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Research Report

Volatile Flavor Compounds in the Leaves of Fifteen Taxa of Korean Native *Chrysanthemum* Species

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Abstract: This study was conducted to compare the volatile flavor compounds found in the leaves of 15 taxa of Korean native *Chrysanthemum* species. The volatile flavor compounds from the taxa were collected using a simultaneous steam distillation and extraction technique and were analyzed using gas chromatography/mass selective detector (GC/MSD). A total of 45 volatile flavor compounds were identified with six functional groups: 14 alcohols, 4 ketones, 19 hydrocarbons, 5 esters, 2 acids, and 1 aldehyde. The main functional group in 15 taxa of *Chrysanthemum* species was alcohols, accounting for 28.7% of volatile flavor compounds, followed by ketones (21.2%) and hydrocarbons (13.2%). Camphor, which is known for its antimicrobial properties, was the most abundant volatile compound (30%) in *C. zawadskii* ssp. *latilobum* and var. *leiophyllum.* In particular, *C. indicum* subspecies and *C. boreale* contained α-thujone, which has outstanding anti-bacterial, anti-cancer, anti-inflammatory, anti-ulcer, and anti-diabetic efficacies. *C. indicum* var. *albescens* could be used in perfumes, since it showed 21 times more camphene than *C. indicum.* In addition, *C. indicum* var. *acuta* contained a fairly high content of 1,8-cineole, which has an inhibitory effect on mutagenesis. *C. lineare* contained only pentadecanoic acid compounds, whereas other taxa hexadecanoic acids. Overall, the Korean native *Chrysanthemum* species had considerable variation in volatile flavor compounds in their leaves. This study provides a good indication of specific potential use for various applications.

Additional key words: borneol, camphor, GC/MSD, ketone, native plant

Introduction

Korean native *Chrysanthemum* species are perennial Asteraceae plants that have been used as natural medicine or food as well as an ornamental since the ancient times (Choi, 1992). Since Korean native *Chrysanthemum* species contain a lot of terpene compounds, they have been used as ingredients in cosmetics (Kim et al., 1995; Matsuda et al., 2002), foods (Ko and Jeon, 2003), and folk medicines (Kim et al., 1998; Nam and Yang, 1995) for a long time. Recently, the antioxidant activation and anti-inflammatory effects of the flavor compounds in Korean native *Chrysanthemum* species have been reported (Bae and Lee, 2008; Sung et al., 2007; Yoon and Cho, 2007), and food processing studies with its healthful benefits have been conducted, such as utilizing *Chrysanthemum* species for cookie powder (Bae et al., 2009) and rice cake powder (Park and Shin, 1998; Park et al., 2000). *Chrysanthemum* species also have been proposed as a potential ingredient for herbal cosmetics involving the effects of tyrosinase inhibitory activity associated

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with anti-oxidant activation, whitening, and moisturizing effects (Bae et al., 2009; Sung et al., 2007; Yoon and Cho, 2007).

There are three main native *Chrysanthemum* species in Korea: *C. zawadskii*, *C. indicum*, and *C. boreale*, with each species having several subspecies (Lee, 2006). In particular, *C. zawadskii* has been used for treating various women's diseases, menstrual irregularity, cold stomach, indigestion, pneumonia, bronchitis, urinary disorders, and neurodegenerative diseases (Lee et al., 2008). *C. zawadskii* also has a great potential as a pharmacological ingredient in shampoo products due to its high anti-bacterial activity against dandruff (Lee and Lee, 2007). Previous research indicated that *C. indicum* also contains several healthful volatile flavor compounds and various vitamins, which benefit blood flow in long-term use, refresh the body, help digestion, alleviate fever and headaches, lower blood pressure, and offer efficacy such as inhibitory effects against tuberculosis and various viruses (Shin et al., 2004; Sung et al., 2007). *C. indicum* had been used to make chrysanthemum wine for celebrations during the Chosun Dynasty (AD 1392 to 1897). *C. indicum* has also been used as a medicinal wine for patients with hypertension (Choi, 1992) and a natural flavoring ingredient in traditional foods (Bae et al., 2009) in Korea. Dried *C. indicum* was also used to make tea or added to foods (Yoon and Cho, 2007). The benefits of *C. boreale* were reported to be associated with sedation of the central nervous system, hypotensive effects (Nam et al., 1997), and anti-microbial effects (Cha et al., 2000; Jang et al., 1999; Nam and Yang, 1995). Additional benefits include reductase inhibitory effects of enzyme aldose (Shin et al., 1995), related to anti-cancer (Nam et al., 1997), and diabetic complications (Shin et al., 1995).

With the increasing interest and use of Korean native *Chrysanthemum* species as pharmacological ingredients, cosmetics ingredients, and in health food, several studies on volatile flavor compounds of *Chrysanthemum* species have been conducted. Byun et al. (2006) reported that anti-oxidant compounds such as flavonoids, terpenoids, and phenolic compounds were the main functional compounds of the Korean native *Chrysanthemum* species. Through previous research, it was reported that the main compounds of volatile flavors derived from *C. indicum* and *C. boreale* were 1,8-cineole, germacrene-D, camphor, α-pinene, and camphene, whereas those from *C. zawadskii* ssp. *latilobum* were mainly terpenoids such as camphene and ocimene (Hong, 2002; Jiang et al., 2005; Kim, 1997; Wang and Yang, 2006; Wang et al., 2008). However, despite these studies, species-specific volatile flavor compounds found in Korean native *Chrysanthemum* species are not clearly compared yet.

This study aims to provide a comparison of volatile flavor compounds among 15 taxa of Korean native *Chrysanthemum* species by analyzing and comparing the species-specific volatile flavor compounds. Results of the study provide clear species-specific volatile flavor compound resource, which could be used as basic data for the germplasms and potential ingredient for functional foods, medicines, and cosmetics.

Materials and Methods

Plant Materials

Fifteen taxa of Korean native *Chrysanthemum* species were collected from different regions, and grown in greenhouse at Highland Agriculture Research Center (37°40′N, 128°43′E, altitute 722 m), Rural Development Administration in Pyeongchang, Korea since 2010 (Table 1). From July 12-14, 2011, 20 g of leaves from each *Chrysanthemum* species were collected. The leaves were stored in -70°C deep freezer and freeze-dried using freezing dryer (FDT-12012, Operon Co., Gimpo, Korea).

Extraction of Volatile Flavor Compounds

To extract the volatile flavor compounds, simultaneous steam distillation (SDE; Likens & Nickerson type) were done following the extraction method of Schultz et al. (1977). Two grams of freeze-dried leaf sample and 500 mL of distilled water were added to one of the flasks, and heated-reflux was performed for 2 h on a 100°C heating mantle. Meanwhile, the essential oils were extracted by adding 50 mL of n-pentane:diethyl ether mixture (1:1 = v:v) in the other flask and heated-refluxing to 40°C. The extracted volatile flavor compounds were dehydrated using anhydrous sodium sulfate (Na_2SO_4). Afterwards, they were filtered using filter paper (No. 1, Whatman Limited, Maidstone-kent, UK), and concentrated using 99.9% nitrogen gas. Finally, the concentrated compounds were dissolved in 0.2 mL of diethyl ether and analyzed using gas chromatography/mass selective detector (GC/MSD) as below.

