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Comparative analysis of the composition of essential oils from the needles, twigs and berries of *Juniperus chinensis* L. in Korea

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ABSTRACT

The essential oil constituents from the needles, twigs and berries of *Juniperus chinensis* from Korea were investigated by gas chromatography-mass spectrometry (GC-MS). The essential oils from the different plant parts were obtained by steam distillation and the yields were 0.34, 0.11 and 0.12% (v/w), respectively. The GC-MS analysis revealed the identification of 36 different components from needles, twigs and berries, which were mostly monoterpene hydrocarbons (42.05-48.15%) followed by oxygenated monoterpenes (28.53-39.92%).Among the 36 components, 17 components were identified in all the three essential oils. The components such as bornyl acetate (2.85 – 20.70%), sabinene (10.23 – 18.13%), α-pinene (5.80 – 16.26%), terpinen-4-ol (5.98 – 31.10%), limonene (3.98 – 6.96%), β-pinene (3.05 – 4.39%), γ-terpinene (2.24 – 8.36%), α-elemol (1.74 – 4.77%) and α-cadinol (2.49 – 3.39%) were detected as the major components in the essential oils from the three different parts of *J. chinensis*. The main differences between the three essential oils can be referred to terpinen-4-ol and bornyl acetate. The essential oil of the berries contained the highest level of terpinen-4-ol (31.10%) than needles (7.51%) and twigs (5.98%). On the other hand, bornyl acetate content was very less in berries (2.85%) when compared with twigs (20.70%) and needles (16.43%).

INTRODUCTION

The genus *Juniperus* (Cupressaceae) comprises about 70 species of evergreen shrubs or trees, widely distributed in the Northern Hemisphere (Foudil-Cherif and Yassaa, 2012). The essential oils from the needles and berries of various *Juniperus* species have been used for cosmetic and therapeutical purposes in many centuries. Among them, *Juniperus chinensis* L. (Chinese juniper) is a well-known species in China, Korea, Japan, Myanmar and eastern parts of Russia (Jin *et al.*, 2015).In the traditional medicine, *J. chinensis* has been used for the treatment of gout, rheumatism, diarrhea, and chronic tracheitis (Ali *et al.*, 1996; Lee *et al.*, 2009).Previous studies have reported that *J. chinensis* possesses antioxidant, anti-inflammatory, anti-obesity, antitumor, antimicrobial, acaricidal, and repellent activities (Ali *et al.*, 1996; Lim *et al.*, 2002; Kim *et al.*, 2008; Lee *et al.*, 2009; Carroll *et al.*, 2011). Jin *et al.* (2015) studied the anti-melanogenic activities of methanol extracts of *J. chinensis* and isolation of functional compounds such as cedrol and widdrol. This plant contains various bioactive components such as terpenes, lignins, and flavones (Lim *et al.*, 2002). Several studies have reported the chemical composition of essential oils from the needles of *J. chinensis*. The results showed a variation in the chemical composition and concentration of major components (sabinene, bornyl acetate, α-pinene, limonene and elemol) between the samples (Raina *et al.*, 2005; Afsharypuor *et al.*, 2007; Lee *et al.*, 2009; Carroll *et al.*, 2011; Kim *et al.*, 2015). The essential oil composition and its contents are mainly influenced by geographical location, weather parameters, plant part and age of the plant (Dhouioui *et al.*, 2016). To the best of our knowledge, there has been no previous study on the comparison of essential oil composition from the different plant parts of *J. chinensis.* Hence, the present study was carried out to investigate the composition of essential oils obtained by steam distillation from different plant organs (needles, twigs and berries) of *J. chinensis*.

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MATERIALS AND METHODS

Plant material and extraction of essential oil

The different parts such as needles, twigs and berries of *J. chinensis* (Fig. 1) were collected from Kangwon National University campus, Chuncheon, Republic of Korea during the month of July 2015. The plant was authenticated and deposited in the Herbarium, Daejin University, Pocheon, Gyeonggi-do, Republic of Korea with voucher number DJU- 20160869.

