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Seasonal Variation in Concentration and Composition of Monoterpenes from *Artemisia princeps* var. *orientalis*

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쑥에 함유된 monoterpenes의 함량과 조성의 계절적 변이

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ABSTRACT

The profile and concentration of monoterpene metabolites in the leaf and stem of *Artemisia princeps* var. *orientalis* were quantified, and seasonal variation in monoterpenes of *Artemisia* plant was investigated. Samples were taken from five sites at the campus of Kyungnam University during maturing season. Monoterpenes in leaf and stem were analyzed using gas chromatography-mass spectrometry (GC-MS). The major constituents of *A. princeps* var. *orientalis* in both the leaf and stem were 21 monoterpenes. α-pinene, β-pinene, β-myrcene, dl-limonene, naphthalene and unknown monoterpenes with 5.49 and 16.27 of retention time were present in high concentrations of compounds identified on the leaf and stem of *A. princeps* var. *orientalis*. The amounts of total monoterpenes of leaf were from two to five times higher than stem and rapidly decreased with the time, while that of stem was constant except early spring. Most of the high percentage of monoterpenes in the leaf were those with later retention time. These results indicated that monoterpenes yields are considered to be more variable than monoterpene composition in responding to the time in both the leaf and stem.

Key words: *Artemisia princeps* var. *orientalis*, Gas chromatography-Mass spectrometry, Monoterpenes, Retention time

INTRODUCTION

The terpenes or terpenoids comprise the largest class of secondary plant products. Among them, monoterpenes and their derivatives are important allelopathic agents in ecology. Prior terpenes researches have suggested that microbial (White 1990, 1991), fungal (Espinosa-Garcia and Langenheim 1991), insect, herbivorial behavior (Halligan

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1975) and germination and growth of neighbor plants (Kil *et al.* 1994) were severely inhibited by the terpenes.

The genus *Artemisia* has been the object of numerous chemical studies (Marco *et al.* 1994a,b, Kil *et al.* 1991). There is much evidence that mixtures of volatile monoterpenes and sesquiterpenes, called essential oils, are found in *Artemisia* species (Ahmad and Misra 1994). The volatile and somewhat water soluble terpenes from *A. tridentata* (Groves and Anderson 1981, Weaver and Klarich 1977) and *A. argyi* (Kil *et al.* 1994) were strong inhibitors of germination of other plants. *Artemisia* plants are distributed all over the country and contributed to traditional biomedicines. The *Artemisia* plant has recently acquired further importance in Korea because of its attractive monoterpenes and essential oil. The composition of the oil varies considerably from one phytogeographical region to another (Hall and Langenheim 1987, Estell *et al.* 1994) and also depends on the time of harvest (Hall and Langenheim 1987). Li and Madden (1995) reported that large differences in leaf oils between and within species observed.

In spite of this, little is known about the amount or constituents of seasonal variation within and between the *Artemisia* species in Korea. The objective of this study is to identify and quantify monoterpenes and to evaluate intraspecific seasonal variation in monoterpenes from leaf and stem in *Artemisia princeps* var. *orientalis*.

MATERIALS AND METHOD

The study site on the campus of Kyungnam University, was heavily covered with *Artemisia princeps* var. *orientalis*. Samples were taken from five sites, sealed in plastic bags, and transported to the laboratory during maturing period approximately one week interval. All leaf materials and stems were removed from each plant, and immediately three grams of subsamples were ground with pure sand and extracted with n-pentane (approximately 50 ml) and one ml internal standard (1% tetradecane). The pentanes were steam distilled three times, using a glass distillation unit for 6 hours, which were used for all chemical analyses to increase the purification. Leaves and stem extracts were filtered with sodium sulfate and concentrated by evaporation with a gentle stream of nitrogen gas. All of the oils were analyzed by combined GC-MS using HP-5890GC. Gas Chromatography used a 30m HP-5MS capillary column with an inside diameter of 0.25mm and a flame ionization detector. The injector temperature, dectector temperature, and flow rate were 220℃, 320℃, and 1.8ml/min, respectively. The initial oven temperature was 37℃ for five minutes, and increased to 180℃ at a rate of 5℃ per minute, then by 20℃ per minute until 320℃. One μl of the resulting extract was injected to an injector of the GC.

