

Effects drying and harvest season on the essential oil composition from foliage and berries of *Juniperus excelsa*

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ABSTRACT

The volatile constituents of the foliage and berries (female cones) of *Juniperus excelsa* trees from Iran were analyzed by GC and GC–MS. Twenty six constituents were identified and quantitated. Considerable seasonal variation was found in the berry oils, in contrast with the foliage oils. The oil yields in berries increased by 162% from spring to autumn as the berries matured. The amount of α -pinene decreased in the foliage oil during the summer; however it increased in the berry oil. Seasonal variation of other constituents showed various patterns. Seasonal variation in the berry oil was much greater than in the foliage oil. A comparison of oils from fresh vs. dried foliage and berries revealed changes in the amounts of several constituents. α -Pinene decreased after drying, whereas the amounts of several other constituents in the foliage and berry oils increased. This study shows that for the industrial utilization of *J. excelsa* berry oils, the harvesting period is a critical factor. However, either fresh or dried foliage can be utilized for leaf oil production.

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1. Introduction

The genus *Juniperus* (Cupressaceae) contains more than 60 species is quite widespread in the northern hemisphere (Adams, 2008). The Flora of Iran (Assadi, 1998) lists six *Juniperus* species (*J. communis* L., *J. excelsa* M.-Bieb., *J. foetidissima* Willd., *J. oblonga* M.-Bieb. (= *J. communis* in Adams, 2008), *J. oxycedrus* L., *J. sabina* L.). Farjon (2005) maps *J. excelsa* subsp. *excelsa* in northwestern and northern Iran and *J. e.* subsp. *polycarpus* K. Koch. in both northeastern and southern Iran. Adams (2008) maps *J. excelsa* in northwestern Iran, *J. polycarpus* K. Koch var. *polycarpus* in northwestern and southern Iran, and *J. polycarpus* var. *turcomanica* (B. Fedtsch.) R.P. Adams in northeastern Iran (and neighboring Turkmenistan).

Among these, *J. excelsa* grows on the mountainous regions of Iran. Analytical data on volatile components of foliage oils of *J. excelsa* has been reported by Sadri and Assadi (1994). However, no information is available on the berry oil composition or season variation. It should be noted that the use of 'berry' or 'berries' oil is commonly applied to commercial juniper oil, but, of course, 'berry' is a misnomer as the 'berry' is actually a seed cone (often called female cone). *Juniperus*, being a conifer, does not produce true berries, but seed and pollen cones. However, in congruence

with common nomenclature the terms 'berry' and 'berries' will be used for seed cones.

The potential applications of juniper oils include aromatherapy, mood scents, room fresheners, scent masks, insect repellents, soaps and candles, cosmetics and fragrances, lotions and creams and naturopathic remedies (Yesenofski, 1996). Furthermore, there has been considerable local interest in this species for the commercial production of essential oil. Information on the effects of seasonal variation on oil composition is crucial to optimize harvesting protocols.

The quantity and quality of volatile oils reflect the influence of genetic as well as certain non-genetic variables such as climatic and edaphic conditions (Fluck, 1963; Powell and Adams, 1973; Adams et al., 1992). The season and even the number of hours of sunlight may influence the phytochemistry of the plant, because some compounds may be accumulated at a particular period to respond to environmental changes (Koenen, 2001). Burbott and Loomis (1967) suggested that, in peppermint, the monoterpenes are utilized as substrates for energy metabolism when more suitable stored substrates become depleted within the secretory cells.

Several investigations on seasonal variations in the volatile oil composition in *Juniperus* have been reported. The results are contradictory and reflect strongly the taxa and tissues examined. A year long seasonal variation analysis of the volatile oil extracted from the foliage of *J. ashei* tree sampled at monthly interval throughout a 1-year period (Adams, 1969) and *J. pinchotii* (Adams, 1970) revealed that the relative percentage of 25 of 70 terpenoids com-

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Table 1
Soil characteristics at study site.