Analysis of Volatile Flavor Compounds

The extracted volatile flavor compounds were analyzed and identified using GC/MSD as described in Table 2. About 1.0 μL of the extracted volatile flavor compounds was injected into GC (Agilent 7890A, Agilent Co., Wilmington, DE, USA) equipped with HP-5MS capillary column (30 m

Table 1. List of 15 taxa of Korean native *Chrysanthemum* species used in this study.

Scientific name	KMRH[z] voucher	Cites (Natural habitats)
C. zawadskii Herbich ssp. *acutilobum* (DC.) Kitagawa	MPS003031	Mt. Yumyeong, Gyeonggi-do, Korea
C. zawadskii Herbich ssp. *acutilobum* (DC.) Kitagawa var. *tenuisectum* (Kitagawa) Y. Lee	MPS003032	Pocheon, Gyeonggi-do, Korea
C. zawadskii Herbich ssp. *acutilobum* (DC.) Kitagawa var. *alpinum* (Nak.) Y. Lee	MPS003033	Mt. Baekdu, Hamgyeongbook-do, Korea
C. zawadskii Herbich ssp. *lucidum* (NAK.) Y. Lee	MPS003034	Ullung, Gyeongsangbook-do, Korea
C. zawadskii Herbich ssp. *coreanum* (NAK.) Y. Lee	MPS003035	Mt. Halla, Jeju-do, Korea
C. zawadskii Herbich ssp. *naktongense* (NAK.) Y. Lee	MPS003037	Gimhae, Gyeongsangnam-do, Korea
C. zawadskii Herbich ssp. *yezoense* (Maekawa.) Y. Lee	MPS003039	Goheung, Jeollanam-do, Korea
C. zawadskii Herbich ssp. *latilobum* (Maxim.) Kitagawa	MPS003041	Wando, Jeollanam-do, Korea
C. zawadskii Herbich ssp. *latilobum* (Maxim.) Kitagawa var. *leiophyllum* (Nak.) Y. Lee	MPS003043	Gangneung, Gangwon-do, Korea
C. indicum Linné	MPS003044	Anmyeondo, Chungcheongnam-do, Korea
C. indicum Linné var. *albescens* Makino	MPS003046	Jeongseon, Gangwon-do, Korea
C. indicum var. *acuta* (Uyeki) Kitam.	MPS003047	Byeonsanbando, Jeollabuk-do, Korea
C. boreale (Mak.) Makino	MPS003049	Pyeongchang, Gangwon-do, Korea
C. lineare Matsumura	MPS003050	Mt. Chilbo, Gyeonggi-do, Korea
C. makinoi Matsumura et Nakai	MPS003051	Daegu, Korea

[z]Korea Medicinal Resource Herbarium at Rural Development Administration in Korea.

Table 2. Analytical condition of GC-MS for the identification of volatile flavor compounds in 15 taxa of Korean native *Chrysanthemum* species.

Instrument	Gas chromatography/mass spectroscopy
Model	Agilent 7890A model (Agilent Technologies 7890A GC System, Wilmington, DE, USA)
Column	HP-5MS (30 m × 0.25 mm, film thickness: 0.25 μm)
Detector	Triple-Axis detector Temperature: 250°C
Carrier gas	He (1 mL·min^{-1}) Initial temperature: 50°C Initial time: 5 min
Oven temp.	Rate: 7°C·min^{-1} Final temperature: 250°C Final time: 30°C·min^{-1}
Injection	Port Temperature: 250°C Injection volume: 1 μL (split ratio 50:1)
Electron voltage	70 eV
Temperature priming	initial 50°C $\xrightarrow{5\ min}$ 50°C $\xrightarrow{7°C\cdot min^{-1}}$ 250°C $\xrightarrow{30\ min}$ 250°C
Data	Wiley 70 database system

× 0.25 mm i.d., 0.25 μm film thickness) and was analyzed by split mode (split ratio = 50:1). As for GC analysis conditions, the inlet and detector temperatures were maintained at 250°C, and the helium carrier gas flow rate at 1.0 mL per minute. The oven temperature was kept at 50°C for 5 min, raised by 7°C per min, and then maintained for 30 min at 250°C. For GC/MSD conditions, the electron ionization energy was 70 eV, the ion source temperature was 250°C, and the mass range was 20-400 a.m.u. Regarding verification of the compounds, the mass spectrum obtained using GC/MSD was compared with Wiley 275L database system (Wiley 70, Agilent Co., Wilmington, DE, USA) and mass spectral data from the previous literature (Kondjoyan and Berdague, 1996) for the identification. C_5-C_{20} of alkane (Aldrich Chemical Co., Milwaukee, WI, USA) were used as reference flavor compound materials and expressed in terms of relative peak area (%).

Statistical Analysis

In order to compare the volatile flavor compounds among *Chrysanthemum* species, analysis of variance (ANOVA) was used to compare the means (n = 4) of the peak area from GC/MSD using SAS program (SAS institute, ver. 9.2, Cary, NC, USA). Levels of significance were calculated using ANOVA test at $p \leq 0.05$, 0.01, and 0.001. Values of $p \leq 0.05$ were considered to be statistically significant.

Results and Discussion

Volatile Flavor Compounds Found in Korean native *Chrysanthemum* Species

Identification of the volatile flavor compounds in 15 taxa of Korean native *Chrysanthemum* species was accomplished by comparing the mass spectra of their components with the Wiley database, and their GC retention time with GC/MSD spectral data (data not shown).

The flavor compounds of 15 taxa of *Chrysanthemum* species are constituted of six large functional groups, such as alcohols, ketones, hydrocarbons, esters, acids, and aldehyde. On the average, alcohols were the highest flavor compound group (28.7%) in 15 taxa of *Chrysanthemum* species, followed by ketones (21.2%), and hydrocarbons (13.2%) based on peak area (Table 3). Three functional groups consisted of 63.1% of the total flavor compounds in 15 taxa of *Chrysanthemum* species. These compositions were dependent on the taxa; alcohols were the most abundant flavor compounds in 8 taxa, and ketones were the most abundant in the other 7 taxa. All the taxa showed negligible amount (< 1%) or no aldehyde compound. Although most taxa contained little (< 2%) to none acid compound, *C. indicum* and *C. lineare* contained particularly high acid contents at 4.95% and 10.88% of peak area, respectively. Esters consisted of 0.0-11.3% of peak area depending on the species, and *C. zawadskii* ssp. *acutilobum* had the highest esters contents among the taxa.

