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Volatiles of *Chrysanthemum zawadskii* var. *latilobum* K.

− Research Note −

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Abstract

The volatile aroma constituents of *Chrysanthemum zawadskii* var. *latilobum* K. were separated by hydro distillation extraction (HDE) method using a Clevenger-type apparatus, and analyzed by gas chromatography-mass spectrometry (GC/MS). The yield of *C. zawadskii* var. *latilobum* K. flower essential oil (FEO) was 0.12% (w/w) and the color was light green. Fifty-five volatile chemical components, which make up 88.38% of the total aroma composition, were tentatively characterized. *C. zawadskii* var. *latilobum* K. FEOs contained 27 hydrocarbons, 12 alcohols, 7 ketones, 4 esters, 1 aldehyde, 1 amine, and 3 miscellaneous components. The major functional groups were terpene alcohol and ketone. Borneol (12.96), (±)-7-epi-amiteol (12.60), and camphor (10.54%) were the predominant volatiles. These compounds can be used in food and pharmaceutical industries due to their active bio-functional properties.

Key words: *Chrysanthemum zawadskii* var. *latilobum* K., aroma constituent, hydro distillation extraction, flower essential oil, borneol

INTRODUCTION

Chrysanthemum zawadskii var. *latilobum* K., '*gujeolcho*' or '*sunmocho*' in Korean for its typical shape, belongs to the tribe *Anthemideae* of the family *Asteraceae* (1,2), and has been used as a traditional folk medicine to treat various diseases such as pneumonia, bronchitis, cough, common cold, pharyngitis, bladder-related disorders, woman's diseases, gastroenteric disorders, and hypertension (3). The plant essential oils (PEOs) are very rich in terpenoids, which exert an inhibitory mechanism against microorganisms by disrupting their membranes (4). PEOs are a complex mixture of volatile flavor compounds consisting of terpenes and their oxygenated derivatives (5). With the growing interest in the use of aromatic medicinal PEOs in both the food and pharmaceutical industries, a systemic examination on the volatile components of the *C. zawadskii* var. *latilobum* K. plant has become increasingly important. *Chrysanthemum* species are known to be a rich source of bioactive compounds such as flavonoids and terpenoids (6-8). *Chrysanthemum* species such as *C. indicum* L., *C. boreale* Makino, *C. morifolium* R., and *C. coronarium* L., have been studied (6-13); however, studies on the volatile aroma flavor components from *C. zawadskii* var. *latilobum* K. has been limited (14,15). In this study, the hydro distillation extraction (HDE) method, a modified simultaneous steam distillation extraction (SSDE) method, was used since organic solvents are capable of contaminating the plant aroma (16). The purpose of this experiment was to characterize the volatiles and functional groups of *C. zawadskii* var. *latilobum* K. flower oils as a basis to study potential bio-active components.

MATERIALS AND METHODS

Plant materials

Chrysanthemum zawadskii var. *latilobum* K. were either harvested in Fall 2008 from Jecheon Province (Chungbuk, Korea) or purchased at Gyungdong Herbal Market (Seoul, Korea) in Spring, 2009 and deposited in the Plant Resources Research Institute, Duksung Women's University, Korea. Samples were stored at -70°C in air-tight bags until further analysis.

Separation of PEOs

The dried *Chrysanthemum zawadskii* var. *latilobum* K. were crushed for 10 s by a blender (NJ-8060SM, NUC Electronics, Seoul, Korea) and 1 kg samples were extracted by HDE for 3 h from setting using a Clevenger-type apparatus (Hanil Lab Tech Ltd, Incheon, Korea) (17). The PEOs obtained were dried over anhydrous sodium sulfate overnight, measured, and stored in hermetically sealed dark-glass containers in a freezer at -4°C until analytical testing by GC/MS.

