Variability of the Volatile Composition of *Agastache rugosa* in South Korea

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Abstract

Equilibrium in combination with gas chromatography/mass spectrometry (GC/MS) was used to identify volatile compounds from the leaves of 76 individual plants of *Agastache rugosa* representing 16 regions in South Korea. Chemometric investigation of the intraspecific variability in volatiles led to identify five main chemotypes; estragole (Type 1) as the major component, and the other four chemotypes; methyleugenol (Type2), methyleugenol+limonene (Type 3), menthone (Type 4) and menthone+pulegone (Type 5). The Soraksan region collection was classified as methyeugenol type; Hambaeksan region collection, as methyleugenol +limonene type; Bongpyeong and Inje regions (two of three sample) collection as menthone type; Hongcheon and Inje regions (one of three sample) collection as menthone+pulegone type; and all of the others collections as estragole type.

INTRODUCTION

The species *Agastache rugosa* Kuntze (Syn. *Lophanthu rugosus*) is a perennial herb and belongs to the *Labiatae*. The plant is widely distributed in Korea, China, India, Japan, and other East Asian countries and used in the Chinese folk medicine. Antitumor and cytotoxic activities of the plant were reported (Weverstahl et al., 1992) and the whole plant has been used as an agent for the treatment of cholera, vomiting, and miasma. Leaves can be used as a spice. The species is traditionally used as a medicinal plant in Korea (Jung and Shin, 1990).

Previous studies showed that estragole was as a major component (56-94%) of the leaves in natural populations of *A. rugosa* grown in Iowa (Charles et al., 1991; Wilson et al., 1992) and in Ishikawa, Hyogo Prefecture, Japan (Fujita and Fujita, 1965). However, a population of *A. rugosa* (*A. rugosa* O. Kuntze var. *methyleugenolifera*) collected in Kitami, Hokkaido, contained 84-92% of methyl eugenol and only 2-6% of estragole (Fujita and Fujita, 1973). Svoboda et al. (1995) and Wilson et al. (1992) analyzed the essential oil composition using the individual samples collected in Scotland and USA, respectively, and indicated the presence of chemotypes. However, the compositional character of the chemotypes was not detailed. Essential oil content of *A. rugosa* from Korea was reported that the leaves contained 0.29%, the inflorescence 0.38% of essential oil. Estragole comprised approximately 80% of the essential oil (Ahn and Yang, 1991; Lee et al., 1994).

In our study, we compared the characteristics of volatile components of different *A. rugosa* strains collected from 16 individual regions of Korea.

MATERIALS AND METHODS

Plant Materials

Eight accession (Damyang, Garyeongsan, Gurye, Gwangyang, Jindo, Jinju, Mokpo, Suncheon) of *A. rugosa* collected from the southern parts of Korea were obtained from Gene Bank, RDA in Korea. These accessions were field grown at the College Experimental Farm, Seoul National University, Suwon and harvested at flowering stage.

Samples from other eight accessions (Bongpyeong, Geumyongsa, Hambaeksan, Hongcheon, Inje, Soraksan, Seonamsa, Tongdosa) were harvested at reproductive stage directly from the eight regions.

Headspace Sample Collection

From the individual plant samples, two to three grams of leaves were weighed and placed into the bottles without damaging the tissue. The leaves were immediately freeze-dried and sealed with teflon-coated septa and aluminum seals.

GC-MS Analysis

Volatile analysis was performed on equilibrium headspace autosampler (Tekmar 7000) connected with gas chromatography/mass spectrometry (Hewlett-Packard 6890/5973) equipped with "Chemstation" software. A fused-silica HP-5 capillary column (1.0 film thickness, 0.25 mm (id)×30 m, Hewlett-Packard, USA) was used through this study. Helium carrier gas was applied and the injector and detector temperatures were set at 250 and 280°C, respectively. The oven temperature was held isothermal at 80°C for 3 min and then programmed to increase at 5°C/min to 230°C. Identification of the headspace volatile compound of individual plants within each *A. rugosa* population was performed by comparison retention time and mass spectrum with standard compounds [α-pinene, 1-octen-3-ol, myrcene, menthone, isomenthone, estragole, pulegone, β-caryophyllene, germacrene, methyleugenol] and Wiley 273 library (Wiley, USA).