The preliminary identification of monoterpene components was decided by comparison of spectral data of the internal spectral library of the instrument and retention times, based on references. The positive identification of monoterpenes was based on acquisition of standard terpene compounds for comparison with unknown retention times. Because of the complexity of chromatograms, a limited number of unidentified peaks were examined. The concentrations of peaks at selected retention times were estimated from peak area using the internal standard curve of tetradecane. Although estimates derived in this manner do not yield absolute concentrations, relative differences and an assessment of seasonal variation are valid.

The ANOVA for variation in oil components was calculated following Sokal and Rohlf (1973) and computed using the Excel program (ver. 4.0). T-testing was performed for the difference of terpenenes in the leaf and stem.

RESULITS AND DISCUSSION

Approximately 21 monoterpenes and other compounds were identified in *Artemisia primceps* var. *orientalis,* however many were present only in small or trace amounts (Table 1). α-pinene, β-pinene, β-myrcene, dl-limonene, naphthalene, and unknown monoterpenes with 5.49, and 16.27 of retention times were present in high concentrations of compounds identified in the *Artemisia* plants (Fig. 1).

Fig. 2 shows that the total amount of monterpenes in the leaf was significantly higher than in the stem ($t = 2.863$, $p<0.05$). The total amount of monoterpenes ranged from 0.034~0.230 (mg/g f.w.) of the leaf and 0.011~0.102 (mg/g f.w.) of the stem in *Artemisia princeps* var. *orientalis.* The amounts of both leaf and stem rapidly decreased with the time since approximately two weeks, while that of later time (after 29, May) was constant in both plant parts.

Several terpenes identified in *Artemisia* exhibit antimicrobial activity in herbivores (Yashphe *et al.* 1987, Bicchi *et al.* 1985). Volatile compounds are effective deterrents because they repel herbivores prior to defoliation (Levin 1976). Volatile aromatic compounds

Table 1. The major monoterpenes of *Artemisia princeps* var. *orientalis* in the leaf and stem

No.	Compound	R.T.	Tent. I.D.(q)	No.	Compound	R.T.	Tent. I.D.(q)
1	unknown	4.75		12	dl-limonene	12.57	95%
2	unknown	5.07		13	unknown	13.38	
3	unknown	5.49		14	unknown	13.78	
4	unknown	6.96		15	unknown	16.27	
5	unknown	7.43		16	naphtalene	16.93	95%
6	α-pinene	9.39	96%	17	unknown	17.46	
7	unknown	9.86	camphene	18	unknown	18.73	
8	unknown	10.77	(+)-sabinene(60%)	19	bvornyl acetate	20.64	94%
9	β-pinene	10.87	95%	20	unknown	22.32	
10	β-myrcene	11.25	95%	21	geranyl acetate	22.89	90%
11	β-ocimene	12.47	cyclohexene(90%)	22	tetradecane	23.77	standard

R.T. : Retention time of gas chromatography

Tent. I.D.(q): Tentative identification of unknown compounds based on Wiley library from mass instrument. (q) means quality.