Depth (cm)	EC (ds m ⁻¹)	pH	Sand (%)	Clay (%)	Silt (%)	Texture
0–20	0.81	7.21	20.72	34.28	45	Sandy/clay/silty
20–40	0.20	7.33	14.72	51.44	33.84	Clay
40–60	0.14	7.26	14.72	51.44	33.84	Clay

posing the foliage volatile oil were quite significantly different when summer and winter collections were compared. Tatro et al. (1973) in a study of the volatile oil of *J. occidentalis*, *J. osteosperma* and *J. californica* concluded that significant seasonal variation was not occurring in these taxa, yet they present evidence that diurnal variations occurred. Powell and Adams (1973) found significant seasonal variations in both the relative percentage and weight data for oil components from the foliage of *J. scopulorum*.

It is a well accepted that plant drying introduces flavor changes to the oil (Bartly and Jacobs, 2000; Achak et al., 2008) and there may be a substantial loss of oil during the drying operation. Thus, it is important to understand the differences in the essential oils from fresh and dried berries and foliage of *J. excelsa* in determining the potential of these oils for commercial utilization.

The purposes of this study were to: (1) analyze the volatile oil compositions from different plant organs (foliage, berries), (2) determine the amount of seasonal variation in the volatile oil composition of both foliage and berries of *J. excelsa* and (3) report on changes in the volatile oil compositions fresh vs. dried foliage and berries of *J. excelsa*.

2. Materials and methods

2.1. Plant materials

The samples were taken from three permanently tagged *J. excelsa* trees in a natural stand on the southern slope of the Alborz (Elburz) Mountains, Iran, 2000 m, at 11 am on each sampling date. Voucher specimens are deposited at the herbarium, Research Institute of Forests and Rangelands, Tehran, Iran (Salehi Shanjani 95113). Soil and climatic characteristics of studied area are shown in Table 1 and Fig. 1, respectively. For the study of seasonal variation, foliage and berries were collected from 3 trees in three different seasons (April, August and November) and dried in room temperature (in shade) for a week, respectively. For the comparison of volatile fresh and dried foliage and berries oils constituents,

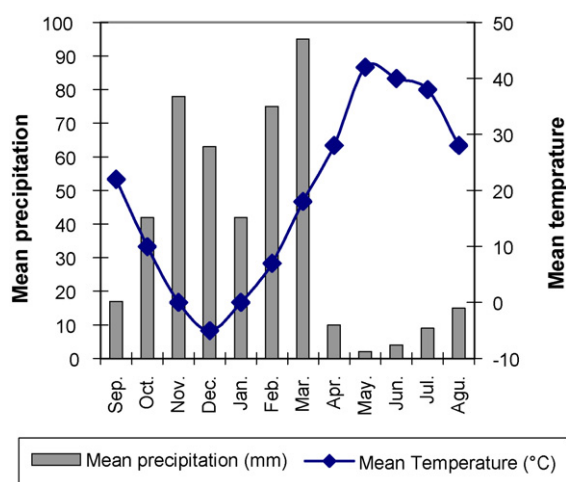


Fig. 1. Graphs of temperature (°C) and precipitation (mm) throughout the study year at the collection site. Seasonal samples were taken in April, August and November (notice the extreme drought after).

a portion of the fresh plant material was extracted and analyzed immediately and equal portion dried at room temperature for 1 week, then extracted and analyzed.

2.2. Isolation and yield of the essential oil

The volatile oils were isolated by steam distillation (200 g foliage/100 g berries) for 1 h. The lighter than water, slightly yellow oils were dried over anhydrous Na₂SO₄ and stored under refrigeration (+4 °C). Oil yields were determined on an oven-dry weight basis (48 h in 70 °C).

2.3. Gas chromatography

GC analysis was performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was 50 °C for 5 min and then programmed to 270 °C at a rate of 4 °C/min, injector and detector (FID) temperature were 280 °C, carrier gas: helium with a linear velocity of 32 cm/s.