Fig. 1: The different plant parts of *Juniperus chinensis*. A, needles; B, twigs; C, berries.

Extraction of essential oils

The essential oil was separately extracted from the needles, twigs (without needles) and berries (unripe) by steam distillation (HanilLabTech., Republic of Korea) at 100 ºC for 90 min (1 kg sample) using a Clevenger-type apparatus. The collected essential oils were dried with anhydrous sodium sulfate and stored under refrigeration (4ºC).

Gas chromatography/mass spectrometry (GC/MS) analysis

GC-MS analysis was performed with a Varian CP 3800 gas chromatography equipped with a VF-5 MS polydimethylsiloxane capillary column (30 × 0.25 mm x 0.25 µm) and a Varian 1200 L mass detector (Varian, CA, USA). Helium was used as a carrier gas at the rate of 1 mL/min. Oven temperature was kept at 50ºC for 5 min initially, and then raised with rate of 5ºC/min to 250ºC/min. The injected volume of essential oil was 10 µL with a split ratio of 1:10. The injector temperature was set at 250ºC. The mass spectra were recorded in the electrospray ionization mode at 70 eV in a scan range of 50 - 600 m/z. The components of essential oils were identified by comparing the retention indices of the GC peaks obtained using homologous series of n-alkanes (C_8-C_{20}) with those reported in literature (Adams, 2007). The mass spectra of the peaks were also matched with standards reported in literature and National Institute of Standards and Technology (NIST, 3.0) library.

RESULTS AND DISCUSSION

The essential oil yields were 0.34%, 0.11% and 0.12% (v/w) for the needles, twigs and berries, respectively, and their compositions are presented in Table 1.The yield of essential oil from juniper needles (0.34%, v/w) was almost thrice that from twigs as well as berries. A total of 36 components were identified in the essential oils, accounting for 98.39% (needles), 98.18% (twigs) and 98.24% (berries) of the total compositions. Out of 36 components, 17 components were detected in all the three essential oils. From the results, qualitative and quantitative differences were observed in the composition of the essential oils obtained from different organs of *J. chinensis*. In the overall results, all the three essential oils showed a higher percentage of monoterpenes (72.87 – 88.07%) than sesquiterpenes (9.29% – 23.68%) (Fig. 2). Although these three essential oils contained higher amount of monoterpenes, there were differences in composition between the detected components. Among the three essential oils, the essential oil from the berries contained the highest amount of monoterpene hydrocarbons (48.15%) and oxygenated monoterpenes (39.92%) than twigs (45.06 and 28.53, respectively) and needles (42.05 and 30.82%, respectively).On the other hand, the amount of sesquiterpene hydrocarbons were found to be higher in the essential oils from the twigs (15.16%) and needles (7.37%) than berries (0.57%)(Fig. 2).For oxygenated sesquiterpenes, the highest percentages were detected in needles (15.95%), followed by berries (8.52%) and twigs (8.52%). Further, the three essential oils also contained a small amount oxygenated diterpenes (0.88 – 2.2%).

