes, phytochemical analysis.

* **Corresponding author:** hsadeghi@jia.ac.ir

Introduction

Francoeuria undulata (L.) LACK. is a perennial aromatic herb, from the Asteraceae family (Tribe: Inuleae), producing small bright yellow flowers. The plant is often synonymous with two species of the genus Pulicaria, i.e. *P. undulata* (L.) C.A. Mey. and *P. crispa* (Forssk.) olive, which are often included under the genus Francoeuria (1-3). This species is found distributed in Saudi Arabia, Kuwait, Iran, Iraq, Egypt, Afghanistan, Pakistan, India and parts of northern and western tropical Africa (4).

Due to the strong smell of the essential oil in the aerial parts of this medicinal plant, it has been used from ancient times in the treatment of sinusitis and respiratory tract infections in the traditional medicine system in southern Iran.

Besides, these essential oils play an crucial part in plant protection as an insect repellent, anti-bacterial, anti-viral and anti-fungal (5). The volatile terpenes act as pollinator attractants, and provide an important defense mechanism against the herbivores and pathogenic fungi; they also play a significant role in plant–plant interactions, revealing an evolutionary relationship with their functional roles (6, 7). Although the biosynthesis of these compounds in plants has strong genetic control, environmental conditions also exert a significant impact on their production rates (8, 9).

Different Pulicaria species have been extensively studied to establish the composition of their essential oils. One of the earliest studies on the extract of the *P. crispa* aerial parts by Bohlmann *et al*., (1979), resulted in the identification of a new pseudoguaianolide epoxide and a seco-sesquiterpene lactone compound, besides the identification of three xanthanolide compounds. Later, Bohlmann *et al*., (1982), introduced seven new compounds of caryophyllene derivatives in Et_2-O petrol extract from the *P. scabra* shoots (11). Further studies by Abdel-Mogib *et al*., (1990), enabled the identification of two new sesquiterpene lactones. Overall, the previous studies have indicated that the extractions of the Pulicaria species are rich sources of the sesquiterpene lactones (2, 13). Apart from these, Hanbali *et al*., (2005), identified twenty-seven components in the *P. odora* essential oils (14), and recently Znini *et al*., (2013), have identified twenty-five different volatile compounds in the *P. mauritanica* essential oils (15).

However, the vast majority of phytochemical studies suggest that the Pulicaria species essential oils contain about 90% carvotanacetone (15, 16). Despite numerous researches conducted on the essential oils of the aromatic species of genus Pulicaria (14, 15, 17), to the best of our knowledge no reports have been produced on the constituents of the essential oils of *F. undulata*. Population masses of *F. undulata* thrive in the wasteland surrounding the roadways, in Fars province, in southern Iran. In the present work, we report the oil yield and chemical compositions of *F. undulata*. Further, we focused on the intraspecific variation of the essential oil components among the five populations of this medicinal plant in the geographical distribution range in the Fars Province.

Methods and Materials

Plant materials

The aerial parts (i.e. stems, leaves and flowers) of *F. undulata* (individual plants) were collected at the full bloom stage in late May 2013 in five locations from 9 AM to 13

PM local time. The site details are listed in Table 1. Healthy and dominant plants, growing 50 meters apart from each other, were sampled. Botanical identification of the materials collected was verified by the Plant Biology Department of Islamic Azad University, Jahrom Branch, Jahrom, Iran. Moreover, species identification was also verified by the Fars Agriculture Research Center and the voucher specimen deposited in the herbarium of this institute (no. 3312). About 100 g of the shade-dried aerial parts (ten samples of each population) were submitted to hydrodistillation for 5 h using a Clevenger-type apparatus. The separated oils were then dried using anhydrous sodium sulfate (Merck, Darmstadt, Germany) and maintained at 4°C until analysis using GC and GC–MS spectrometry.

Table 1. Plant collection site (in the case of annual temp. and rainfall the average of 25 years was considered)

Site	Pop. Id.	Coordinates	Alt. (m)	min/max temp (°C)	rainfall (mm)
Kakh-E-Sasan	P1	29°12'19"N, 53°15'25"E	1500	-2.0/33.0	300.0
Nazar Abad	P2	29°11'54"N, 53°15'35"E	1350	-2.0/33.0	300.0
Tol-E-Chitgar	P3	29°11'35"N, 53°16'16"E	1250	-1.0/32.0	300.0
Jangal-E-Shadid	P4	29°07'57"N, 53°29'06"E	1130	-1.0/32.0	300.0
Jahrom	P5	28°34'44"N, 53°35'57"E	1000	1.0/36.0	282.0