2.4. Gas chromatography and mass spectrometry

Mass spectral analyzes were performed on a Varian Ion Trap (ITD) mass Spectrometer, Saturn II, directly coupled to a Varian 3400 gas chromatograph, using a J&W DB-5, 30 m × 0.25 mm (0.25 μm film thickness) fused silica capillary column. Oven temperature: 60–260 °C (4 °C/min); injection temperature: 220 °C.

2.5. Identification of components

The components of both oils were identified by comparison of their mass spectra with those of a computer library (Adams, 2007), comparison with published spectra (Pand and Bicchi, 1987) or by co-elution with authentic compounds.

2.6. Statistical analysis

Differences in the percentages of individual compounds of foliage and berries during different seasons and in fresh vs. dried samples were analyzed using one-way repeated measures ANOVA using the SAS statistical software package (SA System 6.12, SAS institute Inc., Cary, NC). Means of foliage and berries oil components were compared by Duncan multiple range tests ($p < 0.05$). Variations of foliage and berries compounds among individuals were analyzed by Principal Components Analysis (PCA) using NTSYS-pc 2.02e package (Rohlf, 2004). PCA was also used to reveal the relationships among oils from dried and fresh oils.

3. Results and discussion

The yields of essential oils from the foliage varied from spring (0.6%), summer (0.5%) to autumn (0.85%). These changes may be due to growth during the growing season. The increase in oil concentration parallels is similar to *J. scopulorum* in which Powell and Adams (1973) found the concentration of leaf oils to be lowest in the spring and summer, then increasing in the autumn. The berry oil yields varied considerably from spring (0.8%), summer (1.8%) to

Table 2Seasonal variation in the essential oil compositions (% total oil) obtained from air dried foliage and berries of *J. excelsa* sampled in spring, summer or autumn.

Compound	RI	Avg. value in foliage			F-value	Avg. value in berries			F-value
		Spring	Summer	Autumn		Spring	Summer	Autumn	
α -Pinene	935	67.3 a	14.2 c	56.6 b	119.8**	15.2 c	75.6 a	57.2 b	906.5**
β -Pinene	947	1.1 a	0 b	0.96 a	184.3**	0 b	1.6 a	2.0 a	9.4*
Myrcene	986	2.6 a	0.5 b	2.3 a	10.7*	0 b	3.5 a	4.2 a	6.3*
Limonene	1025	2.1 a	1.5 a	2.1 a	0.6 ns	0 b	1.5 ab	3.1 a	5.4*
γ -Terpinene	1057	0.2 a	0 a	0 a	1.0 ns	0 b	0.4 b	3.2 a	21.35*
Terpinolene	1087	0.8 a	0 b	0.7 a	3.98 ns	0 b	1.6 a	0 b	30.72**
Linalool	1099	0 b	1.9 a	0 b	17.8**	0	0	0	
α -Campholenal	1125	1.2 a	1.1 a	1.5 a	0.31 ns	5.3 a	0 c	1.5 b	77.2**
Trans-pinocarveol	1139	1.4 b	3.4 a	1.7 b	71.6**	10.4 a	0 b	1.1 b	84.6**
Cis-verbenol	1140	0.8 a	1.7 a	1.5 a	0.2 ns	5.6 a	0.7 b	0 b	57.9**
Trans-verbenol	1144	5.9 c	16.3 a	7.9 b	100.1**	24.1 a	0 c	4.6 b	187.9**
Pinocaryvone	1163	0	0	0		2.8 a	0 b	0 b	14.9**
Cymen-8-ol (p-)	1185	0	0	0		4.2 a	0 b	0 b	65.3**
Myrtenal	1196	0	0	0		2.3 a	0 b	0 b	13.1**
Verbenone	1208	3.6 b	5.7 a	4.3 ab	3.2 ns	9.3 a	0 b	1.7 b	65.0**
Trans-carveol	1219	0.9 b	2.4 a	0.8 b	11.5**	3.6 a	0 b	0 b	69.1**
Hexyl 2-methyl butyrate	1235	0.3 a	1.2 a	0.4 a	0.47 ns	0	0	0	
Bornyl-acetate	1285	0.4 b	2.4 a	0 b	11.8**	3.4 a	0.8 b	1.4 b	6.4*
Cumin alcohol	1303	0.3 a	1.2 a	0.7 a	0.28 ns	0	0	0	
Methyl carvacrol	1344	1.5 a	6.3 a	2.0 a	1.7 ns	0	0	0	
β -Caryophyllene	1414	1.4 a	3.3 a	1.2 a	3.2 ns	0 c	1.4 b	2.8 a	13.5**
Germacrene D	1478	1.4 a	3.3 a	2.1 a	2.9 ns	0 c	1.5 b	2.9 a	13.4**
γ -Cadinene	1516	0.3 a	0 a	0.6 a	0.59 ns	0	0	0	
Δ -Cadinene	1526	0.6 a	2.3 a	1.9 a	1.2 ns	0 b	0.8 a	0.0 b	7.7*
Elemol	1552	0.5 b	2.8 a	0.3 b	25.5**	6.3 a	1.0 b	1.5 b	29.1**
Germacrene B	1559	4.4 b	13.9 a	7.1 ab	6.0*	8.2 b	7.2 b	11.6 a	6.5*