Comparison of the flavor compounds in 15 taxa of *Chrysanthemum* species is shown in Table 4. GC chromatogram showed 45 volatile flavor compounds from 12 to 32 volatile flavors each depending on taxa. The six flavor compounds, including sabinene, *cis*-chrysanthenol, borneol, *m*-thymol, chrysanthenone, and camphor showed the most significant differences among the taxa ($p \leq 0.001$). Sabinene was detected at a significant amount only in *C. zawadskii* subspecies and *C. makinoi*. *cis*-Chrysanthenol was detected only in four taxa of *C. zawadskii* subspecies such as ssp. *naktongense*, *yezoense*, *latilobum*, and *latilobum* var. *leiophyllum*. Significant differences ($p \leq 0.01$) in γ-terpinene, 1-octen-3-ol, linalool, α-terpineol, hinesol, and *trans*-chrysanthenyl acetate were found among the taxa. In particular, γ-terpinene, 1-octen-3-ol and α-terpineol were detected only in *C. zawadskii* subspecies and *C. makinoi*, while hinesol was detected only in *C. indicum* subspecies and *C. boreale*. To investigate the differences in flavor compounds among 15 taxa of *Chrysanthemum* species, we compared the flavor compounds within the six functional categories of the compounds below.

Table 3. Variation in the functional group of volatile flavor compounds among 15 taxa of Korean native *Chrysanthemum* species in greenhouse of highland area.

Functional group	Peak area (%) of volatile flavor compounds in *Chrysanthemum* species[z]															Mean
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	
Alcohols	24.3	22.8	34.4	35.9	31.3	27.4	25.8	28.5	26.2	24.2	43.0	20.6	41.3	19.7	25.0	28.7
Ketones	30.9	28.7	21.6	18.3	10.6	31.2	26.2	31.7	36.1	7.4	8.4	9.4	19.2	13.1	25.8	21.2
Hydrocarbons	9.4	12.4	13.8	21.3	15.4	18.2	18.4	11.1	11.2	11.5	18.9	15.5	9.2	3.3	8.1	13.2
Esters	11.3	9.5	3.8	4.2	4.2	7.0	2.0	5.3	2.9	0.0	0.6	3.5	0.3	2.2	0.2	3.8
Acids	0.0	0.0	0.2	0.2	0.0	0.0	0.6	0.0	0.3	5.0	1.5	0.5	0.6	10.9	1.5	2.1
Aldehyde	0.1	0.1	0.2	0.0	0.2	0.3	0.2	0.8	0.6	0.0	0.4	0.3	0.3	0.0	0.1	0.3

[z]A, *C. zawadskii* ssp. *acutilobum*; B, *C. zawadskii* ssp. *acutilobum* var. *tenuisectum*; C, *C. zawadskii* ssp. *acutilobum* var. *alpinum*; D, *C. zawadskii* ssp. *lucidum*; E, *C. zawadskii* ssp. *coreanum*; F, *C. zawadskii* ssp. *naktongense*; G, *C. zawadskii* ssp. *yezoense*; H, *C. zawadskii* ssp. *latilobum*; I, *C. zawadskii* ssp. *latilobum* var. *leiophyllum*; J, *C. indicum*; K, *C. indicum* var. *albescens*; L, *C. indicum* var. *acuta*; M, *C. boreale*; N, *C. lineare*; O, *C. makinoi*.

Table 4. Components of the volatile flavor compounds obtained from 15 taxa of Korean native *Chrysanthemum* species in greenhouse at highland areas.

Volatile flavor compounds	Peak area (%) of volatile flavor compounds in *Chrysanthemum* species[z]															*p* value
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	
Alcohols																NS
1-Octen-3-ol	0.20	0.07	0.30	0.09	0.39	0.58	0.06	0.38	0.25						0.37	**
1-Decanol														1.84		NS
Linalool						0.36	0.07	0.17	0.76						0.44	**
cis-Chrysanthenol						9.24	13.98	14.81	14.13						9.41	***
Borneol	11.32	12.48	18.52	20.94	18.96	1.10	0.91	0.66	0.62	5.40	18.29	2.86	3.27	1.70	1.82	***
Epoxylinalol															0.03	NS
α-Terpineol	1.49	0.86	1.08	0.36	1.13	0.86	0.28	0.52	0.66						1.53	**
Myrtenol	1.05	0.57	1.72	0.32	0.39	1.24	1.34	0.81	0.60	1.68	0.54	2.85	0.51		0.23	NS
Piperitol										0.15	0.14	4.12	12.39			NS
m-Thymol	2.85	2.98	1.97	4.26	3.39											***
Eugenol						1.31	1.80	0.06	0.17							NS
Hinesol										4.79	2.95	6.73	3.49			**
α-Cadinol														2.72		NS
Phytol	7.36	5.86	10.84	9.92	7.03	12.72	7.37	11.12	9.02	12.21	21.08	4.05	21.62	13.48	11.12	NS
Ketones																
Chrysanthenone						1.15	1.02	0.25	1.99						0.88	***
Camphor	28.61	25.67	19.61	18.01	10.07	29.77	24.88	30.79	33.22	6.44	8.39	8.91	18.25	13.11	24.88	***
Pinocarvone	1.07	0.04	0.83	0.02	0.16	0.30	0.29	0.62	0.91	0.99		0.49	0.99			NS
Thyme camphor	1.22	2.98	1.16	0.26	0.40											*
Hydrocarbons																
2,4-Dimethyl-heptene	0.04	0.02	0.10	0.12	0.53	0.03	0.57	0.34	0.32	0.07	0.20		0.04	0.22	0.21	NS
α-Thujene	0.15	0.08	0.14			0.04	0.13	0.03	0.10							NS
α-Pinene	1.27	0.58	0.25	0.86	0.36	0.44	0.80	1.33	0.59	0.01	1.19	0.45	0.21	0.79	0.99	NS
Camphene	2.04	1.53	1.28	1.23	0.12	0.22	0.10	0.06	0.18	0.17	3.57	0.28	0.98	0.42	0.39	NS
Sabinene	0.38	0.07	0.11	0.06	0.19	0.70	0.65	0.56	0.28						0.23	***
β-Pinene	0.95	0.17	0.12	0.27	0.10	0.36	1.41	0.07	0.05		0.25	0.08	0.06		0.95	NS
α-Terpinene	0.02	0.26	0.19	1.30	0.99	0.26	0.08	0.25	0.20							NS
p-Cymene	0.93	1.22	0.09	0.08	1.36	0.99	1.10	1.01	0.26	0.02	0.17	0.13	0.37		1.28	*
Limonene	0.22	0.12	0.09	0.06	0.97										0.23	NS
γ-Terpinene	0.43	0.61	0.84	0.36	1.21	1.01	0.54	0.53	0.11						0.22	**
α-Thujone										0.05	0.28	0.08	0.27			*
trans-Caryophyllene	0.47	0.84	1.27	1.23	1.15	0.32	0.64	0.60	0.29	2.62	0.21	2.39	0.63		1.17	NS
β-Selinene	0.35	0.19	1.38		0.22	0.60	1.79	1.24	0.96							*
Germacrene-D	0.25	0.64	1.16	0.92	1.89	3.71	1.74	0.97	1.11	2.67	2.96	0.18	3.17	1.89	1.01	NS
α-Muurolene										0.29	2.65	3.80	0.15			NS
δ-Cadinene	0.09	0.66	1.66	8.03	2.56	7.31	5.38	1.88	1.49	1.06	2.59	2.07	0.95		1.39	NS
α-Longipinene				3.80												NS
Caryophyllene oxide	1.11	3.69	2.23	2.37	1.94	1.51	2.75	1.35	3.87	2.34	3.65	3.90	1.67			NS
γ-Gurjunene	0.68	1.72	2.84	0.65	1.85	0.66	0.68	0.92	1.40	2.18	1.15	2.09	0.65			NS
Esters																
1,8-Cineole	1.27	0.73	1.13	0.74	1.06	1.03	0.26	0.91	0.29	0.02	0.55	3.50	0.26	0.20	0.05	NS
trans-Sabinene hydrate	6.97	6.20	1.88	0.36	0.40	2.70	0.49	0.37	0.04							NS
cis-Sabiene hydrate	1.85	2.21	0.18	0.42	1.48	2.91	0.84	3.37	2.27							*
trans-Chrysanthenyl acetate					0.27	0.39	0.36	0.68	0.30						0.14	**
Bornyl acetate	1.17	0.40	0.58	2.66	1.00									1.98		NS
Acids																
Pentadecanoic acid														10.88		NS
Hexadecanoic acid			0.24	0.23			0.63		0.25	4.95	1.45	0.48	0.64		1.50	NS
Aldehyde																
trans-2-Hexenal	0.07	0.10	0.16	0.02	0.23	0.33	0.16	0.80	0.64	0.01	0.37	0.28	0.25		0.13	NS
No. of total compound	30	30	31	30	30	31	32	31	32	21	21	21	22	12	25	