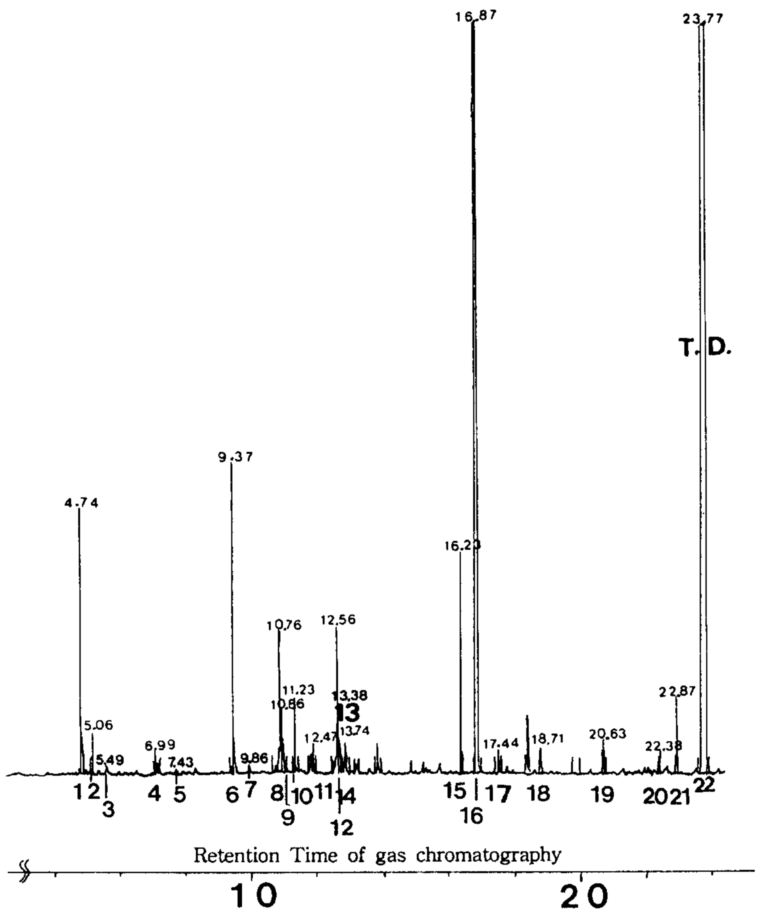

Fig. 1. Gas chromatographic assessment of the extraction from *Artemisia princeps* var. *orientalis* leaf. Many monoterpenes are present only in small or trace amounts. Number means monoterpene compound with the same No. in Table 1. Compounds represented after tetradecane (T.D.) are sesquiterpenes.

may be involved in olfaction whereas leaf surface compounds and plant components may affect taste and/or pain receptors that influence preference. Especially, the high concentrations of monoterpene in early stage of *Artemisia* plant might be advantageous for developing mechanisms to overcome herbivores, because most of monoterpenes are volatile. And since monoterpenes are easily volatilized, volatilization are also rapidly increased by the high temperature (Kim and Langenheim 1994). Therefore the amounts of total monoterpenes might be constant in both leaf and stem after the end of June, whereas sesquiterpenes might be increased at higher temperatures compared to monoterpenes because sesquiterpenes are less volatile than these compounds with early retention times.

Fig. 3 suggests that the total percentage of each compound has no significant difference

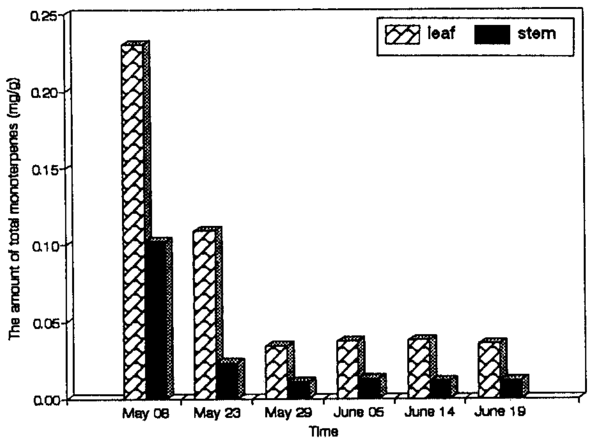

Fig. 2. Seasonal variation of total monoterpene concentration between the leaf and stem in *A. princeps* var. *orientalis*. Means in each column came from five measurements belong to five sites.