GC and GC–MS analysis

GC analysis was conducted using an Agilent-technology chromatograph with HP-5 column (30m ×0.32 mm i.d.× 0.25 µm). Oven temperature was maintained as follows: 60°C to 210°C at 3°/min; 210°C to 240°C at 20/min and held for 8.5 min, injector temperature 280°C; detector temperature, 290°C; carrier gas, N_2 (1 ml/min); split ratio of 1:50. The GC-MS analysis was performed using an Agilent 7890 operating at 70 eV ionization energy, equipped with a HP-5 MS capillary column (phenylmethylsiloxane, 30m × 0.25 mm i.d.× 25µm.) with He as the carrier gas in a split ratio of 1:50. The retention indices were determined using the retention times of the n-alkanes under the same chromatographic conditions. The retention indices for all the components were determined based on the procedure using n-alkanes as standard. The compounds were identified by comparison of the retention indices (RI, HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley GC/MS Library, Adams Library and Mass Finder 2.1 Library data published mass spectra data (18-20).

Statistical analysis

Statistical analysis was performed according to Sadeghi *et al.*, (2014). Moreover, in the MANOVA procedure the significance levels were adjusted for multiple comparisons using the Bonferroni correction. Using this method the significance level of 0.05 was divided by the number of dependent variables and the result was considered the significant level. Statistical analyses were performed using the SPSS software, version 21 (SPSS Inc., Chicago, USA) and Minitab software, version 16 (Minitab Inc., State College, Pennsylvania) in order to show up the hidden structures among the populations more clearly.

Results and Discussion

Oil yield and chemical composition

The yields of the *F. undulata* essential oils ranged from 0.34 to 0.52% (w/w) with a mean of 0.42%, SD=0.16 in the five populations studied. The normally distributed data of oil yield along with the equal variance in the five populations showed no significant change among them. The oil yield of the *F. undulata* populations in this study was in the same range as *P. mauritanica* (0.45%) (15), lower than the yield of *P. undulata* (2.1%) (16), but higher than the yield reported for *P. jaubertii* (0.15%) (21).

From the analysis of the essential oils of *F. undulata* fifty-six compounds were identified, representing 92.9% of the total oil (Table 2).

Table 2. Composition of the essential oils of *Francoeuria undulata* (RI,s are experimental values)

Entry	Compound name	RI[a]	Content [%]	Entry	Compound name	RI	Content [%]
1	α-Thujene	925	0.3	29	Geranial	1269	0.3
2	α-Pinene	933	5.0	30	Neryl formate	1283	0.1
3	Camphene	947	0.1	31	Thymol	1290	0.3
4	Sabinene	972	0.1	32	(*Z*)-Jasmone	1394	0.4
5	β-Pinene	976	0.7	33	Methyl eugenol	1404	0.5
6	dehydro-1,8-Cineole	990	0.1	34	(*E*)-Caryophyllene	1418	0.6
7	α-Phellandrene	1005	0.4	35	Unknown	1489	0.3
8	*p*-Cymene	1023	0.8	36	γ-Cadinene	1517	0.1
9	Limonene	1026	0.5	37	δ-Cadinene	1526	0.2
10	1,8-Cineole	1028	21.1	38	Unknown	1529	0.2
11	(*E*)-β-Ocimene	1045	0.1	39	(*E*)-γ-Bisabolene	1532	0.3
12	γ-Terpinene	1057	1.8	40	*trans*-Cadina-1(2),4-diene	1536	0.5
13	*cis*-Sabinene hydrate	1068	0.8	41	*cis*-Cadinene ether	1554	0.1
14	Terpinolene	1087	0.4	42	*trans*-Cadinene ether	1561	0.7
15	*trans*-Sabinene	1100	1.2	43	Spathulenol	1569	0.5
16	*cis*-*p*-Menth-2-en-1-ol	1120	0.3	44	Caryophyllene oxide	1582	0.1
17	Chrysanthenone	1127	0.3	45	viridiflorol	1592	0.6
18	1-Terpineol	1131	0.7	46	Unknown	1608	0.2
19	*trans*-Verbenol	1143	0.2	47	epoxide-*allo*-	1642	16.9
20	*cis*-Chrysanthenol	1162	0.4	48	Unknown	1655	0.3
21	δ-Terpineol	1166	0.3	49	14-hydroxy-(*Z*)-	1666	1.4
22	Terpinene-4-ol	1177	5.0	50	*trans*-methyl	1682	1.1
23	α-Terpineol	1187	8.1	51	Unknown	1688	0.5
24	*trans*-Piperitol	1205	0.2	52	Eudesma-4(15),7-dien-1-β-	1689	15.7
25	*trans*-Carveol	1213	0.2	53	Unknown	1718	0.6
26	Nerol	1229	1.5	54	(*E*)-Nuciferal	1725	1.9
27	Neral	1239	0.3	55	(*E*)-Nuciferol	1733	0.5
28	Geraniol	1254	0.8	56	(*Z*)-Nuciferol acetate	1830	0.8