[z]For species name, refer to Table 3.
[NS,*,**,***]Non-significant or significant at $p \leq 0.05$, 0.01, or 0.001, respectively within the same row.

Alcohols

Alcohols were the most abundant flavor compounds (19.7-43.0% of peak area) in 8 taxa of *Chrysanthemum* species, which include three subspecies of *C. zawadskii*, and *C. indicum* subspecies, *C. boreale*, and *C. lineare* (Table 4). Although a total of 14 alcohols were detected from all taxa, their contents were different depending on the taxa. Chang and Kim (2009) reported that 12 alcohols were detected in flower of *C. indicum*, including 1,8-cineole, chrysanthenol, isopinocarveol, borneol, terpinen-4-ol, endo-borneol, carveol, eugenol, spathulenol, cedrol, vulgarol B, and α-bisabolol. Choi et al. (2006) reported 11 alcohols in flower of *C. boreale*, which was more than the number of alcohols detected from the same species in this study. These different results were mostly due to the plant parts where the flavor compounds were extracted.

All 15 taxa of the common *Chrysanthemum* species contained two alcohols (phytol and borneol), which consisted of 32-92% alcohols, depending on the species (Fig. 1). Phytol was one of the most abundant alcohol compounds in 6 taxa, with an average of 50% of the total alcohols. In particular, the phytol content in *C. lineare* was 67.6% of total alcohols. Phytol was identified as an antioxidant compound in *Melittis melissophyllum* (Maggi et al., 2010) and *Eriobotrya japonica* (Ham et al., 2012), and it was reported having high anti-microbial activity against *Staphylococcus aureus* (Inoue et al., 2005). With high phytol contents, these taxa may potentially be a great source for medicinal terpenic compounds.

Borneol was the most abundant alcohol compound in five *C. zawadskii* subspecies (ssp. *acutilobum*, ssp. *acutilobum* var. *tenuisectum*, ssp. *acutilobum* var. *alpinum*, ssp. *lucidum*, and ssp. *coreanum*) and *C. indicum* var. *albescens*, which had more than 11.5% of peak area (Table 4). Other four *C. zawadskii* species had less than 1.1% of peak area. In previous studies, Shunying et al. (2005) detected borneol (8.3-18.3% of peak area) from *C. indicum* flower, however this study showed borneol was detected at 5.4% of peak area in the leaves of the same species, likely due to different plant parts where the flavor compounds were extracted. Borneol was reported to have an antimicrobial activity (Shunying et al., 2005).

All nine *C. zawadskii* species and *C. makino* contained

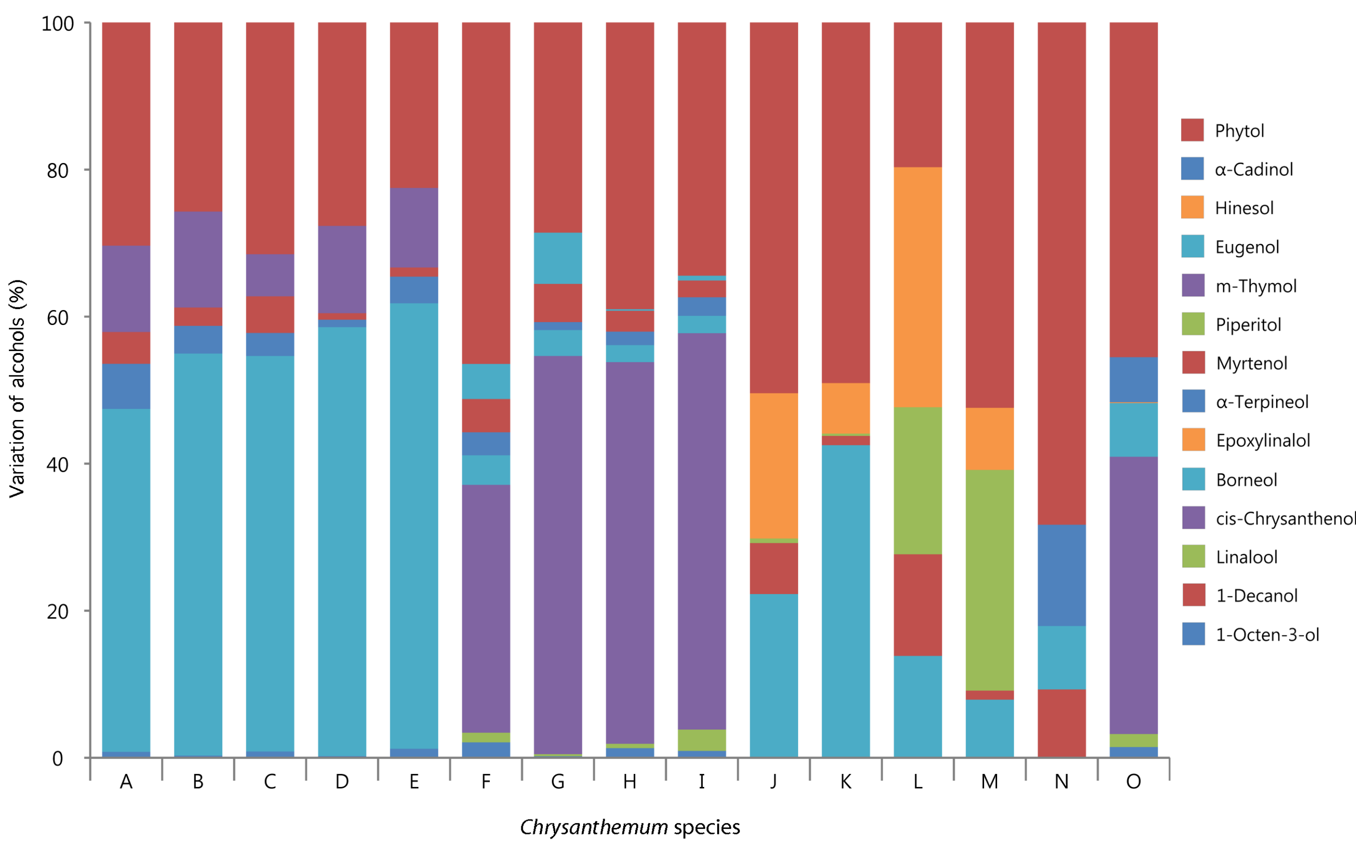

Fig. 1. Variation in the alcohols of volatile flavor compounds among 15 Korean native *Chrysanthemum* species in greenhouse of highland area. For species name, refer to Table 3.

