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Original Research Article

Evaluation of the effects of different environmental factors on the quality and quantity of the essential oil of *Prangos ferulacea* (L.) Lindl.

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ABSTRACT

The present study was carried out to investigate the role of some environmental factors on the quality and quantity of the essential oils of *Prangos ferulacea* (L.) Lindl. grown in four different locations in Iran. Chemical constituents of the essential oil were extracted by hydrodistillation and were identified using GC-MS. Among identified compounds, thirty one compounds were jointly found in the plant EOs of all four sampling locations. Monoterpene hydrocarbons were the significant portion of the essential oils and α-pinene, β-pinene, δ-3-carene, germacrene B and α- humulene were the five main constituents of the plant EOs that were jointly found in EOs of four sampling places. According to obtained results, the amount of *cis*-β-farnesene, germacrene-B and thymol was enhanced by increasing the relative humidity in four sampling places. Moreover, the content of α-pinene and α-terpineol was significantly increased by enhancement of temperature and altitude of the locations.

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1. Introduction

Secondary metabolites of different plant species are a main source of natural products and can be used for different purposes such as food, perfumery and the production of new and effective drugs (Tepe et al., 2005; Bakkali et al., 2008; Roby et al., 2013). Essential oils (EOs) are mixtures of organic ingredients produced by aromatic plants' secondary metabolism and used as important therapeutic components (Bakkali et al., 2008). Due to their unique pharmacological properties, phytochemical investigations about the components have recently gained scientific interest. Hydrodistillation (HD), is the most used conventional technique for EOs extraction from the plants (Kant and Kumar, 2022). Although, due to its some limitations such as the requirement to energy and large amount of samples, time consuming and EO yield, advanced extraction techniques such as solvent free microwave extraction (SFME) (Mohammadhosseini 2015; Mohammadhosseini et al., 2015), solid-phase microextraction (SPME) (Mohammadhosseini and Nekoei, 2014; Torbati et al., 2016), sub-critical extraction liquid and supercritical fluid extraction (Pourmortazavi and Hajimirsadeghi, 2007) have been developed recently that are preferable in terms of low cost, time and energy saving, fewer solvent requirements and shorter extraction time (Kant and Kumar, 2022). *Prangos* sp. is a perennial genus of Apiaceae family and consists of 45 species that were distributed from Portugal to Tibet (Mottaghipisheh et al., 2020). The main anatomical and morphological characters of Apiaceae family are also found in *Prangos* species and the most distinctive morphological characteristics, separating the genus from others, is the number of mesocarp layers and its architecture (Lyskov et al., 2017). Mesocarp in *Prangos ferulacea* (L.) Lindl. divided into outer mesocarp "epimesocarp" (consisting of nonlignified cells) and