between leaf and stem (t=0.156, p>0.5). It means that the ratio of composition of monoterpenes between the leaf and stem was not variable. Nevertheless most of the peaks with above of 10% (represented with *, dl-limonene, naphthalene, and 15) in leaf were those with later retention times. While the first, second, and third peaks of stem were No. 1, 2 compound and α-pinene (represented with ▲, above of 8%), which were those with early retention times. These results suggested that monoterpenes with early retention time on the leaf might be more volatile than the stem because leaves are expanded for easy volatilization. Although a large amount of variability was noted in the seasonal concentration of many of the monoterpenes of the *Artemisia* leaves as shown in Fig. 4 (F=6.13), their ratio of composition of monoterpenes was not variable (F=0.035).

Fig. 5 showed that the monoterpene content of stem changed with time. The major differences were compound No. 1 and β-pinene (No. 6); these compounds with early retention times appeared in early spring and were rapidly lost into the atmosphere after two weeks. There is a highly significant difference (F=4.78, p <0.0001) within the total amount of monoterpenes, while there is no significant difference in composition of monoterpenes in the stem (F=0.13, p>0.01). Results from this study indicated that yields are considered to be more variable than monoterpene composition in responding to the time. Li and Madden (1995) suggested that volatile leaf oil has been

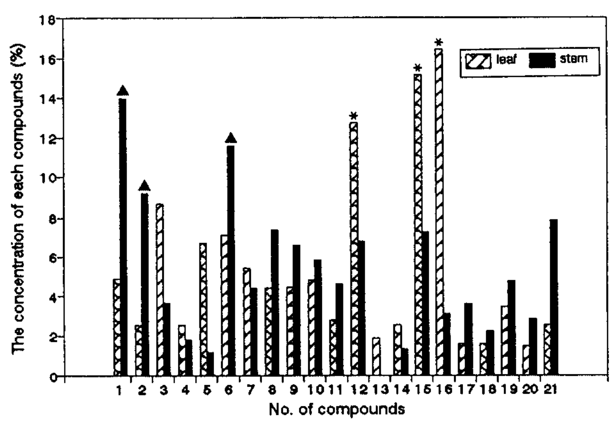

Fig. 3. The total percentage of each compound in the leaf and stem of *A. priceps* var. *orientalis*. There is no significent difference between the leaf and stem (t=0.156, p>0.5). Averages from 6 measurements in each column. ▲ represents the first, second and third peaks of stem. * represents the first, second, and third peaks of leaf.

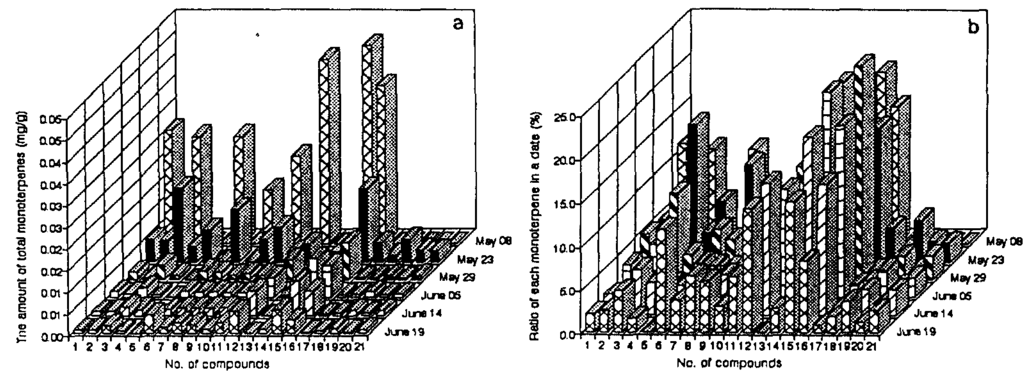

Fig. 4. Seasonal variation of each monoterpene compound in *A. princeps* var. *orientalis* leaf. a. The concentration of each monoterpene with the time. Each data represent the average obtained from five measurements belong to five sites. There is a high significant difference. b. The ratio of composition of each monoterpene with the time. There is no significant.