GC-MS analysis

Analysis was performed on an Agilent 6890 gas chromatography/5973 mass selective detector (Palo Alto, CA,

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USA) equipped with an HP-5MS (5%-phenylmethylpolysiloxane) capillary column (30 m length × 0.25 mm I.d. × 0.25 μm film thickness, Agilent Co.) using a micro syringe. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was maintained at 40°C for 5 min and then programmed to increase as follows: from 40 to 150°C at a rate of 3°C/min, and holding at 150°C for 5 min; then from 150 to 220°C at a rate of 7°C/min, and holding at 220°C for 5 min. The temperatures of the injector and detector were 250 and 280°C, respectively. A 10^{-1} μL sample, previously dissolved in methylene chloride, was injected in split mode with a split ratio of 10:1. The MS conditions were: ionization energy of the mass selective detector was 70 eV, 0.5 sec scanning interval, 1.2 kV detector voltage, and the mass scanning ranges were recorded at m/z 33-330.

Characterization of volatiles

The volatile flavor compounds were identified by comparison of the mass spectra with those in an on-line computer library (Wiley 275) (Agilent Co.). Alkanes were used as reference points in the calculation of relative retention indices (RI). The RI were experimentally determined using the standard method involving retention time of n-alkanes [Alkane Standard Solution (04070, 04071), (C_8-C_{20}, C_{21-40}), Standard for GC, Fluka, USA], injected after the essential oil under the same chromatographic conditions (18). The RIs of the compounds, determined using C_8-C_{22} as external references, were compared with previous published data (19,20). Several compounds were identified, similar to literature (21-23), based on co-injection with authentic compounds (Acoros and Sigma-Aldrich, St. Louis, MO, USA). The quantification of each individual volatile flavor component was performed based on the ratio of the peaks obtained from the mass total ion chromatogram and marked quality percentage of the volatile flavor compounds from the MS data.

RESULTS AND DISCUSSION

Flavor profiles

To identify the aroma components of *Chrysanthemum zawadskii* var. *latilobum* K. flower, hydro distillation extraction (HDE) was initially performed to extract the flower essential oils (FEOs), which were then separated and analyzed. *C. zawadskii* var. *latilobum* K. FEO yielded 0.12% (w/w) and light green in color. The characterized aromatic chemical compounds of *C. zawadskii* var. *latilobum* K. FEOs are shown in Table 1, and listed in order of their elution on the HP-5 column along with retention indices, approximate concentrations (relative peak area percents), quality percentages, and method of identification. A classification based on functional groups is summarized in Table 2. As shown in Tables 1 and 2, fifty-five volatile chemical compounds make up 88.38% of the total aroma composition and were tentatively characterized from *C. zawadskii* var. *latilobum* K. The *C. zawadskii* var. *latilobum* K. FEOs contained 27 hydrocarbons (23.62), 12 alcohols (37.57), 7 ketones (18.63), 4 esters (2.59), 1 aldehyde (1.79), 1 amine (0.83), and 3 miscellaneous components (3.35%). Borneol (12.96) was the most abundant component, followed by (±)-7-epi-amiteol (12.60), camphor (10.54), δ-cadinene (6.29), spathulenol (4.64), propyl sulfone (2.83), thymol (2.64), β-elemene (2.39), germacrene D (2.07), and valencene (2.02%). Isoaromadendrene epoxide, longifolenaldehyde, piperitone, α-gurjunene, β-selinene, nerolidol, umbellulone, filifolone, bornyl acetate, (*E*)-chaveol, and sabinyl acetate were the compounds with concentrations higher than 1% as % peak area.

Alcohols

Twelve terpene alcohol compounds (37.57%) were found in *C. zawadskii* var. *latilobum* K. FEOs, consisting of borneol (most abundant), carveol, eucalyptol (1,8-cineol), α-phallandrene-8-ol (*p*-Mentha-1,5-dien-8-ol), terpinen-4-ol, myrtenol, methyl chavicol, (*E*)-chaveol, thymol, nerolidol, spathulenol, and (±)-7-epi-amiteol. Borneol ($C_{10}H_{18}O$) has dry-camphoraceous and woody-peppery odor, and a woody, minty, and slightly burning taste. Borneol is used in nut and spice flavor compositions, usually in mere traces (24). Borneol, also known as borneo camphor or camphanol, is a bicyclic volatile organic compound containing a hydroxyl group at the endo position, to be easily oxidized to the ketone, camphor. Hence, one historical name for borneol is borneo camphor (25). Borneol can be found in several species of *Artemissia*, *Dipterocarpaceae*, *Blumea balsamifera*, and *Kaempferia galanga*. Borneol is used in traditional Chinese medicine as *Moxa*. Among an important volatile component of oregano and rosemary, borneol is also a natural insect repellent. Previous studies report that borneol has anti-bronchitic, anti-salmonella, and anti-inflammatory activity (25). In this experiment, we also detected terpinen-4-ol (4-terpineol) by GC/MS, a volatile compound, that has been used for aroma therapy with lavender oil and one of the major flavors in the tea tree oil, where its quality is dependent upon the concentration of this compound (26). The main terpene alcohol compounds with concentrations higher than 10% as % peak area were borneol and (±)-7-epi-amiteol. And followed by spathulenol (4.64), thymol (*p*-cymen-3-ol or thyme

