

INFLUENCE OF HARVESTING TIME ON CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *ABIES KOREANA* TWIGS

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ABSTRACT

Objective: In the present study, the influence of harvesting time (April, June, August and October 2015) on the essential oil composition of *Abies koreana* twigs from Korea was investigated.

Methods: The essential oil from the twigs of *A. koreana* was isolated by steam distillation and its chemical composition was determined by gas chromatography-mass spectrometry (GC-MS).

Results: The essential oil yield was found to vary from 0.76 to 1.20% depending on the month of harvesting. The GC-MS analysis revealed the identification of 26 different essential oil components from the twigs harvested in the months of April, June, August and October, which were mostly monoterpene hydrocarbons (57.63–72.38%) followed by oxygenated monoterpenes (18.82–25.96%). Harvesting time mainly influenced on the concentration of the major components of the essential oil from the twigs of *A. koreana*. Limonene (17.38–31.13%), bornyl acetate (13.22–21.17%), camphene (12.56–13.26%), α -pinene (11.05–13.02%), β -pinene (4.55–5.70%), 3-carene (5.21–6.43%) and β -eudesmol (1.49–8.24%) were detected as the major components in the essential oil.

Conclusion: The main differences between the essential oil compositions of four different months can be referred to limonene and bornyl acetate. The results showed considerable variations in the composition of essential oil, particularly quantitative variation during different harvesting months.

Keywords: *Abies koreana*, Essential oil, Harvesting time, Seasonal, Twigs

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INTRODUCTION

The genus *Abies* (Pinaceae) consists of 51 species of coniferous evergreen trees and is widely distributed in temperate and boreal regions of the northern hemisphere, mainly in mountainous regions [1]. Several species of *Abies* are used in the traditional medicine to treat various disorders including colds, stomachache, indigestion and pulmonary diseases [2]. Among them, *Abies koreana* Wilson (Korean fir; Korean name: Kusang namu) is a slow growing shrub or broadly pyramidal evergreen tree and distributed in the alpine regions of Republic of Korea [3]. The needles of this plant are used commercially in the cosmetic and fragrance industries to whiten the skin and to treat atopic dermatitis and asthma. The essential oil from the needles also provides an UV protection effect by inhibiting the melanin synthesis in cells [4]. Kim *et al.* [5–7] reported the presence of lignans and triterpenoids (secocycloartenoid and two lanostane-type) in this plant. In addition, the supercritical carbon dioxide extract from the needles of *A. koreana* exhibited memory enhancing effect in mice [8]. Previously, some authors reported the essential oil composition and its antimicrobial activity of *A. koreana* of different origins [2, 3, 9, 10]. However, the published data about the essential oil composition of *A. koreana* has been extensively varied between the authors.

The essential oils are mainly composed of terpenes and their oxygenated derivatives including alcohols, aldehydes, ketones, acids, esters, etc. The complex mixtures of essential oil components provide a characteristic aroma to plant foliage [11]. In general, the yield and chemical composition of the essential oil producing plants have been greatly associated with the environmental parameters such as temperature, relative humidity, sunlight, harvesting season and soil properties as well as the maturity of the plant and its physiological and biosynthetic pathway [12–16]. Among them, the season and duration of sunlight may influence the chemistry of the plant, since many components may be stored at a specific period to respond to environmental changes [17]. In addition, the biological activity of the essential oil is mainly correlated with its chemical composition. A

number of studies have been reported previously in relation to seasonal influences in the yield and chemical composition of essential oils from various plant species and showed great variations in the concentration of essential oil components [12, 17–19]

The study of the influence of seasonal variation on the essential oil composition of *A. koreana* twigs is required to increase the quality and quantity of essential oil production. Further, there have been no reports on seasonal variation in the essential oil composition of *A. koreana* twigs. With regard to this topic, the present study was carried out to determine the chemical composition of the essential oil of Korean *A. koreana* twigs collected at different months.

MATERIALS AND METHODS

Plant material

The twigs of *A. koreana* were collected from Inje, Gangwon-do, Republic of Korea during the last week of April, June, August and October 2015. The plant was authenticated and deposited in the Herbarium, Daejin University, Pocheon, Gyeonggi-do, Republic of Korea with voucher number DJU-20152382.

Extraction of essential oil

The essential oil from the twigs of *A. koreana* harvested over different months was extracted by steam distillation (Hanil LabTech., Republic of Korea) using a Clevenger-type apparatus for 90 min at 100 °C. The steam distillation was carried out with 1 kg of fresh *A. koreana* twigs. The essential oil extraction was performed immediately after harvesting the plant material. The essential oil yield (% v/w) was calculated in triplicate on a fresh weight basis. The extracted essential oil was dried by using anhydrous sodium sulfate and was then stored at 4 °C until further analysis.