RI = retention index, Kovat's index on DB-5 column (see Adams, 2007); F-value: between groups variance/within groups variance; a, b, c: values followed by different letters differ significantly within each column at $\alpha = 0.05$ according to the Duncan multiple range test; ns: not significant.

* Significant at 0.05.

** Significant at 0.01.

autumn (2.1%). Of course, the berries are very small in the spring as pollination occurs in the spring for *J. excelsa*. The changes in oil yields are likely due to the ripening cycle of the berries. This trend is in contrast with results reported for female berry oil of the shrubby juniper species, *J. communis*, by Chatzopoulou and Katsiotis (1993). However, it should be noted that the berries of *J. excelsa* ripen after 1 year, whereas the berries of *J. communis* ripen in about 1/2 years. It is not clear from Chatzopoulou and Katsiotis (1993) if they were able to analyze only the 1-year-old berries.

GC and GC-MS analysis resulted in the identification of 26 components in the fresh and dried foliage and berries essential oil at different seasons (Tables 2 and 3).

3.1. Seasonal variation in the volatile oil components

ANOVA for 26 non-trace components (Table 2) revealed significant F ratios for 10 compounds in the foliage oils and 21 compounds in the berry oils. Duncan multiple range tests showed different suites of components are significantly different in foliage and berries oil (Table 2). β -Pinene and myrcene were highest in essential oil in spring (for foliage) and summer (for berries). Trans-pinocarveol, trans-verbenol, trans-carveol, bornyl-acetate and elemol, were highest in the summer (for foliage) and spring (for berries). PCA, based on analytical data of foliage and berries oils for different seasons, showed that almost all the juniper samples were clearly separated according the oil source (foliage, berries) and collection season (Fig. 2).

Patterns of seasonal variation in essential oils of foliage and berries are different. In the berry oil, α -pinene increased from spring (15.2%) to summer (75.8%) then decreased in autumn (54.6%). However, in the foliage oil, a considerable decrease was observed in α -pinene during the growing season (67.3%, spring–14.2%, summer). Although α -pinene (and other monoterpenes) declined in the leaf oil during the summer (as temperatures

increased, see Fig. 1), the decline is far larger than the decline in yields (0.6% spring, 0.5% summer). It seems more likely that biosynthesis/catabolism is also involved in the large change in α -pinene. The amounts of oxygenated terpenes and sesquiterpenes increased during the growing season (e.g. trans-verbenol, and germacrene B, Table 2). The concentrations of the main components of berry oil changed considerably during maturation. α -Campholenal, trans-pinocarveol, cis-verbenol, trans-verbenol, verbenone and trans-carveol were present in considerable amounts in the spring, but they decreased to trace amounts during the summer and autumn. In contrast, β -pinene, myrcene, limonene, γ -terpinene that showed different patterns (Table 2).