The main components included 1,8-cineol (21.1%, SD=±7.7), alloaromadendrene epoxide (16.9%, SD=±5.8), eudesma-4(15),7-dien-1-ß-ol (15.7%, SD=±3.8), α-terpineol (8.1%, SD=±2.5), α-pinene (5.0%, SD=±2.3) and terpinene-4-ol (5.0, SD=±1.4) (Table 3).

Table 3. The mean percentage of the relative amounts based on the total integrated peak area of 18 terpene compounds in the essential oils of five *F. undulata* populations were selected for statistical analyses (a comparison of the means of the abnormally distributed variables and the normally distributed variables was performed employing the non-parametric Kruskal Wallis test and F-test, respectively).

Variable	P1	P2	P3	P4	P5	Sig
α-Pinene	7.7± 0.9	3.5 ± 0.7	4.5 ± 0.8	3.2 ± 2.1	6.3 ± 2.6	0.000^{**KW}
ß-Pinene	1.0 ± 0.2	tr	tr	tr	tr	0.003^{**KW}
p-Cymene	tr	tr	1.0 ± 0.4	tr	tr	ns ^{KW}
1,8- Cineole	28.0 ± 0.9	12.6 ± 2.2	20.9 ± 4.4	18.0 ± 7.0	26.0 ± 8.9	0.000^{**KW}
ɣ-Terpinene	1.7 ± 0.7	1.4 ± 0.7	2.3 ± 0.8	2.1 ± 0.8	1.7 ± 0.4	ns ^{F}
cis-Sabinene hydrate	1.0 ± 0.2	tr	tr	tr	tr	0.020^{*KW}
trans -Sabinene hydrate	1.5 ± 0.7	tr	tr	tr	1.5 ± 0.5	0.012^{*KW}
1-Terpineol	tr	1.6 ± 0.3	0.7 ± 0.3	tr	tr	0.000^{**KW}
Terpinen-4-ol	5.0 ± 0.7	3.6 ± 1.0	5.7 ± 1.0	4.7 ± 1.4	5.8 ±1.7	0.002$^{** F}$
α-Terpineol	8.9 ± 0.8	6.2 ± 1.4	8.6 ± 2.2	7.3 ±2.5	9.3 ± 3.6	0.021^{*KW}
Nerol	1.6 ± 0.8	2.0 ± 0.7	1.3 ± 0.7	tr	1.4 ± 0.5	ns ^{F}
Viridiflorol	0.5±0.2	0.8±0.4	0.8±0.4	0.9±0.4	0.6±0.4	ns ^{F}
epoxide-*allo*-Aromadendrene	11.0 ± 0.8	21.0 ± 4.3	17.1 ± 4.5	19.8 ± 6.7	15.3 ± 5.8	0.001^{**KW}
14-hydroxy-(*Z*)-caryophyllene	1.5 ± 0.5	1.2 ± 0.5	1.7 ± 0.9	1.1 ± 0.6	1.5 ± 1.0	ns ^{F}
trans-methyl dihydrojasmonate	tr	1.1 ± 0.6	0.8 ± 0.4	1.8 ± 1.1	1.2 ± 0.8	ns ^{F}
Eudesma-4(15)7- dien-1-ß-ol	12.7 ± 0.8	18.2 ± 3.1	16.4 ± 2.3	17.3 ± 3.7	14.0 ± 5.2	0.002^{**KW}
(*E*)-Nuciferal	1.2 ± 0.5	2.2 ± 0.7	1.5 ± 0.7	3.7 ± 2.4	Tr	ns ^{F}
(*Z*)- Nuciferol acetate	tr	1.2 ± 0.3	0.7 ± 0.4	tr	tr	0.001$^{** F}$
Monoterpene hydrocarbons	15.6	5.9	9.4	6.7	11.3	
Oxygenated monoterpene	52.5	34.3	44.7	38	50.6	
Sesquiterene hydrocarbons	15.1	29.3	22.6	26.5	20	
Oxygenated sesquiterpenes	18	28.5	22.4	26.6	16.7	

In *F*-test, according to Bonferroni correction, the significant level for normally distributed variables was reduced to 0.006. RI= Retention indices, KW=Kruskal Wallis test, F= *F* test, ns=non-significant, *, **=Significant at 0.05 and 0.01 respectively.