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inner mesocarp. The inner mesocarp is divided into five wholly separated parts and consist of parenchymatous cells with lignified walls (Lyskov et al., 2015). Other features such as the shape of fruits, wings and leaflets are also important taxonomically in the genus *Pangos*. Leaf primary segments in *P. ferulacea* are petiolulate and the length of the basal leaves is 30-50 cm. The plant species have umbels with 12-16 equal, terete, glabrous rays, 8–10 cm long and with 5-8 glabrous bracts similar to upper leaves (Lyskov et al., 2015). *Prangos* species have been commonly used in traditional medicine in the Mediterranean region and the Middle East. Indeed, they are known for their pharmacological effects including anti-flatulent (Mneimne et al., 2016), anti-haemorrhoidal (Mneimne et al., 2016), anthelmintic (Ulubelen et al., 1995), anti-spasmodic (Sadraei et al., 2012) and anti-microbial effects (Uzel et al., 2006; Razavi et al., 2010). These plants are also indicated for their anti-HIV effects and antioxidant activities (Shikishima et al., 2001; Çoruh et al., 2007). *P. ferulacea* commonly known as Djashir in Persian, is one of the five endemic *Prangos* species that grow in Iran with high distribution and high ethnobotanical uses (Hadavand Mirzaei et al., 2007; Ghorbani et al., 2014). For instance, aerial parts of the plant were traditionally used as laxative, anti-parasitic, anticancer and carminative agents (Azizi and Keshavarzi, 2015; Amiri and Joharchi, 2016). Phytochemical constituents of EOs of some *Prangos* species were reported in different studies, previously. For instance, α-pinene and *cis*-ocimene were reported as two main compounds in EOs of *P. ferulacea* (Razavi et al., 2010) and *epi*-globulol and β-elemene were introduced as two significant components in EOs of *Prangos scabra* Nabelek. (Nazemiyeh et al., 2007b). Moreover, it was reported that the EO produced by *Prangos pabularia* Lindl. leaves possess a considerable phytotoxic potential and might be used to control of weeds as a bioherbicide (Razavi, 2012b). The major constituents in the flower of *Prangos peucedanifolia* Fenzel. were β-pinene, α-pinene and β-phellandrene, while *m*-cresol was the main volatile fraction of the leaf (Brusotti et al., 2013). Nevertheless, to the best of our knowledge, this is the first study about the effects of environmental factors on quality and quantity of the EOs of *P. ferulacea*. Only in two studies about the EOs of *Prangos* genus, it was indicated that the chemical composition of EOs of the same species could change depending on a variety of the conditions (Akhlaghi 2015; Bagherifar et al., 2019). Hence, the present study was undertaken to evaluate the effects of some environmental factors such as altitude, temperature and relative humidity on composition and amount of ECs of *P. ferulacea* grown in northwest Iran.

2. Experimental

2.1. Plant materials and preparation of the extracts

Aerial parts of *P. ferulacea* populations at the fruiting stage were collected from four different locations (Ivand (S1), Liqvan (S2), Hashtrood (S3) from East Azerbaijan province and Hir (S4) from Ardabil province, Iran) in the summer of 2013. Fig. 1 and Table 1 show the characteristics of collection areas. Metrological information was obtained from applied metrology research center of East Azerbaijan province. The plants were identified by botanist, Dr. Sh. Esmailbegi Kermani, (a voucher specimen of the plants (MIR 3866-69) was preserved) using reliable botanical literature, including Flora Iranica (Rechinger 1963-2010) and then compared with other floras of the region, including Flora of Turkey (Davis 1984) and Flora of Iraq (Townsend and Guest 1966-1985). The plant samples were dried at room temperature in the shade and then powdered.
For the preparation and identification of essential oil composition, related to the collected plant samples from four different places, the samples (50 g) were separately submitted to 3 hours hydrodistillation using Clevenger-type apparatus. The obtained essential oils were dried over Na_2SO_4 and stored in sealed dark vials, at 4 °C up to GC-MS analysis.

2.2. Essential oil analysis

GC-MS analysis was performed using an Agilent 6890 gas chromatograph with a HP-5MS capillary column (30 m-0.25 mm; film thickness 0.32 μm) coupled with an Agilent 5973 mass spectrometer (Agilent Technologies, Palo Alto, Canada) operating in EI mode at 70 eV using the following temperature program: 70 °C for 5 min, 15 °C min^{-1} up to 120 °C and hold time 2 min and 20 °C min^{-1} up to 220 °C and hold time 3 min. The percentage composition was computed from the peak areas, without correction factors. The EOs were identified by matching their mass spectra and retention indices (RI) relative to n-alkane scale, both with those given in the literature and those stored in the Willey library.

2.3. Statistical analysis

The Pearson correlation coefficient by GraphPad software (GraphPad Software, Inc. USA) was used to measure the correlation between environmental variables and some of the plant EOs compounds content in subjected samples.