This study has demonstrated that the essential oils of *J. excelsa* foliage and berries exhibit seasonal changes in yields and compositions. Many factors affect essential oil yields and compositions such as harvest season, temperature and reproductive stage (Kamatou et

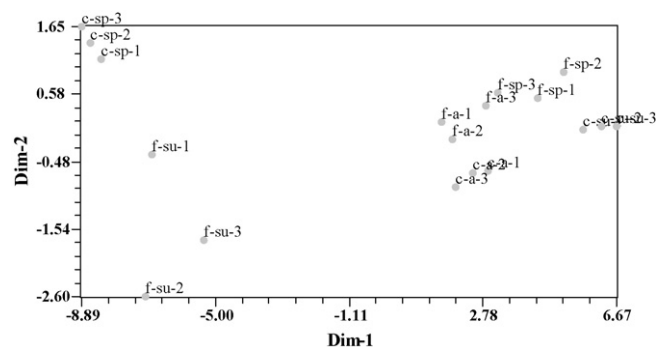


Fig. 2. Principal components analysis (PCA) plot based on berry and foliage essential oils compositions sampled at different seasons (c: berries, f: foliage, sp: spring, su: summer, a: autumn). The first two principal components (Dim-1 and Dim-2) account for 93.8% and 3.2% of the total variance, respectively.

Table 3
Comparisons of oils obtained from fresh vs. air dried foliage and berries of *J. excelsa* (% total oil) sampled in the summer.

Compound	RI	Avg. value in foliage		F-value	Avg. value in berries		F-value
		Fresh	Dry		Fresh	Dry	
α -Pinene	935	23.9 a	14.2 a	7 ns	83.7 a	75.6 b	17.65*
β -Pinene	947	0.37 a	0 a	1.0 ns	0.53 b	1.6 a	16.01*
Myrcene	986	1.7 a	0.53 a	1.4 ns	1.0 b	3.5 a	4.8*
Limonene	1025	4.0 a	1.5 b	10.8*	0.5 b	1.5 a	8.1*
γ -Terpinene	1057	0	0		0.2 a	0.4 a	1.1 ns
Terpinolene	1087	0	0		1.6 a	0.4 b	14.6*
Linalool	1099	1.3 a	1.9 a	0.6 ns	0	0	
α -Campholenal	1125	0.2 a	1.1 a	2.0 ns	0	0	
Trans-pinocarveol	1139	2.1 a	3.4 a	2.2 ns	0	0	
Cis-verbenol	1140	3.4 a	1.7 a	0.5 ns	0 b	0.7 a	10.8*
Trans-verbenol	1144	10.8 a	16.3 a	15 ns	0	0	
Verbenone	1208	4.6 a	5.7 a	0.3 ns	0	0	
Trans-carveol	1219	1.3 a	2.4 a	0.8 ns	0	0	
Bornyl-acetate	1285	3.1 a	2.4 a	0.3 ns	0.3 a	0.8 a	2.7 ns
Cumin alcohol	1303	1.3 a	2.4 a	2.8 ns	0	0	
Methyl carvacrol	1344	3.1 a	6.3 a	0.7 ns	0	0	
β -Caryophyllene	1414	2.3 a	3.3 a	0.7 ns	0.2 b	1.4 a	22.0**
Germacrene D	1478	2.4 a	3.3 a	0.4 ns	0.4 b	1.5 a	10.3*
γ -Cadinene	1516	1.8 a	0 a	1.0 ns	0	0	
Δ -Cadinene	1526	5.1 a	2.3 a	1.2 ns	0.3 a	0.8 a	2.3 ns
Elemol	1552	2.6 a	2.8 a	0.1 ns	0.4 a	1.0 a	3.2 ns
Germacrene B	1559	9.7 a	13.8	8 ns	0.7 b	7.2 a	137.2**

RI = retention index, Kovat's index on DB-5 column (see Adams, 2007); F-value: between groups variance/within groups variance; a, b, c: values followed by different letters differ significantly within each column at $\alpha = 0.05$ according to the Duncan multiple range test; ns: not significant.