The number of compounds identified from the *F. undulata* essential oils was more than double compared with some species of the genus Pulicaria (14, 15, 17), and the identity of the most dominant components differed completely. Identification of essential oil components from a Francoeuria species could be of chemotaxonomic significance. In particular, the taxonomic relationships in the tribe Inuleae are quite problematic with the uncertainty of distinguishing between the genera Pulicaria and Francoeuria (3, 22). From this perspective, the *F. undulata* essential oils in this study showed significant differences in the main components when compared with most species of the genus Pulicaria such as *P. undulata*, which contains carvotanacetone (91.4%) (17), *Pulicaria mauritanica*, with carvonacetone (87.3%) as the main component (15), and *P. odora* essential oil, which is characterized by a combination of thymol (47.83%) and isobutyrate (30.05%)

(14). These phytochemical findings concurred with the molecular outcomes based on the cpDNA and ITS sequences by Englund et al., (2009). They demonstrated that genus *Francoeuria* is completely separate from genus *Pulicaria* (22).

The higher 1,8-cineol content compared with the other volatile compounds in the *F. undulata* essential oils might explain the characteristic and pleasant odor of the aerial parts of the plant. On the other hand, the mean value of the total oxygenated monoterpenes and monoterpene hydrocarbons in the five populations studied was 42.8% and 12.0% of the total oil, respectively. The proportion of oxygenated monoterpenes to monoterpene hydrocarbons hovered in the range of 3.4 to 5.7. The different ratios could be due to the genetic variation among the five populations or to the environmental conditions of the habitats. According to some researchers, essential oils rich in oxygenated terpenes generally possess stronger antimicrobial activity when compared with oils rich in terpene hydrocarbons (15, 17, 23, 24).

However, the prevalence of the oxygenated monoterpenes in the *F. undulata* essential oil is comparable with most species of genus *Pulicaria* (14, 15, 17). Moreover, the mean values of the sesquiterpene hydrocarbons (21.8%) and oxygenated sesquiterpenes (21.9%) in the five populations of *F. undulata* in this study were much higher than the levels they occurred in, in the Pulicaria species such as *P. mauritanica*, which represented only slightly oxygenated sesquiterpenes (1.9%) and an absence of the sesquiterpene hydrocarbons (15). These findings concurred with the findings of Javadinamin and Asgarpanah (2014) in the essential oils of *Francoeuria undulata* (24).

Apart from the terpenoid compounds in the *F. undulata* essential oils, we detected the ester, *trans*-methyl dihydrojasmonate (1.1%, SD=0.8). This compound plays a vital role in defense against herbivores by inducing the synthesis of defensive proteinase inhibitor proteins (25). Moreover, the jasmonate derivatives such as methyl jasmonate may play a role in interplant communication from the leaves of one plant species to those of another species to activate the expression of the defensive genes (25).

Variability of the essential oil components among populations

A comparison of the essential oil compounds in the five *F. undulata* populations revealed they were quite similar in quality with only quantitative differences occurring in the relative amounts of the essential oil components. Eighteen major compounds detected in the oil samples at an average concentration about 1% of the total oil in the five populations have been considered for the statistical analyses (Table 3). These components constituted 75.8 to 84.0% of the total oils. Exploring the normality assumption of the eighteen dependent variables along with the Box-Cox transformation, outlier elimination and Leveneʼs test of equality of error, variances in the populations studied showed that ten terpenoid variables were either not normally distributed or they do not have equal variance in the populations. Therefore, the mean comparison of these ten variables among the populations was calculated with the non-parametric Kruskal-Wallis test. For the vast majority of these ten variables a significant change was noted among the five populations (Table 3).

Variations in the relative amounts of these compounds in different environmental conditions indicated their adaptive ecological

value. Similarly, non-normal distribution of some terpenoid variables had been observed prior in other species (8, 26). This non-normal distribution was apparently not related to the sample size and was also observed in cases where the sample size is enormous (26). For the remaining eight normally distributed variables, a multivariate analysis of variance (MANOVA) was performed including the Bonferroni correction. The final results showed significant changes in the combined dependent variable among the five populations. Although a separate ANOVA was run in the MANOVA procedure for each normally distributed variable and significant changes were recorded only in the relative amounts of Terpinen-4-ol and (*Z*)-Nuciferol acetate, no significant changes were detected in the other six variables including γ-terpinene, nerol, viridiflorol, 14-hydroxy-(*Z*)-caryophyllene, *trans*-methyl dihydrojasmonate and (*E*)-nuciferal. In total, eleven variables of the eighteen compounds showed significant differences among the populations (Table 3).