3. Results and Discussion

3.1. Chemical composition

Many various factors such as plant developmental stages (Oliveira et al., 2005; Sellami et al., 2009; Javazi et al., 2016), seasonal variation, type of plant secretory structures (Lattoo et al., 2006; Figueiredo et al., 2008), genetic factors (Mahdavi et al., 2013) and environmental conditions (Maksimović et al., 2007) can affect the production and composition of the plant EOs. As shown in Table 1, The essential oil contents (*v/w* %) of the plant samples obtained by hydrodistillation were 1.1, 0.7, 0.2 and 0.5% for the plants grown in S_1, S_2, S_3 and S_4 locations, respectively. In agreement with the results, the essential oil yields of 10 Iranian *P. ferulacea* were determined in the range of 0.5-1.6%, previously (Bagherifar et al., 2019). Identified components of EOs are presented in Table 2. The compounds were identified from EOs of the plants grown in S_1, S_2, S_3 and S_4 locations and monoterpene

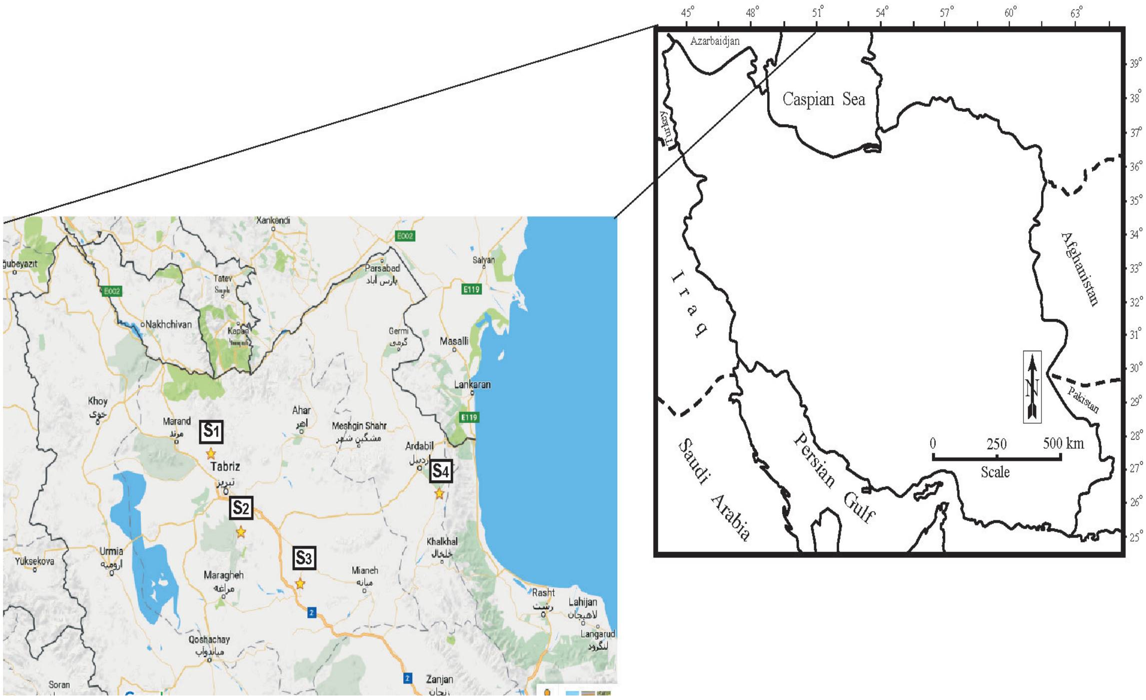

Fig. 1. Four different sampling locations of *P. ferulacea*.