1-octen-3-ol. Interestingly, *m*-thymol was only detected in the five *C. zawadskii* subspecies with high borneol contents, whereas eugenol was only detected in the other four *C. zawadskii* subspecies.

In this study, large quantities of *m*-thymol were also seen in *C. zawadskii* ssp. *lucidum* (4.3% of peak area) and *C. zawadskii* ssp. *coreanum* (3.4% of peak area), indicating a potential as an ingredient for development of bactericidal drugs. Moreover, *m*-thymol has been reported to have a similar medicinal odor as phenol, with antiseptic and disinfectant qualities (Pirbalouti et al., 2013).

Linalool and *cis*-chrysanthenol were also detected from the four *C. zawadskii* subspecies and *C. makinoi* which contained low borneol. Linalool was often used as a raw incense in perfumes and cosmetics (Arctander, 1969), and had been reported to have strong inhibitory effect against 17 bacteria and 10 fungi (Pattnaik et al., 1997). *cis*-Chrysanthenol was not a common component in *Chrysanthemum* species as it had not been found in some *C. zawadskii* and *C. indicum* species (Hong, 2002; Shin et al., 2004; Woo et al., 2008). It has only been reported that *C. indicum* flowers (Jung, 2009) had a minor component of *cis*-chrysanthenol, and aerial part from *C. boreale* had *cis*-chrysanthenol as a major component (Hong, 2002). From the results of linalool and *cis*-chrysanthenol contents, there were two different subgroups within *C. zawadskii* subspecies, with different compositions of alcohols.

Piperitol and hinesol were only detected in three *C. indicum* subspecies and *C. boreale*. These two compounds might be good flavor compounds to identify *C. indicum* or *C. boreale* from other species. 1-decanol and α-cadinol were detected only in *C. lineare*, indicating very specific flavor compounds for *C. lineare.*

Ketones

Ketones were the most abundant volatile flavor compounds in 7 taxa of *Chrysanthemum* species (13.1-36.1% of peak area), including six *C. zawadskii* subspecies, and *C. makinoi*

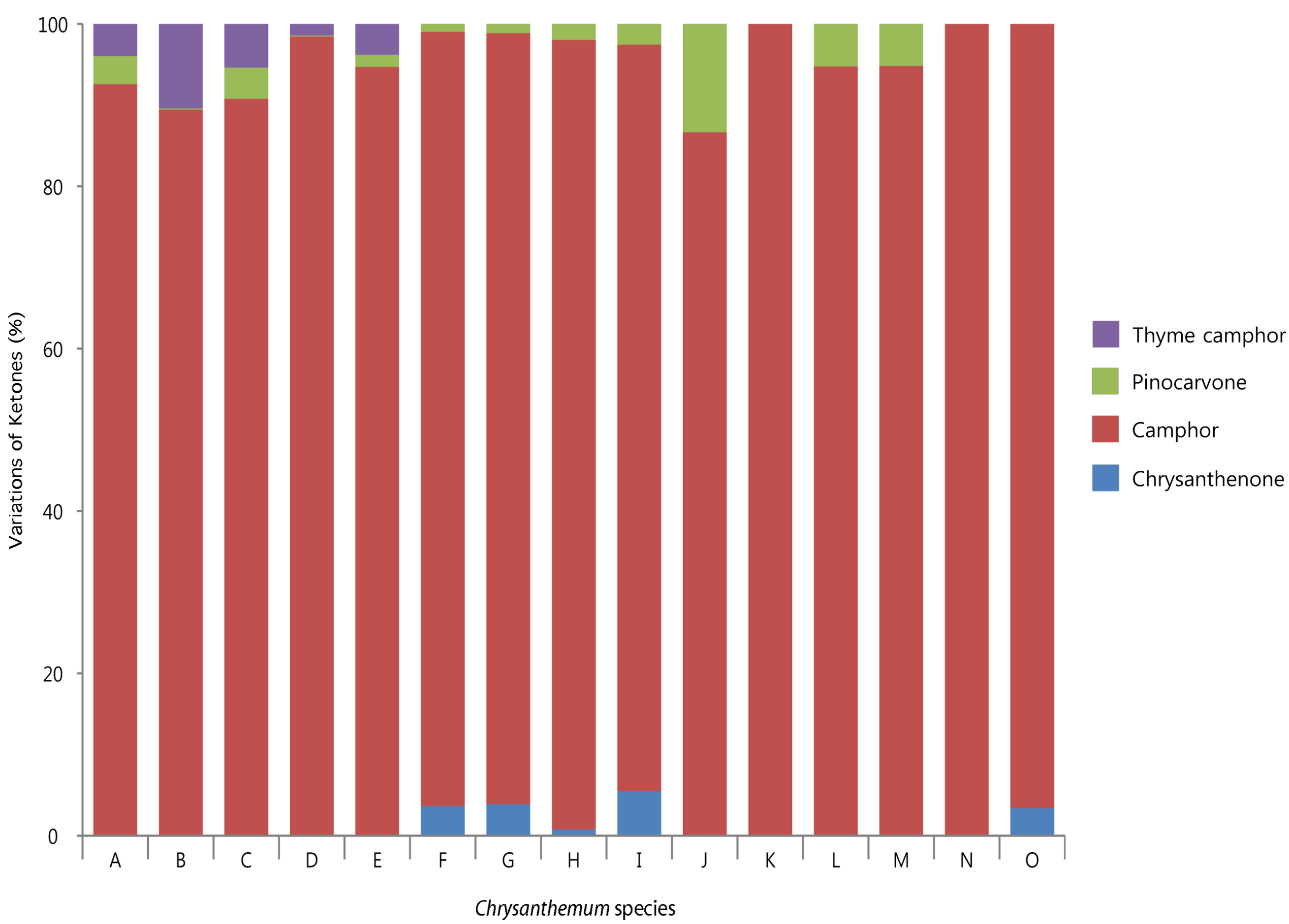

Fig. 2. Variation in the ketones of volatile flavor compounds among 15 taxa of Korean native *Chrysanthemum* species in greenhouse of highland area. For species name, refer to Table 3.

(Table 4). Total of 4 ketones in *Chrysanthemum* species were camphor, pinocarvone, thyme camphor, and chrysanthenone. Camphor was the most common and the most abundant (86.7-100.0% of total ketones) in these species (Fig. 2).