Investigation of the latent relationship among the essential oil variables using the Principal Components Analysis (PCA), based on the correlation matrix between the components and the terpenoid variables indicated that α-pinene and 1,8-cineol were the most significantly correlated with each other and with the first component (Fig. 1). Therefore, the relative amounts of both variables show correlated changes in each individual essential oil (see also Table 3). As the concentration of the individual terpenes appears to be regulated by only a few genes (27), this association between the quantities of the two monoterpenes may be related either to the action of the regulatory genes influencing their chemical pathways or due to the pleiotropic control effect of a gene or a gene linkage over the changes in these variables (28). Similarly, the four sesquiterpene compounds; viridiflorol, alloaromadendrene epoxide, *trans*-methyl dihydrojasmonate and eudesma-4(15), 7-dien-1-ß-ol correlate the most with each other and with the second principal component (Fig. 1).

Moreover, the canonical discriminant functions analysis based on the relative amount of essential oil components of the individual plants showed a clear distinctiveness between the populations in close proximity to each other, P2, P3, and P4 (Fig. 2). The relative effect of each of the discriminant functions according to their eigenvalues showed that the two first discriminant functions have the greatest discrimination ability among those populations with eigenvalues of 11.1 and 6.1 for the first and second discriminant functions, respectively. Thus, 55.7% and 30.7% of the total variance is explained by the first and second canonical discriminant functions, respectively (Fig. 2).

The evaluation of the unstandardized canonical discriminant functions in the group centroids showed the highest mean of the first canonical discriminant function in the center of the population P4 (5.19) and population P2 (-4.19) and separated them as distinct chemotypes based on the absence of nerol and 1-terpineol in population P4 and the highest content of nerol and 1-terpineol in population P2 (see also Table 3 and Fig. 2). In addition, the highest mean of the second canonical discriminant function was in the