hydrocarbons were the significant portion of EOs in the four sampling places (74.1%, 63.8%, 53.9% and 71.2% in EOs of S_1, S_2, S_3 and S_4, respectively). Thirty one compounds were jointly found in the plant EOs of all four sampling locations (Table 2). Sabinene (11.9%), β-ocimene (1.4%), α-cedrene (0.1%) and globulol (0.8%) were only found in S_3 and eremophilene (0.9%) and Myrtenyl acetate (0.1%) were identified just in EO of S_4 (Table 2). Therefore, the composition and yield of essential oil were affected by different ecological factors in the four sampling locations. In accordance with the obtained results, it was confirmed that EOs yield as well as oil composition of the same species were significantly affected by ecological conditions. The finding was also confirmed in *P. ferulacea* samples that were collected from locations in the Northeast and Southwest of Iran (Akhlaghi, 2015; Bagherifar et al., 2019). As shown in Table 2, α-pinene (23.1-29.9%), β-pinene (4.4-26.1%), δ-3-carene (2.6-6.6%), germacrene B (3.4-12.1%) and α-humulene (1.8-3.1%) were five main constituents of the plant EOs that were jointly found in EOs of four sampling places. Fig. 2 illustrates the changes in the content of the main components in the plant EOs in four sampling locations. The obtained results about the chemical profile of the plant EOs are in agreement with some other studies about the genus *Prangos*. For instance, α-pinene was the main compound of *Prangos turcica* A. Duran, M. Sagiroglu & H. Duman. (4.3%) (Özek et al., 2006), *Prangos asperula* Boiss (13.6%) (Hadavand Mirzaei and Ramezani, 2008), *P. pabularia* (4.2%) (Özek et al., 2007), *Prangos latiloba* Korovin. (25.5%) (Akhlaghi and Hashemi, 2005), *P. peucedanifolia* (22.1%) (Brusotti et al., 2013) and *Prangos uloptera* DC. (16.8%). α-pinene was also reported as the main compound of *P. ferulacea* collected from different locations with various contents (Bruno et al., 2019; Bruno et al., 2021; Masoudi et al., 2016; Mohammadhosseini, 2012; Razavi, 2012a). Moreover, β-pinene content was more than 10% in EOs of *P. latiloba*, *P. peucedanifolia* and *P. ferulacea* (Brusotti et al., 2013; Akhlaghi and Hashemi, 2005; Masoudi et al., 2016). Besides, the δ-3-carene was reported as a main component in some other species of *Prangos* such as *P. asperula* (Mneimne et al., 2016), *Prangos acaulis* (DC.) Bornm. (Hadavand Mirzaei et al., 2007), *Prangos uechtrizii* Boiss. et Hausskn. (Özcan et al., 2000) and in other previous studies about EOs of *P. ferulacea* (Delnavazi et al., 2017; Masoudi et al., 2016; Sajjadi et al., 2011). Germacrene B and α- humulene were identified as the main compound of EOs of *P. turcica* (10.6% and 11% respectively) (Özek et al., 2006), *P. pabularia* (5.7% and 16.6%, respectively) (Özek et al., 2007), *P. uloptera* (7.2% and 7.7%, respectively) (Nazemiyeh et al., 2007a).

3.1. Effect of environmental factors

The growth of plants in natural ecosystems and the production of their secondary metabolites in different areas are considerably influenced by environmental conditions (Figueiredo et al., 2008). Relative humidity is one of the important ecological factors that play a significant role in the content and feature of the plants' EO. As shown in Table 1, location S_3 had more percentage of relative humidity and S_1 had the lowest moisture content. According to obtained results, the amount of monoterpene hydrocarbons was decreased with increasing moisture in four sampling locations. In

Table 1
Specifications of sampling locations, mean of EOs yields (%) and the number of identified compounds of each locations.

Place of sampling	Longitude	Latitude	Altitude (m)	Average precipitation (mm)	Relative humidity (%)	Average temperature (°C)	Number of identified compounds	EOs yeilds (%)
Ivand (S1)	46° 14′ 30″	38° 41′ 22″	1785	32.2	50.3	12.3	48	1.1
Liqvan (S2)	46° 42′ 89″	37° 82′ 64″	2240	20.6	51.3	13.4	42	0.7
Hashtrood(S3)	47° 52′ 14″	37° 22′ 34″	1773	25.4	56.8	9.5	43	0.2
Hir (S4)	48° 52′ 45″	38° 88′ 49″	1520	20.1	51.1	8.2	45	0.5

contrast, the contents of sesquiterpene hydrocarbons and oxygenated sesquiterpenes were enhanced by increasing the moisture.

Fig. 3 illustrates the results of the linear regression between relative humidity and content of four molecular groups (monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes) of the EOs of *P. ferulacea*. The correlation coefficient between relative humidity and monoterpene hydrocarbons contents (R = – 0.9) indicated a negative correlation between their content and moisture of sampling locations. It is while the contents of sesquiterpene hydrocarbons and oxygenated sesquiterpenes were increased by enhancement of the relative humidity (R = 0.8 and 0.9, respectively) (Fig. 3). The obtained results are in accordance with some other studies that reported in different manners in monoterpenes and sesquiterpenes emission in response to moisture. For instance, the quantity of the emission of monoterpenes and sesquiterpenes in *Rosmarinus officinalis* L. with water stress was increased and decreased, respectively (Ormeño et al., 2007).