Camphor was detected in all 15 taxa of *Chrysanthemum* species investigated. It was the most abundant compound in most species. In particular, *C. zawadskii* ssp. *latilobum* and ssp. *latilobum* var. *leiophyllum* had high camphor contents with over 30% of peak area (Table 4). Camphor has been reported as an important substance for medicinal purposes (Arctander, 1969); it can alleviate itchiness or suppress cough or food poisoning bacteria (Jang et al., 2010), mostly due to its antimicrobial properties (Tzakou et al., 2001; Viljoen et al., 2003). Previously, several researchers (Chang and Kim, 2008) reported that the most predominant compounds of *Chrysanthemum* species were camphor, although there were some variations of camphor within *Chrysanthemum* species (Chang and Kim, 2008; Huang et al., 2001). These results correspond with the findings of previous research in *Chrysanthemum* species as containing high amounts of camphor.

Pinocarvone was also commonly detected in most *Chrysanthemum* species except for three taxa: *C. indicum* var. *albescens*, *C. lineare*, and *C. makinoi*. Pinocarvone, a similar compound to iso-pinocamphone, was reported to be contained in a large amount in *Artemisia iwayomogi* (Choe et al., 2004).

Interestingly, thyme camphor was detected only in five *C. zawadskii* subspecies with *m*-thymol (also had high borneol contents), and chrysanthenone was detected only in four *C. zawadskii* subspecies and *C. makinoi*, which had linalool and *cis*-chrysanthenol. As previously discussed, nine *C. zawadskii* subspecies could be divided into two groups not only by its alcohols, but also with ketones. Formerly, Chang and Kim (2009) identified 6 ketones (15.3% of peak area) in *C. indicum* flower, which included camphor, filifolone, chrysanthenone, menthone, pinocarvone, and carvone.

Early researches revealed that chrysanthenone was not detected in *Artemisia asictica* or *Matricaria camomilla* in same Asteraceae, and was present only in *C. boreale* (Chang and Kim, 2009; Choi et al., 2006; Kim et al., 1994). However, this study revealed opposite results, suggesting that its content depedns on the plant organs such as leaf, flower or aerial part. Therefore, further research with plant part-specific comparative studies is needed.

Hydrocarbons

Hydrocarbons had the third most flavor compounds in *Chrysanthemum* species, which consisted of an average 13.2% of peak area (Table 2). Among 19 hydrocarbons, α-pinene, camphene, and germacrene-D were the only three compounds that were detected from all species (Table 4 and Fig. 3).

Interestingly, α-thujene were detected only in four *C. zawadskii* subspecies such as ssp. *naktongense*, ssp. *yezoense*, ssp. *latilobum*, and ssp. *latilobum* var. *leiophyllum*, indicating specific flavor compounds in *Chrysanthemum* species. Jang et al. (2010) reported that α-thujone had outstanding anti-bacterial, anticancer, anti-inflammatory, anti-ulcer, and anti-diabetic efficacies, and *C. zawadskii* subspecies may have potential ingredient for medicinal products.

Amount of α-pinene content varied from 0.01% in *C. indicum* to 1.27% in *C. zawadskii* ssp. *acutilobum*, with an average of 0.7% of peak area (Table 4). Choi et al. (2006) noted that small amounts of α-pinene were detected in *C. boreale*, which was consistent with this study (0.2% of peak area). The α-pinene was often used as a perfume ingredient (Arctander, 1969), and it was known to have anti-inflammatory effects (Jang et al., 2010). Since *C. zawadskii* ssp. *acutilobum*, *C. zawadskii* ssp. *latilobum* and *C. indicum* var. *albescens* were rich in α-pinene (peak area > 1%), they could be used as an ingredient for anti-inflammatory treatments. Although α-pinene content of *C. lineare* was the highest (23.8%), it only had four hydrocarbons since the percentage of total hydrocarbons of the species was relatively low (0.79% of peak area).

Camphene was also detected in all 15 taxa of *Chrysanthemum* species, but its content is also dependent on the species. In particular, *C. zawadskii* ssp. *acutilobum* and *C. indicum* var. *albescens* contained high camphene content (2.04 and 3.57% of peak area, respectively), and consisted more than 18% of hydrocarbons. Woo et al. (2008) reported that camphene content of *C. zawadskii* was 5 times higher than the quantity in *C. indicum*. However, this study showed that *C. indicum* var. *albescens* contained high camphene (3.57% of peak area), which was 21 folds of the amount detected in *C. indicum* (0.17% of peak area). *C. zawadskii* ssp. *latilobum* appeared to contain a rather small amount of camphene (0.06% of peak area), indicating that the camphene contents of *C. zawadskii* were also dependent on the subspecies. These results were different from previous report of Woo et al. (2008), and this is likely due to unclear distinction within *C. zawadskii* subspecies.

trans-Caryophyllene was detected in 14 taxa of *Chrysanthemum* species, except in *C. lineare*. Caryophyllene had been used in spices (Yeon et al., 2012), and it also was detected in *Artemisia*, *Caryopteris*, *Cinnamonum*, *Citurs*, *Eucalyptus*, *Juniperus*, *Lavandula*, *Melissa*, *Menthe*, *Pinus*, *Salvia*, *Thymus*,