* Significant at 0.05.

** Significant at 0.01.

al., 2008). Fluck (1963) suggested that some loss of the terpenoids can be expected from volatilization and resinification during the warm summer months. However, metabolism, catabolism and biosynthesis of volatile oil constituents may also explain the seasonal variation of essential oils (Adams, 1970; Adams and Powell, 1976).

3.2. Effects of drying leaves and berries on volatile oil composition

Comparison of the volatile oils from fresh vs. dried materials revealed that the drying has a greater impact on berry oils than foliage oil (Table 3). Analysis of components shows that α -pinene, the largest constituent of foliage and berry oils, decreased following drying from 23.9% in fresh foliage to 14.2% in dried foliage, and from 83.7% (fresh berries) to 75.6% (dried berries). With the loss of the very volatile α -pinene component during drying, the amounts of less volatile constituents in the foliage and berries oil increased after drying, especially trans-pinocarveol, trans-verbenol, trans-carveol and germacrene B (Table 3). However, limonene decreased

in the foliage oil upon drying (4.0–1.5%, Table 3) and increased in the berries oil upon drying (0.5–1.5%, Table 3). So volatility alone cannot explain all the changes. Germacrene B increased the berry oils upon drying (0.7–7.2; Table 3).

These results are in general agreement with other studies showing that the drying process introduces flavor changes to the product (Sankat and Maharaj, 1994; Venskutonis, 1997; Rao et al., 1998). Most of the berry oil constituents have significant differences following drying process, whereas few significant differences were found in the foliage oil constituents (Table 3).

PCA based on analytical data of fresh and dried foliage and berries revealed that the berry oils clustered by fresh vs. dried treatments. In contrast, the foliage oils clustered more by genotype (cf. Tree 1, f and d; Tree 2, f and d, Fig. 3). Achak et al. (2008) found a similar result in fresh vs. air dried foliage of *J. thurifera*.

4. Conclusion

Autumn is the most desirable season for harvesting both foliage and berry essential oils because yields reached their highest concentrations in autumn. Several of the components important in the aroma of the foliage oil (trans-verbenol, methyl carvacrol and germacrene B) reached their highest concentrations in the summer (16.3%, 5.7%, 14%, respectively). However, in berry oil, trans-pinocarveol, trans-verbenol and verbenone reached their highest concentrations in the spring (10.4%, 24%, 9.3%, respectively). For industrial utilization, if a particular aroma profile is desired, then both the harvesting season and the choice of fresh or dried materials of *J. excelsa* must be considered.

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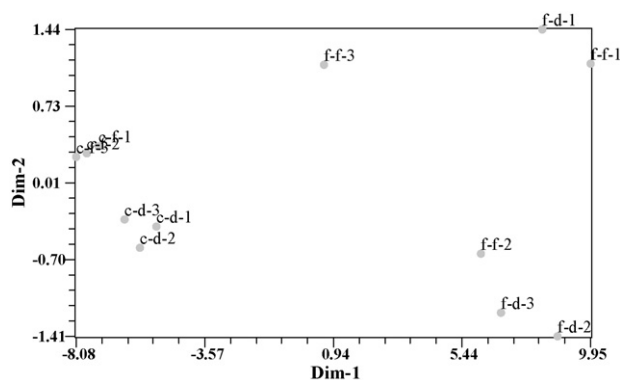


Fig. 3. Principal components analysis (PCA) plot of the essential oils' chemical composition from fresh and dried berries and foliage samples (c: berries, f: foliage, d: dried, f: fresh). The first two principal components (Dim-1 and Dim-2) account for 96.3% and 1.4% of the total variance, respectively.

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