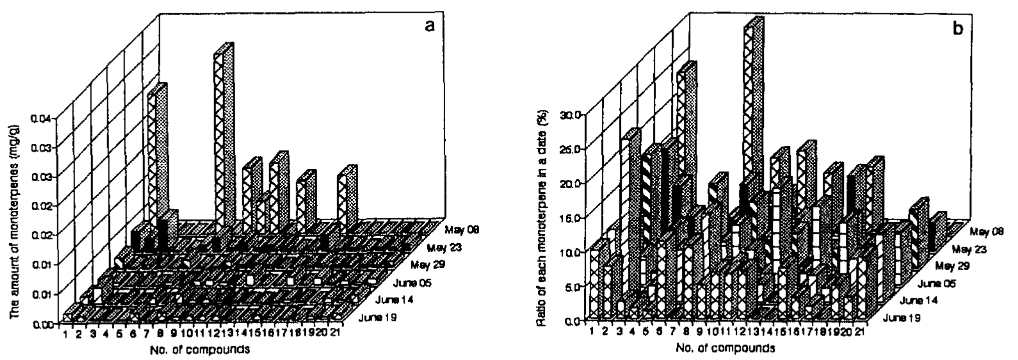

Fig. 5. Seasonal variation of each monoterpene compound in *A. princeps* var. *orientalis* stem. a. The concentration of each monoterpene with the time. Each data represents the average obtained from five measurements belong to five sites. There is a high significant difference. b. The ratio of composition of each monoterpene with the time. There is no significant difference.

used as markers to aid plant taxonomy within *Eucalyptus* species.

The contents of monoterpenes in a species are specific, and are also largely determined by the individual genetics (Barton *et al.* 1991, Marco *et al.* 1994a). Therefore the amounts of monoterpenes of *Artemisia* could support their taxonomic separation. Especially, as most monoterpenes are volatile, olfaction is useful as the primary classification criteria.

Further research is needed to study sesquiterpene variation of the amounts and composition in *A. princeps* var. *orientalis* and to confirm the differences of these researches between *Artemisia* species.

적 요

쑥(*Artemisia princeps* var. *orientalis*)의 잎과 줄기에 함유된 monoterpene의 함량과 조성의 계절적 변이를 연구하였다. Monoterpene은 gas chromatography-mass spectrometry(GC-MS)를 이용하여 분석하였다. 쑥에 함유된 주요한 monoterpene은 21개가 인식되었으나 그중 몇몇만이 동정되었다. 주요한 monoterpene으로는 α-pinene, β-pinene, β-myrcene, dl-limonene, naphthalene과 5.49, 그리고 16.27의 retention time을 지닌 monoterpene이었다. 잎에 있는 monoterpene의 총 함량은 줄기에 비해 2~5배 정도로 높았으며, 시간에 따라 급격히 감소하였고, 줄기의 monoterpene은 이른 봄을 제외하고는 거의 일정하였다. 그러나 각 monoterpene의 조성의 비는 잎과 줄기에서 별 차이가 없었으나 잎에서는 retention time이 늦은 monoterpene이 많은 양을 차지하는 반면 줄기에서는 retention time이 빠른 monoterpene이 많은 양을 차지하는 것으로 나타났다. 또한 잎과 줄기 모두에서, monoterpene의 총 함량은 시간에 따른 변이가 인정되나 그 조성의 비는 변이가 없는 것으로 나타났다. 이와 같은 결과로부터 쑥이 함유하고 있는 monoterpene의 조성은 Li와 Madden(1995)이 제시한 것과 같이 유전적인 것으로 판단되고, 그 조성의 차이로 인한 독특한 냄새가 쑥 식물에서 일차적 분류를 할 수 있는 chemotype이 될 수 있다는 결과를 제시하였다.

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