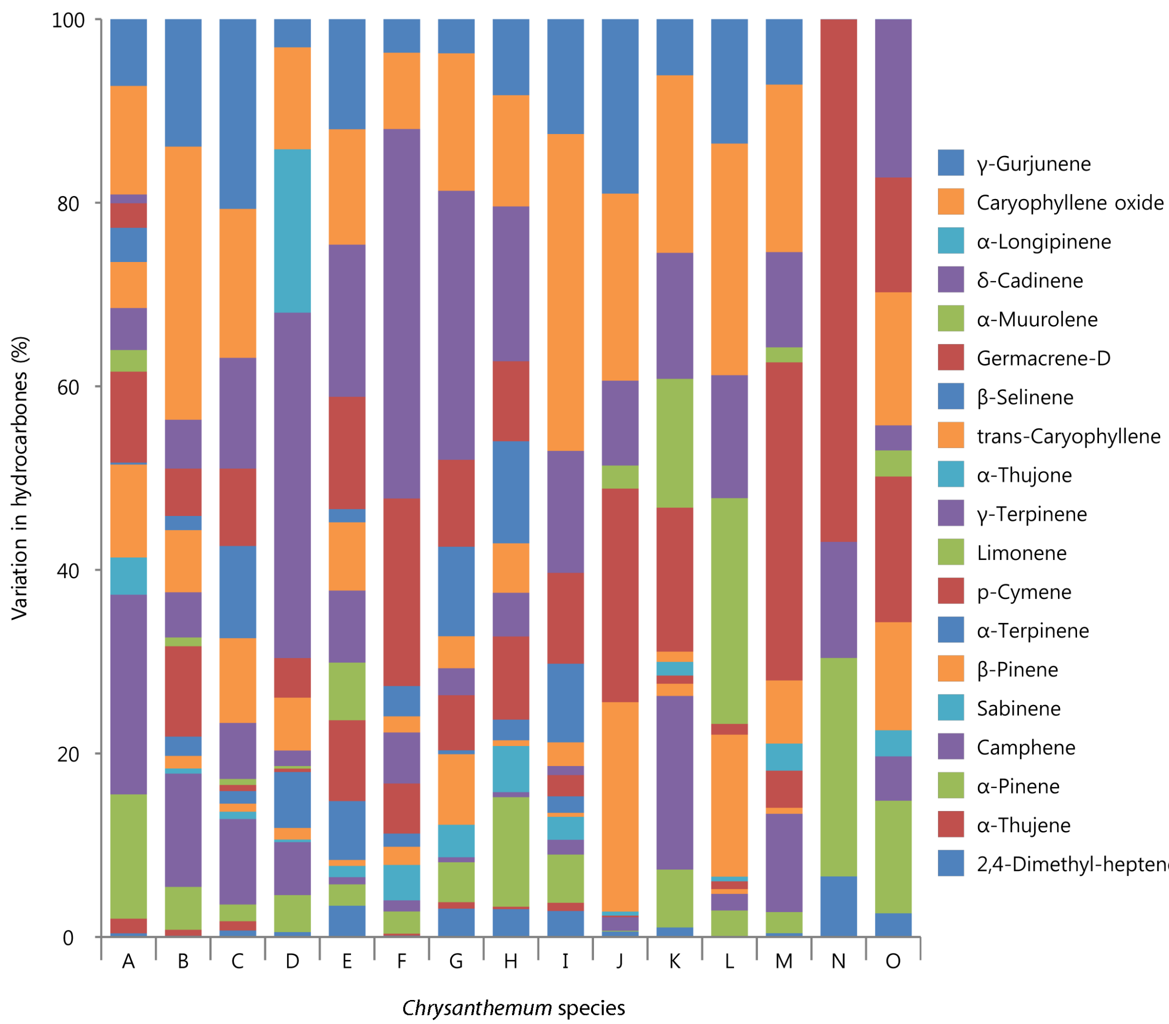

Fig. 3. Variation in the hydrocarbons of volatile flavor compounds among 15 taxa of Korean native *Chrysanthemum* species in greenhouse of highland area. For species name, refer to Table 3.

and *Erigeron* (Yu et al., 2008).

Germacrene-D was detected in all 15 taxa investigated. Its contents ranged from 0.18% in *C. indicum* var. *acuta* to 3.71% of peak area in *C. zawadskii* ssp. *naktongense*. Germacrene is a volatile hydrocarbon that exists in many plants, and it was known to exist as five isomers (A, B, C, D, and E). Germacrene-D was found to be an initial substance from the biosynthesis of sesquiterpene derivative (Ahn et al., 2002), and mainly known as a compound with high antibacterial and antioxidant effects (Rivero-Cruz et al., 2006). Woo et al. (2008) reported that *C. zawadskii* species contained higher germacrene-D than *C. indicum* species or *C. morifolium.* Hong (2002) also reported that the germacrene-D of *C. boreale* was 2.69% of peak area, and this study also showed the high germacrene-D content in *C. zawadskii* ssp. *naktongense* at 3.71% of peak area. Within nine *C. zawadskii* subspecies, germacrene-D contents also varied (0.25-3.71% of peak area) depending on the subspecies. Among three *C. indicum* subspecies, two species had high content of germacrene-D (2.67% and 2.96% of peak area for *C. indicum* and *C. indicum* var. *albescens*, respectively), and *C. indicum* var. *acuta* had the lowest germacrene-D content at 0.18% of peak area.

In this study, cadinene was detected in 14 taxa of *Chrysanthemum* species, except in *C. lineare*. Among all the hydrocarbons, δ-cadinene content was the highest (16.6-40.3% of total hydrocarbons) in 6 taxa, including *C. zawadskii* ssp. *lucidum*, ssp. *coreanum*, ssp. *naktongense*, ssp. *yezoense*, ssp. *latilobum*, and *C. makinoi.* This compound was reported to display bioactivities such as insecticidal, antipyretic, anti-inflammatory, anti-bacterial, and anti-cancer (Lee et al., 2012).

Caryophyllene oxide content was the highest among hydrocarbons in four taxa, including *C. zawadskii* ssp. *acutilobum* var. *tenuisectum* (29.8%), *C. zawadskii* ssp. *latilobum* var. *leiophyllum* (34.5%), *C. indicum* var. *albescens* (19.3%), and *C. indicum* var. *acuta* (25.2%) of total hydrocarbons. *C. indicum* had particularly high *trans*-caryophyllene (22.8%) as well.

Esters

Although esters were relatively few (an average of 3.8% of peak area) compared with other functional groups such as alcohols, ketones, and hydrocarbons, Esters were detected in all 15 taxa of *Chrysanthemum* species (Table 4 and Fig. 4).

Although 1,8-cineole was commonly detected in all 15 taxa of *Chrysanthemum* species, it was a relatively small amount among esters found in this study. Previous research indicated that 1,8-cineole was found in *C. boreale* and *C. indicum,* but not in *Matricaria recutita*, which was another genus in Asteraceae, suggesting it as an index compound for the species classification of *Chrysanthemum* (Chang and Kim, 2008; Choi et al., 2006). In particular, *C. indicum* var. *acuta* had the highest 1,8-cineole content at 3.50% of peak area. 1,8-cineole has been reported to have a suppressive effect on occurrences of mutations (Kim et al., 1992) with the recognition of actual chemical treatment effect on breast cancer in mice (Kubo et al., 1992), suggesting a possible potential ingredient for medicinal use.

trans-Sabinene hydrate and *cis*-sabiene hydrate were detected only in only nine *C. zawadskii* subspecies. *trans*-Chrysanthenyl acetate and bornyl acetate were detected with a little amount depending on *C. zawadskii* subspecies. *C. lineare* contained bornyl acetate at 2.0% of peak area.

Esters synthesized by short-chain acids and alcohols are a large group of flavor and fragrance compounds, which are used extensively in food, cosmetic, beverage, and pharmaceutical industries (Shu et al., 2011). Although one ester from *C. indicum* was detected in this study, four esters in same the species were identified as *trans*-sabinene hydrate, *cis*-sabinene hydrate, *trans*-chrysanthenyl acetate, and bornyl acetate (Chang and Kim, 2009)

Acids

Acids were detected in all *C. indicum* subspecies, *C. boreale, C. lineare*, and *C. makinoi,* but not in all *C. zawadskii*