Statistical Analysis

Principal component analysis (PCA) was done to examine the relationships between the plants from different geographical areas and volatile components.

RESULTS

The volatile compounds extracted from *A. rugosa* leaf samples mainly consisted of estragole, methyleugenol, menthone (including isomenthone), pulegone and limonene (Table 1). The volatile compounds showed large differences between regions, with significant F-values. The major volatile compounds of 11 populations (Damyang, Garyeongsan, Geumyongsa, Gurye, Gwangyang, Jindo, Jinju, Mokpo, Suncheon, Seonamsa, and Tongdosa) had significantly higher percentage of estragole than the population from other regions. Menthone (including isomenthone) was a major volatile component in Bongpyeong and Inje collections while menthone+pulegone in Hongcheon, methyleugenol+limonene in Hambaeksan, and methyleugenol in Soraksan.

It has been noted that the volatile components of *A. rugosa* exhibited significant chemical diversity at the species level. Therefore, phenylprophanoids, monoterpenoids, and sesquiterpenoids which varied among the regions ($P<0.001$) were selected and used for the principal component analysis (PCA). The principal components and the individual tree scores of different collection sites on the first two principal component axes (PCs) are shown in Fig. 1. The first two PCs accounted for 49.5% and 29.8% of the total variance of the components among trees, respectively. The components from these volatile constituents of the individual tree scores of different collection sites (Fig. 1B) indicated that the first two principal components (PC 1 and PC 2) clearly divided the trees into five different groups corresponding to the five chemotypes.

The first principal component (PC 1) separated estragole, from the methyleugenol, limonene, menthone, and pulegone which were the major components of individual samples collected from some collections compared to the 11 populations from Damyang, Garyeongsan, Geumyongsa, Gurye, Gwangyang, Jindo, Jinju, Mokpo, Suncheon, Seonamsa, and Tongdosa.

The second principal component (PC 2) was able to distinguish volatile components according to the positive correlation with menthone and pulegone, simultaneously, negative correlation with limonene and methyleugenol. Therefore PC 2 distinguished Bongpyeong, Inje and Hongcheon collections (high level of menthone and pulegone) from Hambaeksan and Soraksan collections (high level of limonene and methyleugenol).

DISCUSSION

The PCA indicated that the tree of *A. rugosa* species from different regions were clearly classified into the five chemotypes according to their leaf oil components when they were grown in a common environment suggesting the presence of five chemotypes distributed in southern part of Korea. The differences in the amount of estragole, methyleugenol, menthone, pulegone, and limonene among the chemotypes are shown in Table 2. Estragole, one of these phenylprophanoids compound, was the major components in type 1. The other 4 types contain menthone (type 2), menthone + pulegone (type 3), methyleugenol (type 4), and methyleugenol + limonene (type 5) as their major components, respectively (Table 2). Type 1 is widely distributed throughout the southern part of Korea while types 2, 3, 4 and 5 are present only in Kangwondo (Bongpyeong, Hambaeksan, Hongcheom, Inje, Soraksan).

Our study indicates that there was a tremendous diversity in the compounds released by leaves of *A. rugosa* plants collected from South Korea. In the case of USA, the estragole type was also the majority, however, the composition of estragole (55%), menthone (8%), and limonene (10%) in total essential oil were different from the estragole type in Korea native plants (Charles et al. 1991). In the accession from Scotland, quantitatively different estragole type, with estragole (40-50%), menthone (30%), and limonene (10%) were found (Svoboda et al., 1995).

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Literature Cited

Ahn, B. and Yang, C.B. 1991. Volatile flavor components of *Agastache rugosa* O. Kuntze. Kor. J. Food Sci. Technol. 23(5):582-586.

Charles, D.J., Simon, J.E. and Widrlechner, M.P. 1991. Characterization of essential oil of *Agastache* species. J. Agric. Food Chem. 39:1946-1949.

Fujita, Y. and Fujita, S. 1965. Miscellaneous contributions to the essential oils of plants from various territories. Essential oil of *Agastache rugosa* O. Kuntze. Nippon Kagaku Zasshi 86:635-637.