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 m × 0.25 mm × 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 50 °C for 5 min initially and then raised with the rate of 5 °C/min to 250 °C/min. The injected volume of essential oil was 10 μl with a split ratio of 1:10. The injector temperature was set at 250 °C. The mass spectra were recorded in the electrospray ionization mode at 70 eV in a scan range of 50-600 m/z.

Identification of essential oil constituents

The components of essential oils were identified by comparing the retention indices of the GC peaks obtained using homologous series of n-alkanes (C₈-C₂₀) with those reported in the literature [20]. The mass spectra of the peaks were also matched with standards reported in the literature and National Institute of Standards and Technology (NIST, 3.0) library. The relative amount of individual component was calculated based on the GC peak area.

RESULTS AND DISCUSSION

The essential oil obtained from the twigs of *A. koreana* was pale yellow in color with an intensely coniferous, green and woody aroma. The essential oil yield from the twigs of *A. koreana* ranged

from 0.76% to 1.20% during the different harvesting times. The essential oil yield varied with respective harvesting months (April, June, August and October) and the highest yield was obtained from a sample collected in the month of August (1.20% v/w). The qualitative and quantitative composition of the essential oil from the twigs of *A. koreana* at different harvesting times is presented in table 1. Altogether, 26 volatile components, forming 98.35–98.59% of the total oil compositions were identified from the essential oil samples of four different months (In April-23, June-22, August-25, and October-22 components) based on the retention indices and mass spectral data. Among them, 20 components were detected in all the four essential oil samples. The components are listed in order of their elution from a VF-5MS column. The essential oil samples mainly contain monoterpene hydrocarbons (57.63–72.38%) followed by oxygenated monoterpenes (18.82–25.96%), oxygenated sesquiterpenes (2.84–12.2%) and sesquiterpene hydrocarbons (1.1–2.55%) (fig. 1). It was observed that the presence of the highest percentage of monoterpene hydrocarbons in the sample collected in the month of August (72.38%) and the lowest percentage in the month of April (57.63%). The content of monoterpene hydrocarbons increased from April (57.63%) to August (72.38%) and decreased in October (66.65%). The opposite pattern was observed in relation to oxygenated mono-and-sesquiterpenes.

Table 1: Influence of harvesting time on the chemical composition of essential oil from the twigs of *Abies koreana*

S. No.	Component	RI ^a	RI ^b	Area (%)			
				April	June	August	October
1	Santene	888	902	1.75±0.18	1.60±0.46	1.76±0.12	1.55±0.31
2	Tricyclene	926	934	1.91±0.28	2.66±0.76	1.91±0.16	1.88±0.09
3	α-Pinene	939	946	12.92±0.30	12.77±0.84	11.05±0.18	13.02±0.90
4	Camphene	954	959	13.09±0.43	13.26±0.42	12.69±0.45	12.56±0.24
5	β-Pinene	979	980	5.67±0.65	5.70±0.68	4.55±0.20	5.16±0.13
6	3-Carene	1011	1013	5.75±0.05	6.27±0.32	6.43±0.29	5.21±0.09
7	Limonene	1021	1018	17.38±0.47	25.91±0.56	31.13±0.88	28.02±1.06
8	γ-Terpinene	1059	1064	0.15±0.01	0.24±0.01	0.20±0.03	-
9	Terpinolene	1088	1084	0.77±0.10	1.03±0.02	0.87±0.08	0.80±0.04
10	Borneol	1169	1165	0.27±0.02	1.47±0.22	4.43±0.28	1.09±0.19
11	Linalyl formate	1216	1218	0.31±0.04	0.28±0.14	0.77±0.03	0.69±0.13
12	β-Fenchyl acetate	1232	1234	2.47±0.17	2.95±0.77	2.00±0.05	3.08±0.13
13	Bornyl acetate	1285	1286	21.17±1.10	17.59±0.26	13.22±0.05	17.13±0.35
14	α-Terpinyl acetate	1349	1356	1.46±0.09	1.31±0.02	1.27±0.17	1.72±0.22
15	Geranyl acetate	1365	1368	0.29±0.05	0.38±0.17	0.69±0.41	0.21±0.04
16	Caryophyllene	1419	1412	0.25±0.08	0.30±0.09	0.38±0.20	0.45±0.15
17	α-Humulene	1454	1460	0.20±0.09	0.25±0.02	0.27±0.09	0.20±0.04
18	α-Gurjunene	1477	1481	0.22±0.02	0.55±0.04	0.75±0.45	0.28±0.06
19	γ-Murolene	1479	1484	-	-	0.28±0.06	-
20	β-Selinene	1490	1492	0.37±0.11	-	0.24±0.03	0.16±0.02
21	α-Farnesene	1505	1509	-	0.86±0.21	0.32±0.12	-
22	β-Bisabolene	1505	1510	-	-	0.31±0.05	-
23	Nerolidol	1563	1565	1.44±0.30	0.39±0.03	0.56±0.03	0.27±0.06
24	Selina-6-en-4-ol	1624	1625	1.31±0.25	-	-	0.30±0.04
25	β-Eudesmol	1650	1652	8.24±0.75	1.51±0.14	1.49±0.20	4.14±0.42
26	α-Bisabolol	1685	1689	1.21±0.38	1.12±0.33	0.79±0.08	0.55±0.23
	Total identified			98.59±0.27	98.40±0.24	98.35±0.59	98.47±0.48
	Oil yield (v/w)			0.76±0.07	1.11±0.08	1.20±0.09	1.00±0.02