Moreover, according to obtained results, the content of β-pinene and α-phellandrene was reduced by enhancement of the humidity (Fig. 4). Furthermore, three compounds (*cis*-β-farnesene, germacrene-B and thymol) of the plant EO positively correlated with relative humidity (Fig. 4). It means that the amounts of these compounds were enhanced by increasing the moisture. The enhancement of the thymol content by increase of the moisture and altitude was previously confirmed about *Coridothymus capitatus* (L.) Reichenb. fil. and *Satureja thymbra* L. (Karousou et al., 2005). Moreover, the regions with warm and semi-humid climates were reported as the best locations for essential oil production in *Oliveria decumbens* Vent. with high amounts of thymol and carvacrol (Ale Omrani Nejad et al., 2019). Altitude is another important ecological factor that can affect the quantity and quality of the plant EOs and its prominent roles in plants' EO were established in some previous studies. For instance, it was found that the essential oil output of *Artemisia persica* Boiss. was dropped by increasing the altitude (Mohamadi and Rajaei, 2016). In other research, the essential oil level and the number of essential oil components of *Tanacetum polycephalum* (L.) Schultz-Bip. were increased by the increase altitude (Mahdavi et al., 2013). Moreover, temperature, humidity and altitude were introduced as the critical environmental factors in the composition and quantity of EOs of *Achillea milefolium* L. (Foroozeh and Mirdeylami, 2019). Despite the altitude, it was confirmed that the environmental temperature has important effects on the constituent of the plants EO (Ložienė and Venskutonis, 2005; Salgueiro et al., 1997). According to the metrological information (Table 1), altitude and temperature changes in four sampling locations had the same trend. The temperature was increased by enhancement of the altitude in studied locations. Table 3 shows the correlation between the content of some compounds of EOs and altitude/temperature. Accordingly, there were significant positive correlations between the contents

Table 2
Chemical composition of P. ferulacea essential oil in four different sampling locations.