Fujita, Y. and Fujita, S. 1973. Miscellaneous contributions to the essential oils of plants from various territories. Essential oil of *Agastache rugosa* O. Kuntze. Jap. J. Pharm. Soc. 93:1679-1681.

Jung, B.S. and Shin, M.K. 1990. Encyclopedia of Korean crude drugs. Youngrim Sa. 840p.

Lee, J.C., Choi, Y.H. and Kim, Y.H. 1994. Essential oil in aerial parts of *Agastache rugosa* O.Kuntze. Kor. J. Medicinal Crop Sci. 2(2):168-173.

Svoboda, K.P., Gough, J. and Hampson J. 1995. Analysis of the essential oils of some *Agastache* species grown in Scotland from various seed sources. Flavour and Fragrance J. 10:139-145.

Weverstahl, P., Marshall, H., Mantenffel, E. and Humeck, S. 1992. Volatile constituents of *Agastache rugosa*. J. E. O. R. 4:585-587.

Wilson, L.A., Senechal, N.P. and Widrlechner, M.P. 1992. Headspace analysis of the volatile oils of *Agastache*. J. Agric. Food Chem. 40:1362-1366.

Tables

Table 1. The chemical composition of headspace volatile components from individual plant leaves of *A. rugosa* at reproductive stage collected from different regions.

Component	Mean of GC area %								
	Bong-pyeng (6)*	Dam-yang (6)	Garyeong-san (6)	Geum-yongsa (4)	Gurye (6)	Gwang-yang (6)	Ham-baeksan (3)	Hong-cheon (3)	Inje (3)
α-pinene	-	0.08	0.06	-	0.14	0.10	-	-	-
1-octen-3-ol	3.34	0.26	0.19	0.86	0.15	0.32	4.84	2.27	1.39
3-octanone	0.94	0.22	0.19	0.12	0.23	0.40	2.27	-	0.82
Myrcene	2.24	0.03	-	-	0.02	0.06	-	2.59	1.28
Limonene	17.98	3.42	2.70	3.30	3.24	6.05	44.09	5.41	7.12
Oct-1-en-3yl acetate	1.55	0.91	0.34	0.15	0.49	0.36	2.26	0.35	0.44
Menthone	70.98	-	-	-	-	-	-	50.52	78.03
Estragole	0.46	93.25	93.60	92.45	92.34	90.38	2.00	-	0.25
Pulegone	-	-	-	0.93	-	-	5.64	36.69	6.65
Methyleugenol	0.82	-	-	2.21	-	-	35.13	0.46	2.88
β-caryophyllene	0.67	0.66	0.66	-	1.71	0.95	3.11	0.28	0.30
Germacrene-D	-	0.04	0.04	-	0.36	0.05	0.27	0.13	-
others	1.01	1.12	1.12	0.01	1.33	1.36	0.40	1.29	0.85

Component	Mean of GC area %							Between regions	
	Jindo (6)	Jinju (6)	Mokpo (6)	Suncheon (6)	Sorak -san (3)	Seonam -sa (3)	Tongdo -sa (3)	F-values	Sign.
α-pinene	0.11	0.08	0.05	0.16	-	-	-	5.35	***
1-octen-3-ol	0.30	0.36	0.68	0.48	1.01	0.83	0.68	16.10	***
3-octanone	0.17	0.24	0.35	0.46	1.02	0.97	0.45	2.27	*
Myrcene	0.07	0.02	0.11	0.10	-	-	-	6.99	***
Limonene	5.89	2.83	9.04	8.28	21.89	5.13	4.11	14.31	***
Oct-1-en-3yl acetate	0.37	0.36	0.30	0.46	2.34	0.69	0.27	2.92	**
Menthone	-	0.04	-	-	-	0.07	-	295.70	***
Estragole	90.71	93.30	87.42	88.13	0.76	90.44	93.36	32.57	***
Pulegone	-	-	-	-	-	0.72	-	17.10	***
Methyleugenol	-	-	-	-	69.50	0.80	-	12.87	***
β-caryophyllene	1.10	1.49	0.73	0.79	3.07	0.29	0.30	2.94	**
Germacrene-D	0.07	0.31	0.12	0.11	0.41	0.04	-	1.58	Ns
others	1.24	0.96	1.19	1.04	-	0.02	0.84	6.96	***

The figure in the parenthesis indicates the sample number analyzed.
Significance: *** $P < 0.001$; ** $0.001 < P < 0.01$; * $0.01 < P < 0.5$; ns $P > 0.5$
- : not detected

Table 2. Chemical composition of five chemotypes based on volatile composition of *A. rugosa*.