RI^a-Comparison of retention indices with those reported in the literature (Adams, 2007), RI^b-Retention indices relative to n-alkanes (C₈-C₂₀) on the VF-5ms column, Values are mean of three replicate determinations (n=3)±standard deviation.

Limonene (17.38–31.13%), bornyl acetate (13.22–21.17%), camphene (12.56–13.26%), α-pinene (11.05–13.02%), β-pinene (4.55–5.70%), 3-carene (5.21–6.43%) and β-eudesmol (1.49–8.24%) were registered as the major components of the essential oil. In addition, the essential oil contains an unsaturated terpene, santene (1.55–1.76%). The results revealed considerable variations in the essential oil composition of *A. koreana* twigs, especially quantitative variation at different harvesting months. When compared to all the four essential oils, the main differences between samples were noticed especially on the level of a

monoterpene hydrocarbon, limonene and an oxygenated monoterpene, bornyl acetate (fig. 2). The accumulation of limonene increased during the harvesting months from April (17.38%) to August (31.13%) and decreased in October (28.02%). In contrast, the amount of bornyl acetate was decreased during the harvesting months from April (21.17%) to August (13.22%) and increased in October (17.13%). The highest level of β-eudesmol content (8.24%) was detected in the month of April. The variations in other components at different harvesting months were irregular (table 1).

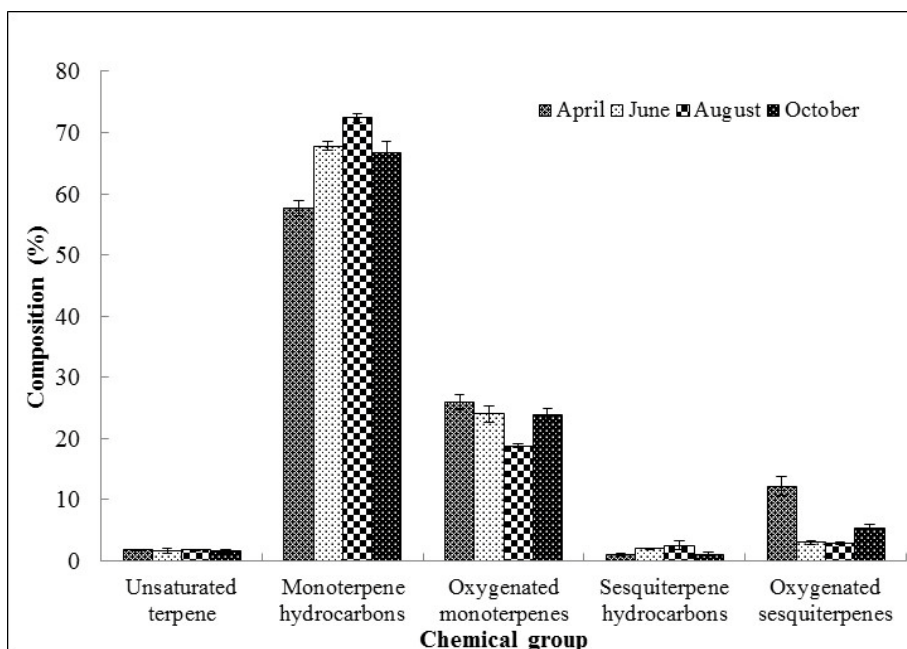


Fig. 1: Percentage concentration of different chemical groups in the essential oils from the twigs of *Abies koreana* collected at different months