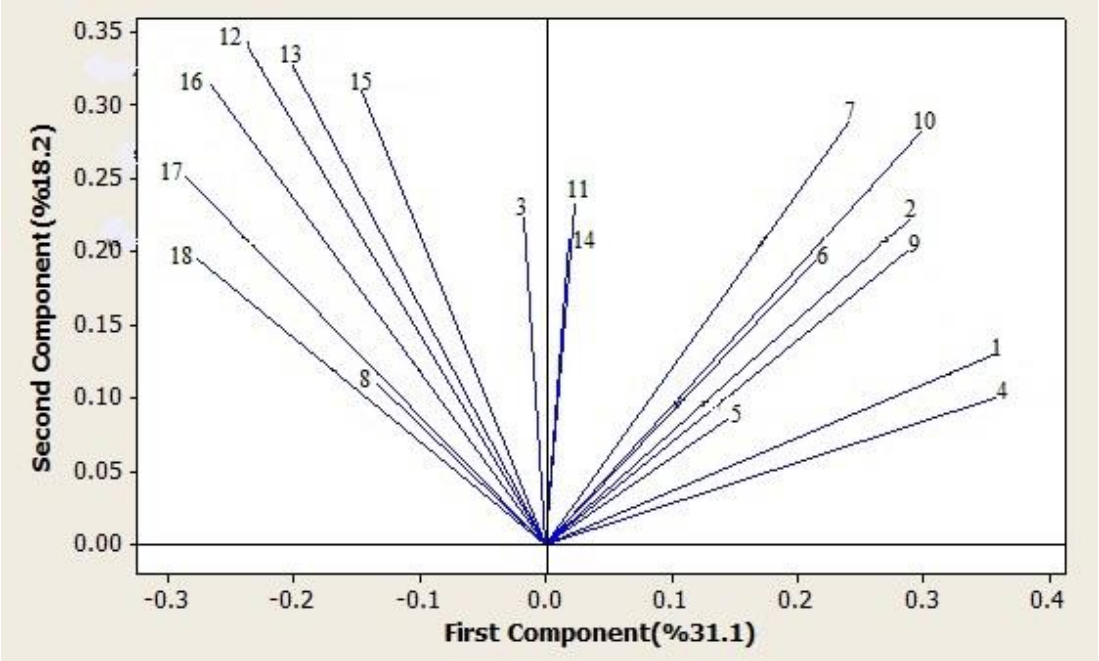

Figure 1. The relationship between the 18 essential oil components isolated from 50 individuals in five *Francoeuria undulata* populations in the space of the first two principal components. The proportion of the eigenvalue for each principal component is listed in parentheses. Terpenes: 1=α-Pinene, 2=β-pinene, 3=p-Cymene, 4=1,8-Cineol, 5=γ-terpinene, 6=cis-sabinene hydrate, 7=trans-sabinene hydrate, 8=1-Terpineol, 9=terpinene-4-ol, 10=α-terpineol, 11=nerol, 12=viridiflorol, 13=epoxy-allo-aromadendrene, 14=14-hydroxy-(Z)-caryophyllene, 15=trans-methyl dihydrojasmonate, 16=eudesma-4(15),7-diene-1-β-ol, 17=(E)-Nuciferol, 18=(Z)-Nuciferol acetate.

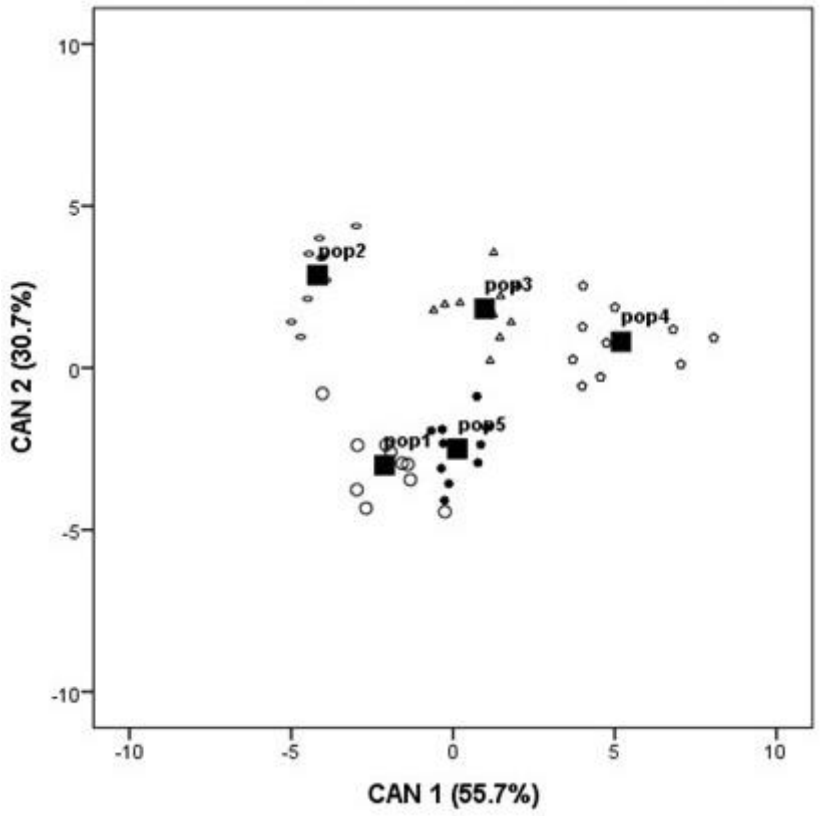

Figure 2. Distribution of the population centroids in the canonical discriminant functions analysis based on the essential oil components in 50 individual plants

center of population P1 (-3.01) and population P5 (-2.50) which are located at the extremes of the range. Therefore, the second canonical discriminant function associated both the P1 and P5 populations in the same chemotype characterized on the one hand by the highest α-pinene and 1,8-cineol content, and on the other hand by the lowest percentage of the allo-aromadendrene epoxide, eudesma-4(15),7- dien-1-ß-ol and viridiflorol (see Table 3 and Fig. 2).

The highest canonical discriminant function in the population P3 centroid (2.21) is related to the third canonical discriminant function which showed a result of 8.6% of total variance. This population formed a distinct chemotype characterized by the highest percentage of 14-hydroxy-(*Z*)-caryophyllene and high content of two oxygenated monoterpenes; terpinen-4-ol and α-terpineol (also see Table 3). The three most recent compounds were among the variables that represented the largest absolute correlation with the third canonical discriminant function. In general, the plot established according to the first two canonical discriminant functions based on the individual plant essential oils suggests the existence of four chemotypes (Fig. 2).