Components	Mean of GC area %				
	Estragole type	Menthone type	Menthone + Pulegone type	Methyl-eugenone type	Methyl-eugenol + limonene type
α-pinene	0.10	-	-	-	-
1-octen-3-ol	0.41	2.36	2.27	1.12	4.30
3-octanone	0.34	0.88	-	0.96	2.36
Myrcene	0.04	1.76	2.59	-	-
Limonene	5.02	12.55	5.41	18.14	41.04
Oct-1-en-3yl acetate	0.43	1.00	0.35	2.78	2.84
Menthone	0.01	74.51	50.52	-	-
Estragole	91.27	0.36	-	0.57	1.66
Pulegone	0.09	3.32	36.69	-	8.24
Methyleugenol	0.15	1.85	0.46	71.71	35.80
β-caryophyllene	1.00	0.48	0.28	3.79	3.01
Germacrene-D	0.15	-	0.13	0.93	0.42
No. of accessions	11	2	2	1	1
No. of plants	58a	8b	4c	3d	3e

- : not detected; a) Damyang (6), Garyeongsan (6), Geumyongsa (4), Gurye (6), Gwangyang (6), Jindo (6), Jinju (6), Mokpo (6), Suncheon (6), Seonamsa (3), Tongdosa (3), b) Bongpyeong (6), Inje(2), c) Hongcheon (3), Inje (1), d) Soraksan (3), e) Hambaeksan (3)

Figures

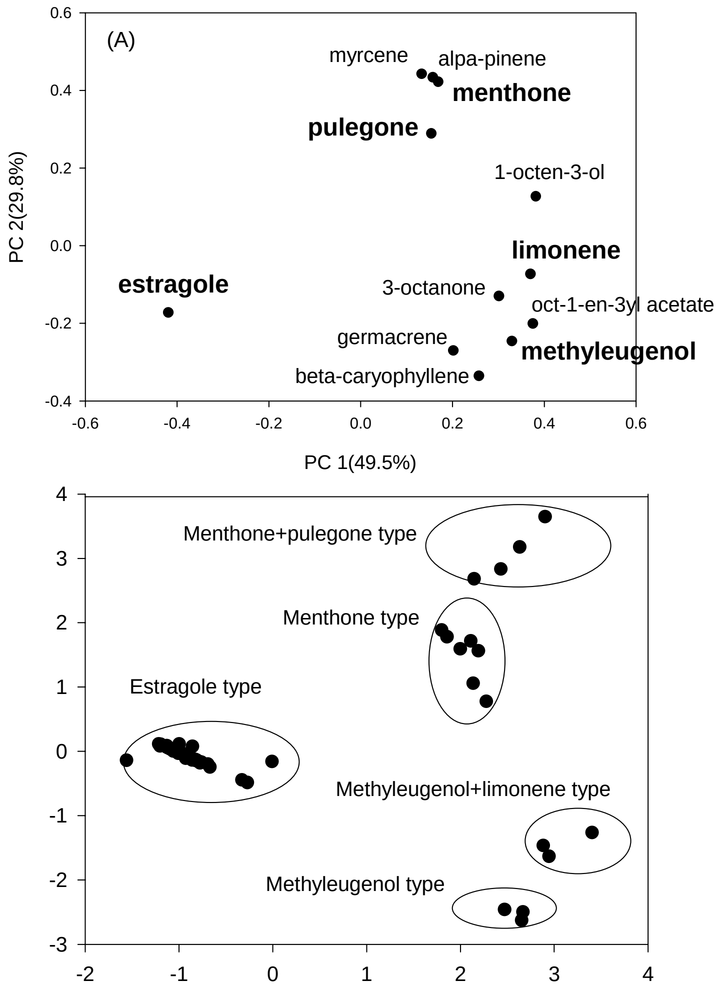

Fig. 1. Scatter plot of the essential oil components (A) and 76 individual samples (B) on the axes of the first two principle components (PC 1 and PC 2).