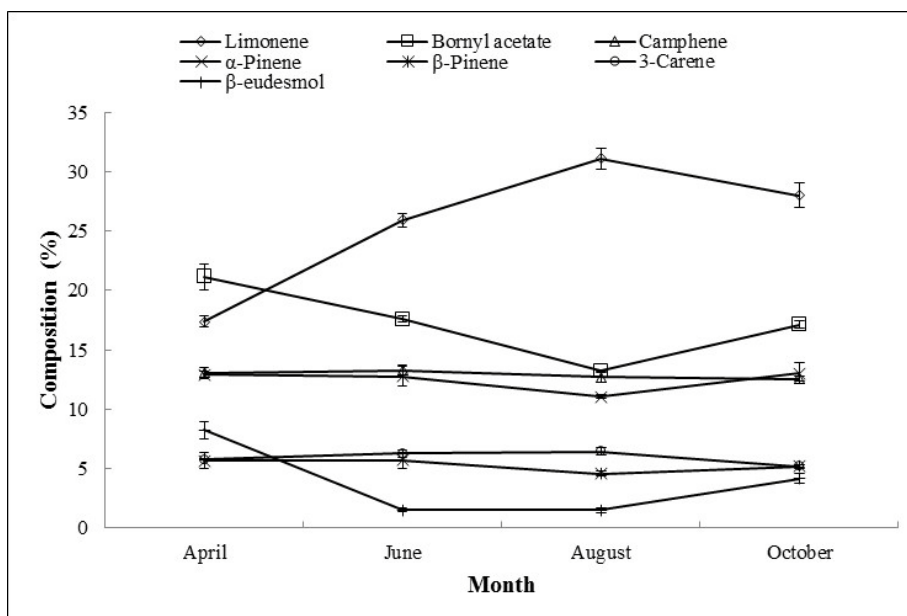


Fig. 2: Variation in the major components of the essential oils from the twigs of *Abies koreana* collected at different months

In the previous reports, the yields of essential oils from the needles, twigs and cones, as well as twigs with needles of *A. koreana* (by after 5 h of hydrodistillation) were 1.0%, 0.64%, 0.42% and 0.83%, respectively [9]. In other studies, 0.6%, 0.53%, and 0.9% of essential oil yields were obtained from the needles of *A. koreana* by Lee and Hong [2], Oh *et al.* [3] and Jeong *et al.* [10] respectively. In the present study, it was observed that the harvesting times highly influenced on the essential oil yield with the highest value in August (1.20%) and the lowest value in April (0.76%). The result showed that the yield was higher in the summer season than spring and autumn seasons. The variations in the essential oil yield of same species may be due to the time duration for distillation and collection time of plant materials. In this context, Rivoal *et al.* [21] also reported the terpene emission rate was significantly higher in spring and summer than in autumn and winter.

Previously, the essential oil compositions from the needles and twigs of *A. koreana* were reported by some authors. The chemical composition of *A. koreana* twigs from the present investigation is also in agreement with previous studies. The *A. koreana* essential oil contains complex mixture consisting mainly by monoterpene hydrocarbons (57.63–72.38%). In conformity with the present findings, Oh *et al.* [3] reported that limonene (23.5%), bornyl acetate (17.9%), α-pinene (11.1%) and camphene (10.2%) were the major components of essential oil from the needles of *A. koreana*. The results of the present study and previous studies show the high variation in the number of components in the essential oil of *A. koreana*. The concentration of major components such as limonene (8.58–23.5%), bornyl acetate/bornyl ester (3.4–41.79%), camphene (10.2–22.5%), α-pinene (6.07–23.2%) and β-pinene (0.46–5.80%) were varied among the previously reported results [2, 3, 9, 10].

Numerous authors have reported the variations in the chemical composition of essential oil due to the environmental conditions (climatic, seasonal and geographical), genetic differences, nutritional status of the plants, extraction methods and analytical techniques [18, 22–24]. In addition, previous studies have reported that the essential oil composition of various medicinal and aromatic plants, when collected at different times varied significantly and the changes were mainly quantitative rather than qualitative [17, 18, 25]. The results revealed that the changes of essential oil content and composition of *A. koreana* twigs are possibly correlated with the various environmental factors commencing from different seasons.

CONCLUSION

Results of the present study clearly indicate the variations in the yield and composition of essential oils from different harvesting times. The major constituents of *A. koreana* essential oil such as limonene, bornyl acetate, camphene, α -pinene, β -pinene, 3-carene and β -eudesmol were considerably varied during the different harvesting times. According to the yield and concentration of monoterpene hydrocarbons, harvesting of *A. koreana* twigs during the summer season (June–August) was found to be the best. The findings of the present study could be useful for further commercial utilization of the essential oil of *A. koreana* twigs.

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CONFLICT OF INTERESTS

We declare that we have no conflict of interest.

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