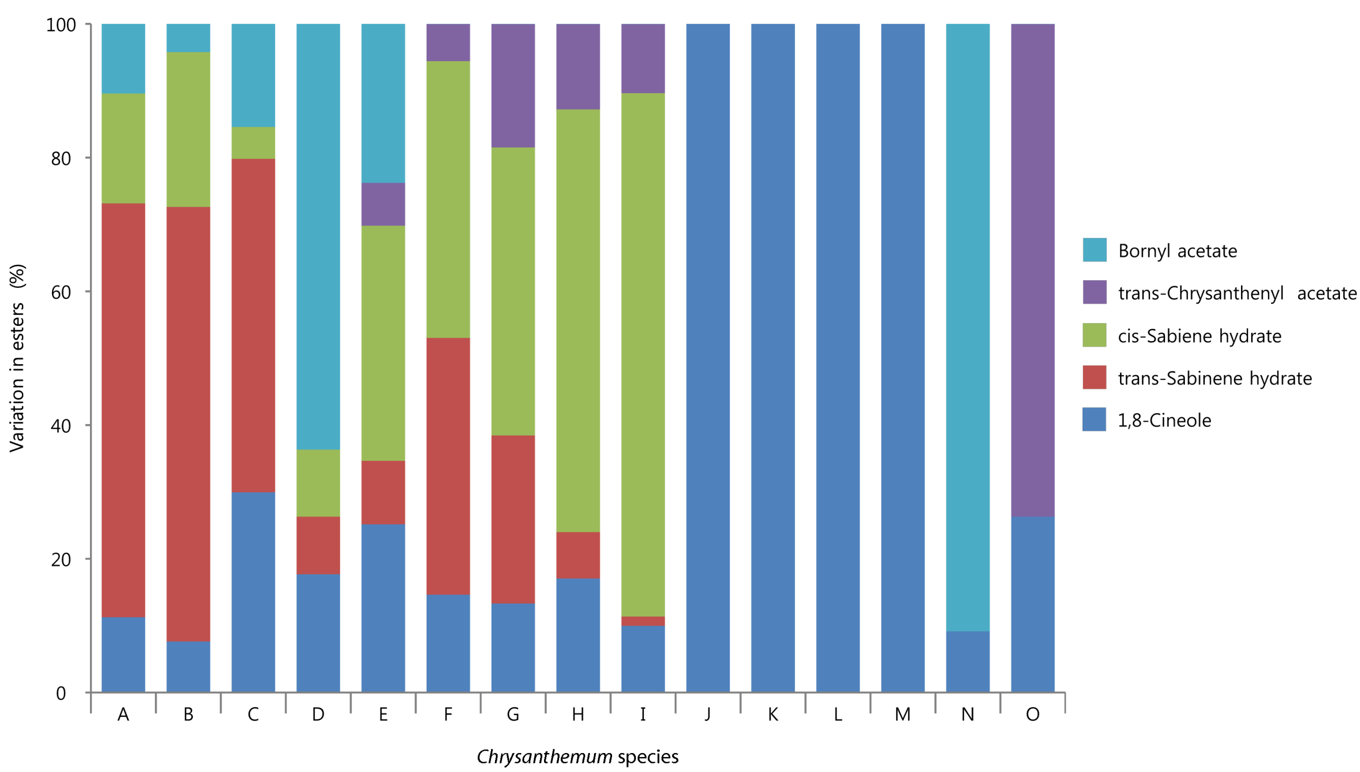

Fig. 4. Variation in the esters of volatile flavor compounds among 15 taxa of Korean native *Chrysanthemum* species in greenhouse of highland area. For species name, refer to Table 3.

subspecies. Although acid content was low in most of *Chrysanthemum* species (average 2.1% of peak area), *C. indicum* had hexadecanic acid at 5.0% of peak area and *C. lineare* had pentadecanoic acid at 10.9% of peak area (Table 4). In particular, only *C. lineare* contained pentadecanoic acid compound, whereas other species contained hexadecanoic acid. This specific compound was very unique to *C. lineare*, and this species had also unique morphological characteristics with different leaf and seed shapes from the other 14 taxa of *Chrysanthemum* species (Kim et al., 2011). Woo et al. (2008) reported trace amount of acids was detected in *C. indicum*, but it was not detected in *C. zawadskii*. However, our results showed that four *C. zawadskii* subspecies contained hexadecanoic acid, indicating subspecies specific volatile flavor compound contents.

Aldehyde

Aldehyde was detected in all *Chrysanthemum* species except *C. lineare*, and only one type (*trans*-2-hexenal) was identified (Table 4). All the *Chrysanthemum* species contained very little amount of aldehyde (less than 0.3% of peak area). Choi et al. (2006) also reported that aldehyde content in *Chrysanthemum* was negligible, which was consistent with this study. Chang and Kim (2009) identified five aldehydes from *C. indicum* flowers with major compounds being 2-hexanal, safranal, benzealdehyde, and phenylacetaldehyde. However, this study found only one aldehyde from *C. indicum* leaves, indicating difference in flavor compounds from different part of organ.

Potential Usage of Korean Native *Chrysanthemum* Species

This study was conducted to compare flavor compounds among 15 taxa of Korean native *Chrysanthemum* species by analyzing and comparing specific flavor compounds, which could be used as baseline data for the germplasms and potential ingredient for functional foods, medicines, and cosmetics. Korean native *Chrysanthemum* species showed a considerable variation in volatile flavor compounds in their leaves, and this study may provide a good indication of species specific potential usage for various applications.

Most *C. zawadskii* subspecies contained β-selinene, which is effective in simulating appetite and a treating nausea and diarrhea (Ravindran et al., 2004). Two *C. zawadskii* subspecies (ssp. *acutilobum* var. *alpinum,* and ssp. *lucidum*) had the most abundant borneol content (18.5-20.9%), which was reported as a volatile compound with antimicrobial activity (Shunying et al., 2005). *C. zawadskii* ssp. *latilobum* and *C. zawadskii* ssp. *latilobum* var. *leiophyllum* contained over 30% peak area of camphor, which was reported as a volatile compound with antimicrobial properties (Tzakou et al., 2001; Viljoen et al., 2003). In particular, *C. zawadskii* ssp. *lucidum* and *C. zawadskii* ssp. *coreanum* contained higher contents of *m*-thymol, which had antiseptic and antibacterial qualities. So, both species can be potentially used as a disinfectant in medical fields. Four *C. zawadskii* subspecies (ssp. *naktongense*, ssp. *yezoense*, ssp. *latilobum*, and ssp. *latilobum* var. *leiophyllum*) contained chrysanthenone, which may have additional use as raw material for cosmetics (Arctander, 1969).

C. indicum subspecies and *C. boreale* contained α-thujone, which had outstanding anti-bacterial, anti-cancer, anti-inflammatory, anti-ulcer, and anti-diabetic efficacies (Jang et al., 2010). In particular, *C. indicum* var. *albescens* have a high utility in making perfumes, since it showed camphene content that was 21 times higher than *C. indicum*. Since *C. indicum* var. *albescens* also contains α-pinene and offers anti-inflammatory effect, its value in cosmetics is expected to increase. *C. indicum* var. *acuta* contained a fairly high content of 1,8-cineole, which has an inhibitory effect on mutagenesis. Also, since 1,8-cineole has outstanding chemical effect (Kubo et al., 1992) in actual treatment of mice with breast cancer, this species may have a great potential as a medicinal plant material. *C. lineare* contained only pentadecanoic acid compound, whereas other species contained hexadecanoic acid. This species had also unique morphological characteristics from other 14 taxa of *Chrysanthemum* species. *C. lineare* was the most different species in hydrocarbon contents among the 15 taxa of *Chrysanthemum* species. It only contained three common compounds (α-pinene, camphene, and germacrene-D) and 2,4-dimethyl-heptene, with the least amount of hydrocarbons at 3.3% of the peak area.

Overall, *Chrysanthemum* species were found to have great potential in multiple applications such as cosmetic raw material or food and medicinal ingredients; especially, Korean native *Chrysanthemum* species had species-specific volatile flavor compounds in the leaves. Therefore, selection of the right species is important to extracting specific volatile compounds. Nevertheless, comparing volatile flavor compounds from different plant organs and finding proper processing methods to extract the useful volatile compound would be done in the future.

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