No.	Constituents [a]	Formula	KI [b]	Content (%)			
				Ivand	Liqvan	Hashtrood	Hir
1	α-Thujone	$C_{10}H_{16}$	928	0.1	0.1	0.1	0.1
2	α-Pinene	$C_{10}H_{16}$	931	27.2	30	25.4	23.1
3	Sabinene	$C_{10}H_{16}$	964	-	-	11.9	-
4	β-Pinene	$C_{10}H_{16}$	970	26.1	20.8	4.4	24.6
5	α-Phellanderene	$C_{10}H_{16}$	997	3.6	0.4	-	3.1
6	δ-3-Carene	$C_{10}H_{16}$	1005	5.9	4.9	2.6	6.6
7	*p*-Cymene	$C_{10}H_{16}$	1011	9.6	0.6	1.8	2.5
8	D-Limonene	$C_{10}H_{16}$	1020	-	3.1	1	-
9	β-Phellanderene	$C_{10}H_{16}$	1030	-	-	3.8	7.2
10	β-trans-Ocimene	$C_{10}H_{16}$	1034	0.1	-	-	1.3
11	β-Ocimene	$C_{10}H_{16}$	1041	-	-	1.4	-
12	γ-Terpinene	$C_{10}H_{16}$	1047	0.2	0.2	1.1	1.2
13	α-Terpinolene	$C_{10}H_{16}$	1078	1.3	3.7	0.4	1.5
Monoterpene hyrocarbones (%)				**74.1**	**63.8**	**53.9**	**71.2**
14	*m*-Cresol	C_7H_8O	1053	0.6	0.6	-	0.8
15	α-Campholenal	$C_{10}H_{16}O$	1114	0.2	0.3	0.3	0.2
16	Verbenol	$C_{10}H_{16}O$	1128	1.4	2	1.3	0.8
17	α-Phellanderen,8-ol	$C_{10}H_{16}O$	1140	0.1	-	0.4	0.1
18	Pinocarvone	$C_{10}H_{16}O$	1158	5.2	0.9	-	0.8
19	4-Terpinenol	$C_{10}H_{16}O$	1161	0.2	0.2	0.9	0.2
20	α-Terpinenol	$C_{10}H_{16}O$	1172	0.5	0.7	0.4	0.2
21	Myrtenal	$C_{10}H_{16}O$	1187	0.7	0.1	-	0.2
22	*trans*-Carveol	$C_{10}H_{16}O$	1206	0.1	0.3	0.2	0.1
23	Propanal-methyl-phenyl	$C_{10}H_{16}O$	1245	0.1	-	0.1	-
24	Thymol	$C_{10}H_{16}O$	1266	-	1.3	1.5	0.8
25	Carvacrol	$C_{10}H_{16}O$	1278	0.1	0.1	0.1	0.2
26	Myrtenyl acetate	$C_{10}H_{16}O$	1299	-	-	-	0.1
Oxygenated monoterpenes (%)				**9.3**	**6.5**	**5.2**	**4.5**
27	Copaene	$C_{15}H_{24}$	1376	-	0.2	0.1	0.2
28	β-Burbonene	$C_{15}H_{24}$	1386	0.2	-	0.2	0.1
29	β-Elemene	$C_{15}H_{24}$	1387	0.5	0.2	0.5	0.4
30	α-Cedrene	$C_{15}H_{24}$	1405	-	-	0.1	-
31	Caryophyllene	$C_{15}H_{24}$	1424	0.2	0.2	0.4	0.1
32	Elixene	$C_{15}H_{24}$	1431	0.1	0.1	0.3	0.1
33	α-Guaiene	$C_{15}H_{24}$	1436	0.1	0.1	1.2	0.1
34	*cis*-β-Farnesene	$C_{15}H_{24}$	1449	0.3	0.2	0.5	0.2
35	α-Humulene	$C_{15}H_{24}$	1456	1.7	2.4	1.8	3.1
36	γ-Muurolene	$C_{15}H_{24}$	1471	0.3	0.3	0.8	0.2
37	Germacerence-D	$C_{15}H_{24}$	1480	1	1	2.3	1.8
38	Bicyclogermacerene	$C_{15}H_{24}$	1496	0.3	-	0.6	0.3
39	α-Selinene	$C_{15}H_{24}$	1500	0.8	1.3	0.5	1.3

Table 2 Continued.

No.	Constituents [a]	Formula	KI [b]	Content (%)			
				Ivand	Liqvan	Hashtrood	Hir
40	δ-Cadinene	$C_{15}H_{24}$	1514	0.4	0.4	3.2	0.8
41	α-Bisabolene	$C_{15}H_{24}$	1518	0.5	8.1	1.4	3.3
42	Globulol	$C_{15}H_{24}$	1530	-	-	0.8	-
43	Germacerene-B	$C_{15}H_{24}$	1554	3.4	7.1	12.1	6.1
Sesquiterpene hydrocarbons (%)				**9.8**	**21.6**	**26.8**	**18.1**
44	Germacerene-D-4-ol	$C_{15}H_{26}O$	1570	0.2	0.9	1.7	2.1
45	τ-Muurolol	$C_{15}H_{26}O$	1628	0.1	1.3	1.3	0.9
46	α-Cadinol	$C_{15}H_{26}O$	1641	0.1	-	0.8	0.4
47	α-(-)-Bisabolol	$C_{15}H_{26}O$	1660	0.3	0.9	2.1	-
48	Eremophilene	$C_{15}H_{26}O$	1486	-	-	-	0.9
49	Isoaromanderene epoxide	$C_{15}H_{24}O$	1731	0.1	0.6	2.1	0.8
Oxygenated sesquiterpenes (%)				0.8	3.7	8	5.1
Total identified content (%)				**94**	**95.6**	**93.9**	**98.9**
Others (%)				**6**	**4.4**	**6.1**	**1.1**

[a]The constituents were identified according to their retention indices (related to *n*-alkanes scale) on an HP-5MS capillary column; [b]Kovatz retention indices (KI) given in the literature.