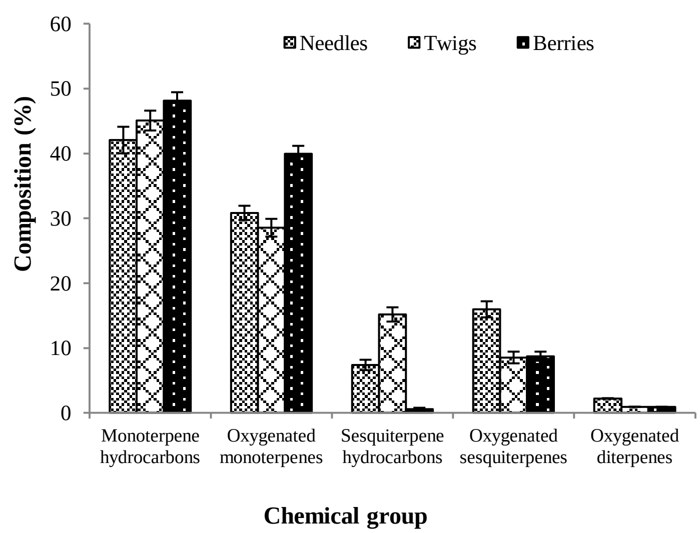

Fig. 2: Percentage concentration of different chemical groups in the essential oils from the needles, twigs and berries of *Juniperus chinensis*. Values are mean of three replicate determinations (n=3) ± standard deviation.

Table 1: Essential oil composition from the needles, twigs and berries of *Juniperus chinensis.*

No.[a]	Components[b]	RI[c]	Area (%)		
			Needles	Twigs	Berries
1	Tricyclene	926	1.66 ± 0.08	-	
2	α-Thujene	930	-	-	0.86 ± 0.04
3	α-Pinene	939	5.80 ± 0.19	16.26 ± 0.76	6.88 ± 0.46
4	Camphene	954	0.92 ± 0.33	0.78 ± 0.15	0.16 ± 0.03
5	Sabinene	975	18.13 ± 0.87	10.23 ± 1.01	14.28 ± 0.77
6	β-pinene	979	3.05 ± 0.14	3.72 ± 0.34	4.39 ± 0.06
7	2-Carene	1002	1.64 ± 0.13	1.30 ± 0.22	-
8	α-Phellandrene	1002	-	-	0.46 ± 0.04
9	3-Carene	1011	-	1.97 ± 0.34	-
10	α-Terpinene	1017	-	-	5.39 ± 0.14
11	o-Cymene	1026	-	-	0.33 ± 0.17
12	Limonene	1029	6.80 ± 0.83	6.96 ± 0.13	3.98 ± 0.06
13	γ-Terpinene	1059	2.52 ± 0.09	2.24 ± 0.30	8.36 ± 0.13
14	Terpinolene	1088	1.53 ± 0.09	1.60 ± 0.13	3.06 ± 0.07
15	Linalool	1096	2.90 ± 0.43	-	-
16	β-Terpineol	1140	1.34 ± 0.07	-	2.72 ± 0.36
17	Camphor	1146	0.91 ± 0.15	0.66 ± 0.08	-
18	Camphene hydrate	1149	0.85 ± 0.08	-	-
19	Borneol	1169	-	0.55 ± 0.05	-
20	Terpinen-4-ol	1177	7.51 ± 0.24	5.98 ± 0.12	31.10 ± 0.21
21	α-Terpineol	1188	0.88 ± 0.30	0.64 ± 0.03	2.32 ± 0.07
22	Piperitol	1196	-	-	0.93 ± 0.06
23	Bornyl acetate	1286	16.43 ± 0.31	20.70 ± 0.87	2.85 ± 0.21
24	Caryophyllene	1419	0.24 ± 0.01	2.55 ± 0.43	-
25	α-Humulene	1454	1.59 ± 0.10	9.90 ± 1.08	-
26	α-Muurolene	1500	1.65 ± 0.11	-	-
27	γ-Cadinene	1513	3.89 ± 0.14	2.71 ± 0.28	0.57 ± 0.02
28	α-Elemol	1549	4.77 ± 0.53	1.74 ± 0.06	3.79 ± 0.19
29	Germacrene D-4-ol	1575	-	1.17 ± 0.27	-
30	Ledol	1602	3.65 ± 0.50	-	-
31	Humulene epoxide II	1608	1.51 ± 0.12	0.93 ± 0.06	1.01 ± 0.05
32	T-Cadinol	1640	2.63 ± 0.06	0.70 ± 0.03	0.53 ± 0.06
33	α-Muurolol	1646	-	0.63 ± 0.06	0.90 ± 0.08
34	α-Cadinol	1654	3.39 ± 0.45	3.35 ± 0.52	2.49 ± 0.23
35	Epimanool	2060	1.37 ± 0.26	0.56 ± 0.02	0.34 ± 0.14
36	Abietal	2313	0.83 ± 0.14	0.35 ± 0.04	0.54 ± 0.05
	Total Identified		**98.39 ± 0.06**	**98.18 ± 0.24**	**98.24 ± 0.29**
	Oil yield (v/w)		**0.34**	**0.11**	**0.12**