It can thus be inferred that the geographical distribution of some oil chemotypes in this narrow distribution area could be explained to be due to the high intraspecific genetic variation associated with low gene flow levels throughout the populations (29). Besides, the presence of various chemotypes indicates that the chemical variation could result from the adaptation to complex biotic interactions, as those found to influence the chemical variability in thyme (30). However, the environmental factors and the short distance from the urban center appear to be the most important factors that affect the biochemical similarity of populations P5 and P1. Moreover, this may imply that the latitude together with altitude could be a factor influencing the plant chemistry. Therefore, the P1 population at the highest latitude along with highest altitude P5 and population at the lowest latitude combined with the lowest altitude exhibited the highest level of oxygenated monoterpenes. This finding concurred with the different latitudinal populations of *Teucrium polium* (8).

Conclusion

The *F. undulata* essential oils in this study are characterized by high levels of oxygenated mono- and sesquiterpenes. The completely different essential oil components of this plant species compared with most species of the genus Pulicaria can verify the separation of this species from the genus Pulicaria.

On the other hand, significant differences were observed in the main components of the *F. undulata* essential oils from five different locations showing four different chemotypes in the five populations. Although some individual populations are quite isolated from one another in the middle of the region, similar variations exist in the oil composition among the populations occurring at the extremes of the geographical distribution range. These findings revealed a high intraspecific genetic variation in *F. undulata*. Moreover, the existence of *trans-methyl dihydrojasmonate* and high oxygenated mono- and sesquiterpenes levels in the essential oils of the plant indicates a high degree of defense in *F. undulata*. This is probably one of the reasons that this plant thrives in areas devoid of other plant species.

Consequently, this can provide opportunities for appropriate developmental strategies, genetic resource management and the selection of *F. undulata* as a suitable candidate for preservation of the germplasm and genetic amelioration of sensitive plants.

Acknowledgement

We are grateful to Mrs. Sheryl Nikpoor for the editorial comments and English revision. We warmly thank Dr. A. Borjian and A. Hatami for plant material identification.

REFERENCES

1. Anderberg, A.A., Eldenäs, P., Bayer, R.J. and Englund, M. (2005) Evolutionary relationships in the Asteraceae tribe Inuleae (incl. Plucheeae) evidenced by DNA sequences of *ndh* F; with notes on the systematic positions of some aberrant genera. *Organisms Diversity & Evolution*, **5**, 135-146.
2. Dendougui, H., Benayache, S., Benayache, F. and Connoly, J.D. (2000) Sesquiterpene lactones from *Pulicaria crispa*. *Fitoterapia*, **71**, 373-378.
3. Zareh, M. (2005) Systematic and anatomical studies of Inuleae and Plucheeae in Egypt. *Feddes Repertorium*, **116**, 43-53.
4. Rechinger, K. (1982) *Flora Iranica: no. 145: 120*. Akademische Druck, Graz.
5. Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. (2008) Biological effects of essential oils–A review. *Food and Chemical Toxicology*, **46**, 446-475.
6. Langenheim, J.H. (1994) Higher plant terpenoids: a phytocentric overview of their ecological roles. *Journal of chemical ecology*, **20**, 1223-1280.
7. Batish, D.R., Singh, H.P., Kohli, R.K. and Kaur, S. (2008) Eucalyptus essential oil as a natural pesticide. *Forest Ecology and Management*, **256**, 2166-2174.
8. Sadeghi, H., Jamalpoor, S. and Shirzadi, M.H. (2014) Variability in essential oil of *Teucrium polium* L. of different latitudinal populations. *Industrial Crops and Products*, **54**, 130-134.
9. McKiernan, A.B., O'Reilly-Wapstra, J.M., Price, C., Davies, N.W., Potts, B.M. and Hovenden, M.J. (2012) Stability of plant defensive traits among populations in two Eucalyptus species under elevated carbon dioxide. *Journal of chemical ecology*, **38**, 204-212.
10. Bohlmann, F., Knoll, K.-H. and El-Emary, N.A. (1979) Neuartige sesquiterpenlactone aus *Pulicaria crispa*. *Phytochemistry*, **18**, 1231-1233.
11. Bohlmann, F., Ahmed, M. and Jakupovic, J. (1982) Caryophyllane derivatives from *Pulicaria scabra*. *phytochemistry*, **21**, 1659-1661.
12. Abdel-Mogib, M., Jakupovic, J., Dawidar, A., Metwally, M. and Abou-Elzahab, M. (1990) Sesquiterpene lactones and kaurane glycosides from *Francoeuria crispa*. *Phytochemistry*, **29**, 2581-2584.
13. Stavri, M., Mathew, K., Gordon, A., Shnyder, S.D., Falconer, R.A. and Gibbons, S. (2008) Guaianolide sesquiterpenes from *Pulicaria crispa* (Forssk.) Oliv. *Phytochemistry*, **69**, 1915-1918.
14. Hanbali, F.E., Akssira, M., Ezoubeiri, A., Mellouki, F., Benherraf, A., Blazquez, A.M. and Boira, H. (2005) Chemical composition and antibacterial activity of essential oil of *Pulicaria odora* L. *Journal of Ethnopharmacology*, **99**, 399-401.
15. Znini, M., Cristofari, G., Majidi, L., Paolini, J., Desjobert, J. and Costa, J. (2013) Essential oil composition and antifungal activity of *Pulicaria mauritanica* Coss., against postharvest phytopathogenic fungi in apples. *LWT-Food Science and Technology*, **54**, 564-569.
16. EL-Kamali, H.H., Yousif, M.O., Ahmed, O.I. and Sabir, S.S. (2009) Phytochemical analysis