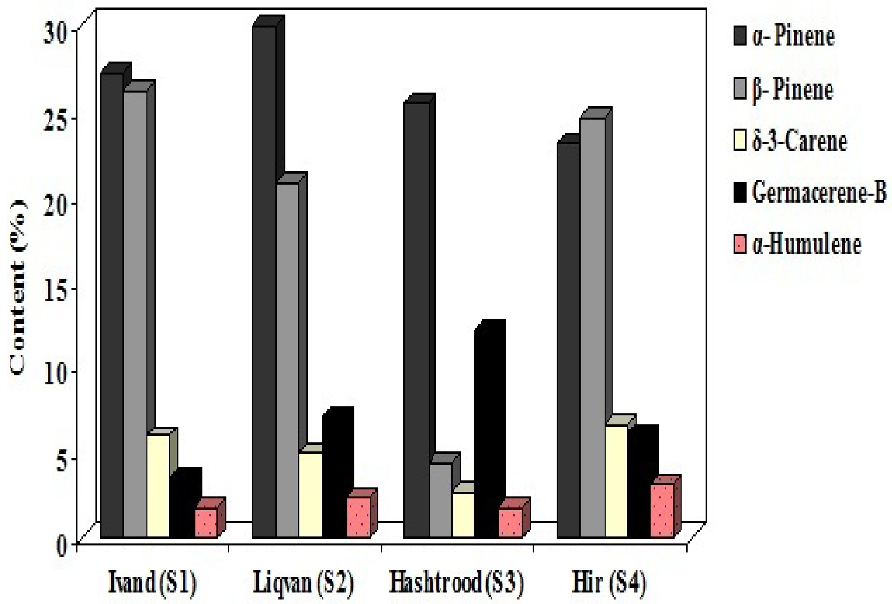

Fig. 2. Changes in the content of five main components of *P. ferulacea* essential oil in four sampling places.

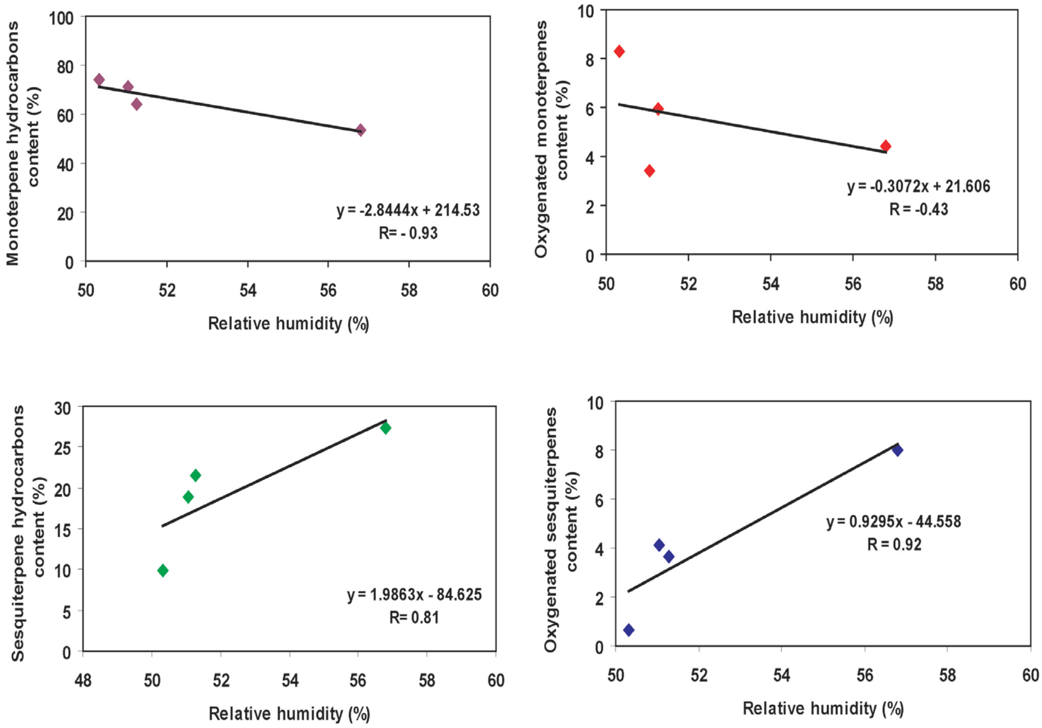

Fig. 3. The effect of relative humidity on the content of four molecular groups (monoterpene hydrocarbones, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes) of the EOs of *P. ferulacea*.