[a]In order of elution on VF-5ms; [b]Components identified based on mass spectra and retention indices; [c]RI, Retention indices reported in the literature. Values are mean of three replicate determinations (n=3) ± standard deviation.

The components such as bornyl acetate (2.85 – 20.70%), sabinene (10.23 – 18.13%), α-pinene (5.80 – 16.26), terpinen-4-ol (5.98 – 31.10), limonene (3.98 – 6.96%), β-pinene (3.05 – 4.39%), γ-terpinene (2.24 – 8.36%), α-elemol (1.74 – 4.77%) and α-cadinol (2.49 – 3.39%) were detected as the major components in the essential oils from the three different parts of *J. chinensis.* The major differences between the three essential oils were related to terpinen-4-ol and bornyl acetate. The essential oil of the berries contained the highest level of terpinen-4-ol (31.10%) than needles (7.51%) and twigs (5.98%). In regards tobornyl acetate, the highest percentage was found in the twigs (20.70%), followed by the needles (16.43%) and considerably lowest percentage in the berries (2.85%). The essential oil of twigs registered the highest amount of α-pinene (16.26%) than berries (6.88%) and needles (5.80%). The twigs also contained a higher amount of α-humulene (9.90%) than needles (1.59%).Whereas, sabinene content was found to be higher in the essential oil of needles (18.13%) followed by berries (14.28%) and twigs (10.23%). In addition, a considerable amount of α-terpinene (5.39%) was found only in the essential oil of berries. Among the three essential oils, ledol (3.65%) and linalool (2.90%) were detected only in the essential oil of needles. The major components from the different parts of *J. chinensis* have also been identified as important bioactive components in the essential oils of various plant species. Among the major components, bornyl acetate has various pharmacological properties including depressing spasm, sedative, antidiarrheal, analgesic, anti-inflammatory and anti-abortive properties (Wang *et al.,* 2011; Chen *et al.,* 2014). Terpinen-4-ol has been reported to have several pharmacological activities such as anti-inflammatory, antimicrobial and vascular muscle relaxation (Hart *et al*., 2000; Loughlin *et al*., 2008; Maia-Joca *et al.,* 2014). α-Pinene is a broad-spectrum antibiotic and has anti-inflammatory, hypoglycemic, sedative, antioxidant, antiulcer and inhibition of acetylcholinesterase enzyme activities (Pinheiro *et al*., 2015). Limonene is used as an additive in food products, cosmetics and perfumes (Sowndhararajan *et al*., 2015). It has antitumor and anxietic effects and aroma of this compound enhances the mental conditions of human (Crowell and Gould, 1994; Heuberger *et al.*, 2001; Lima *et al.,* 2013). Another major component, sabinene possesses antioxidant, antifungal and anti-inflammatory properties

(Kohzakiet al., 2009; Valente *et al.,* 2013).

Previously, several authors reported that the essential oil composition from the needles of *J. chinensis*. Kim *et al.* (2015) studied the essential oil composition from the leaves of five *Juniperus chinensis* varieties in Korea (*J. chinensis* L., *J. chinensis* var. *globosa*, *J. chinensis* var. *horizontalis*, *J. chinensis* var. *kaizuka*, *J. chinensis* var. *sargentii*) and the essential oils mainly consisted of monoterpenes with bornyl acetate (15.26 – 44.31%) and sabinene (3.61 – 28.35%) as major components. The results of the present study also indicated that sabinene (18.13%) and bornyl acetate (16.43%) were detected as major components in the essential oil of needles. Further, the twigs (without needles) also contained 10.23% of sabinene and 20.70% of bornyl acetate in addition to 16.26% of α-pinene (Table 1). Similarly, Raina *et al.* (2005), Afsharypuora *et al.* (2007) and Lee *et al.* (2009) reported that sabinene (19.80, 18.03 and 21.1%, respectively) and bornyl acetate (17.50, 26.10 and 19.5% respectively) were detected as major components in the essential oil from the needles of *J. chinensis* L.