of the essential oil from aerial parts of Pulicaria undulata (L.) Kostel from Sudan. *Ethnobotanical Leaflets*, **13**, 467-471.

17. Ali, N., Sharopov, F.S., Alhaj, M., Hill, G.M., Porzel, A., Arnold, N., Setzer, W.N., Schmidt, J. and Wessjohann, L. (2012) Chemical composition and biological activity of essential oil from Pulicaria undulata from Yemen. *Natural Product Communications*, **7**, 257-260.
18. Joulain, D., König, W.A. and Hochmuth, D.H. (2001) Terpenoids and related constituents of essential oils. Library of MassFinder, 2.1. *Acta Pharm*, **57**, 90-123.
19. Adams, R.P. (2007) *Identification of essential oil components by gas chromatography/mass spectrometry*. Allured Publishing Corporation, Illinois.
20. McLafferty, F.W., Stauffer, D.B., Stenhagen, E. and Heller, S.R. (1989) *The Wiley/NBS registry of mass spectral data*. Wiley New York.
21. Algabr, M., Ameddah, S., Menad, A., Mekkiou, R., Chalchat, J., Benayache, S. and Benayache, F. (2012) Essential oil composition of *Pulicaria jaubertii* from Yemen. *International Journal of Medicinal and Aromatic Plants*, **2**, 688-690.
22. Englund, M., Pornpongrungrueng, P., Gustafsson, M.H. and Anderberg, A.A. (2009) Phylogenetic relationships and generic delimitation in Inuleae subtribe Inulinae (Asteraceae) based on ITS and cpDNA sequence data. *Cladistics*, **25**, 319-352.
23. Knobloch, K., Pauli, A., Iberl, B., Weigand, H. and Weis, N. (1989) Antibacterial and antifungal properties of essential oil components. *Journal of Essential Oil Research*, **1**, 119-128.
24. Javadinamin, A. and Asgarpanah, J. (2014) Essential Oil Composition of Francoeuria undulata (L.) Lack. Growing Wild in Iran. *Journal of Essential Oil Bearing Plants*, **17**, 875-879.
25. Farmer, E.E. and Ryan, C.A. (1990) Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proceedings of the National Academy of Sciences*, **87**, 7713-7716.
26. Sadeghi, H., Tahery, Y. and Moradi, S. (2013) Intra-and inter-specific variation of turpentine composition in Eldar pine (*Pinus eldarica* Medw.) and black pine (*Pinus nigra* Arnold). *Biochemical Systematics and Ecology*, **48**, 189-193.
27. Phillips, M.A. and Croteau, R.B. (1999) Resin-based defenses in conifers. *Trends in Plant Science*, **4**, 184-190.
28. Yazdani, R., Rudin, D., Alden, T., Lindgren, D., Harbom, B. and Ljung, K. (1982) Inheritance pattern of five monoterpenes in Scots pine (*Pinus sylvestris* L.). *Hereditas*, **97**, 261-272.
29. Muñoz-Bertomeu, J., Arrillaga, I. and Segura, J. (2007) Essential oil variation within and among natural populations of *Lavandula latifolia* and its relation to their ecological areas. *Biochemical Systematics and Ecology*, **35**, 479-488.
30. Linhart, Y.B., Keefover-Ring, K., Mooney, K.A., Breland, B. and Thompson, J.D. (2005) A chemical polymorphism in a multitrophic setting: thyme monoterpene composition and food web structure. *The American Naturalist*, **166**, 517-529.