Table 1. The volatile flavor compounds of *Chrysanthemum zawadskii* var. *latillobum* K.

Compounds	RI[1)]	QA%[2)]	PA%[3)]	Method of ID[4)]
Tricyclene	0891	96	0.22	RT, MS/RI
α-Thujene	0927	96	0.01	RT, MS/RI
α-Pinene	0940	94	0.33	RT, MS/RI
Camphene	0949	97	0.62	RT, MS/RI
Verbenene	0955	94	0.08	RT, MS/RI
Sabinene	0967	94	0.18	RT, MS/RI
Carveol	0978	93	0.05	RT, MS/RI
β-Myrcene	0986	94	0.09	RT, MS/RI
α-Terpinene	1008	98	0.58	RT, MS/RI[5)]
1,8-Cineol	1028	98	0.73	RT, MS/RI
(*E*)-β-Ocimene	1038	97	0.03	RT, MS/RI
γ-Terpinene	1046	97	0.13	RT, MS
(*E*)-Sabinene hydrate	1056	94	0.15	RT, MS
α-Terpinolene	1097	98	0.30	RT, MS/RI
Filifolone	1113	90	1.16	RT, MS
Camphor	1150	98	10.54	RT, MS/RI
α-Phellandrene-8-ol	1158	83	0.81	RT, MS/RI[6)]
Pinocarvone	1171	69	0.34	RT, MS
Borneol	1186	87	12.96	RT, MS/RI[7)]
Terpinen-4-ol	1192	97	0.10	RT, MS/RI[8)]
Myrtenol	1195	63	0.11	RT, MS
Verbenone	1204	78	0.39	RT, MS
Methyl chavicol	1215	95	0.07	RT, MS/RI
(*E*)-Chaveol	1229	98	1.14	RT, MS
Piperitone	1252	97	1.76	RT, MS
Bornyl acetate	1289	98	1.16	RT, MS/RI
Sabinyl acetate	1295	94	1.12	RT, MS
Thymol	1324	97	2.64	RT, MS/RI
α-Cubebene	1354	99	0.05	RT, MS/RI
α-Terpinyl acetate	1360	89	0.16	RT, MS
α-Copaene	1379	98	0.78	RT, MS/RI
β-Elemene	1398	99	2.39	RT, MS/RI
β-Caryophyllene	1437	99	0.65	RT, MS/RI
β-Cubebene	1442	96	0.09	RT, MS
(*E*)-β-Farnesene	1478	98	0.96	RT, MS/RI
Germacrene D	1485	96	2.07	RT, MS/RI
β-Selinene	1498	98	1.67	RT, MS/RI
γ-Elemene	1508	78	0.62	RT, MS
Valencene	1517	89	2.02	RT, MS/RI
(*E,Z*)-α-Farnesene	1522	91	0.13	RT, MS/RI
Germacrene B	1528	97	0.28	RT, MS
δ-Cadinene	1539	99	6.29	RT, MS/RI
Nerolidol	1560	95	1.72	RT, MS/RI
Spathulenol	1572	97	4.64	RT, MS/RI
Umbellulone	1594	76	1.61	RT, MS
Eremophilene	1603	93	0.63	RT, MS
α-Gurjunene	1642	98	1.68	RT, MS
(±)-7-Epi-Amiteol	1665	90	12.60	RT, MS
Isoaromadendrene epoxide	1668	91	1.91	RT, MS
Propyl sulfone	1672	56	2.83	RT, MS
4-Bromo-1-naphthalemine	1682	97	0.83	RT, MS
Camezulene	1696	91	0.74	RT, MS
Longifolenaldehyde	1785	91	1.79	RT, MS
Hexadecanoic acid	1898	98	0.97	RT, MS/RI
14 β-Pregnane	2205	95	0.47	RT, MS