Similar to the present study, Hafi *et al.* (2015) studied the chemical composition from berries, leaves and twigs of Lebanese *J. excelsa*. A total of 30 constituents were identified in the essential oils from the leaves, cones and twigs of *J. excelsa* and the main component in these oils was α-pinene. Further, the amount of major constituents in the different plant organs varied significantly. Angioni *et al.* (2003) investigated the chemical composition of the essential oils from the ripe and unripe berries and leaves of *J. oxycedrus* L. ssp. *oxycedrus, J. phoenicea* ssp. *turbinata* and *J. communis* ssp. *communis* and observed both qualitative and quantitative differences between species and between different plant parts. α-Pinene, β-pinene, δ-3-carene, sabinene, myrcene, β-phellandrene, limonene, and D-germacrene were registered as the major components in these essential oils. In addition, the percentage of monoterpenes was higher in the ripe (95%) and unripe berries (94.83%) than in the leaves (87.78%). In Lithuania, the essential oils from the needles and ripe berries of *J. communis* var. *communis* mainly contained 42.4–67.4% of α-pinene (Butkiene*et al.*, 2007).

The essential oil of *J. communis* L. berries from Bulgaria is largely consisted of monoterpene hydrocarbons such as α-pinene (51.4%), myrcene (8.3%), sabinene (5.8%), limonene (5.1%) and β-pinene (5.0%) (Höferl et al., 2014). Chanotiya and Mathela (2007) reported the essential oils composition from the leaves, berries and twigs of *J. wallichiana*. The essential oil from the leaves mainly contained sabinene (46.7%), α-pinene (6.6%) and terpinen-4-ol (6.5%). Further, the berry essential oil contained higher content of sabinene (50.6%) and α-pinene (8.1%) and the twig essential oil contained α-cadinol (9.8%), terpinen-4-ol (8.4%) and oplopanone (8.0%). Based on the previous studies, it was observed that the sabinene or α-pinene is the most abundant component in the essential oil from the berries of different *Juniperus* species. On the contrary, the results of the present study revealed that the essential oil from the berries of *J. chinensis* was mainly dominated by terpinen-4-ol (31.10%). All these studies evidenced a considerable qualitative and quantitative difference in the composition of the essential oils within the same species. In general, the variations in the essential oil composition and its contents are influenced by various factors such as plant´s geographical region, age of the plant, collection time and weather parameters (temperature, relative humidity, sunshine hours and precipitations) (Rajabi *et al.*, 2014; Dhouioui *et al.*, 2016). It is common to find variation in the essential oil composition in different organs of the same plant. The essential oils are biosynthesized and stored in specialized structures called secretory glandules (glandular trichomes). They widely vary in their morphology, structure, function, and distribution among the plant organs. The development of secretory glands and their essential oil production appears to be regulated by genetic and environmental factors. So, the secretory structures may play a major role in the variation of essential oil composition between different plant organs (Boukhris *et al.*, 2013; El Asbahani *et al.*, 2015).

CONCLUSIONS

The present study revealed that the essential oils obtained from the needles, twigs and berries of *J. chinensis* are rich in monoterpenes including monoterpene hydrocarbons and oxygenated monoterpenes. A comparison of oils from different parts revealed the variation in the amounts of several constituents, especially in the monoterpene components. The findings of the present study could be useful for further research in connection with the isolation of essential oil components.

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