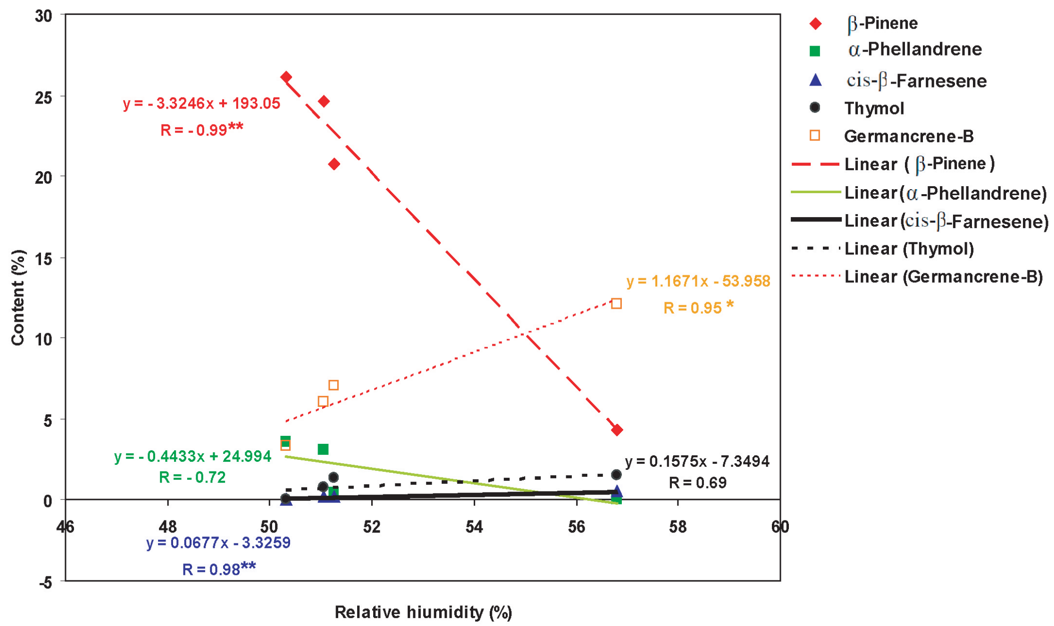

Fig. 4. The correlation between the relative humidity and content of some EO components of *P. ferulacea*.

Table 3
Correlation between the content of some compounds of the plant EOs with altitude and temperature of sampling locations.

Compounds	Correlation (R) by altitude (m)	Correlation (R) by temperature (°C)
α-Pinene	0.96*	0.97*
α-Terpineol	0.97*	0.93*
Verbenol	0.99**	0.9

* Correlation is significant at the 0.05 level.; ** Correlation is significant at the 0.01 level.

the correlation between these compounds' content and temperature, was also significant (Table 3). Actually, by enhancement of altitude and temperature the percentage of α-pinene and α-terpineol was notably increased. In addition, there was a positive relationship between the amount of verbenol and altitude (Table 3).

4. Concluding remarks

The compounds of essential oil of *P. ferulacea* were identified for S_1, S_2, S_3 and S_4 sampling places. The composition of EOs was dominated by the monoterpene hydrocarbons in all four sampling places. α-pinene, β-pinene, δ-3-carene, germacrene B and α- humulene were the five main constituents of obtained the EOs from the plants grown in the four different places. The chemical composition of the EOs and investigation of correlations between examined environmental factors and some of EOs compounds quantities showed notable effects of the environmental factors on the features and content of the plant EOs. For instance, the content of monoterpene hydrocarbons was decreased by enhancement of the relative humidity.

Conflict of interest

The authors declare that there is no conflict of interest.

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