[1)]RI: Retention indices were determined using n-alkanes (C_8-C_{22}) as external references.[2)]PA is peak area %; average of the relative percentage of the peak area in the MS total ion chromatogram (n=3) from *Chrysanthemum zawadskii* var. *latillobum* K. oil. [3)]QA means quality % of the MS data (n=3). [4)]Method of identification based on reference no.19,20. MS, mass spectrum was consistent with that of Wiley mass spectrum database (2001, Hewlett Packard Co., Palo Alto, CA, USA). RI was consistent with that of the literature. [5)]Identification based on reference no.21. [6)]Identification based on reference no.22. [7)]Identification based on reference no.23. [8)]Identification based on co-injection with authentic compounds (Acros, Sigma-Aldrich).

Table 2. Relative constitutions by functional groups of *Chrysanthemum zawadskii* var. *latillobum* K.[1)]

Functional group	No. of peak	% of peak area[2)]
Hydrocarbon	27	23.62
Aldehyde	1	1.79
Ester	4	2.59
Alcohol	12	37.57
Ketone	7	18.63
Amine	1	0.83
Miscelleneous	3	3.35
Total	55	88.38

[1)]Essential oil from the flowers of *Chrysanthemum zawadskii* var. *latillobum* K. by HDE-GC/MS.
[2)]Average (n=3) of the relative percentage of the peak area in MS total ion chromatogram.

camphor) (2.64), and (*E*)-chaveol (1.12%). Terpene alcohols and ketones were the abundant chemical components in *C. zawadskii* var. *latilobum* K. volatile flower aroma.

Ketone, ester, and aldehyde

Seven ketone compounds (18.63%) were identified in *C. zawadskii* var. *latilobum* K. FEOs, consisting of filifolone, camphor, pinocarvone, verbenone, piperitone, umbellanone, and profyl sulfone. Among them, camphor ($C_{10}H_{18}O$) was the most abundant volatile flavor compound (10.54%). Camphor (root bark oil, 2-bornanone, or 2-camphanone) was also detected in *C. indicum* L. making up 2.64～14.10% (27). Camphor has a fresh sweet aroma note like mint and is known as the one of the major volatile flavor compounds of the *Compositae* family and a bio-functional aroma material (26). The ketones give a wide range of aromatic effects and most of them are pleasing. Higher molecular weight ketones have a marked floral character (28). Camphor is a saturated ketone containing a camphene skeleton and is obtained industrially via pinene, camphene, and isoborneol (29). Camphor was initially characterized in *Eclipta prostrata* L. and *Atractylodes japonica* PEOs in small amounts (30,31). Four esters (2.59%), (*E*)-sabinene hydrate, bornyl acetate, sabinyl acetate, and α-terpinyl acetate, were found in *C. zawadskii* var. *latilobum* K. FEOs. Among them, the major ester compounds with concentrations higher than 1% as % peak area were bornyl acetate and sabinyl acetate. Aldehyde compounds constituted 1.79% of the distilled FEOs, with longifolenaldehyde.

Hydrocarbons

Twenty-seven terpene hydrocarbon compounds (23.62), eleven monoterpene hydrocarbons [tricyclene, α-thujene, α-pinene, camphene, verbenene, sabinene, β-myrcene, α-terpinene, (*E*)-β-ocimene, γ-terpinene, and α-terpinolene (2.57)] and sixteen sesquiterpene hydro-carbons [α-cubebene, α-copaene, β-elemene, β-caryophyllene, β-cubebene, (*E*)-β-farnesene, germacrene D, β-selenene, γ-elemene, valencene, (*E,Z*)-α-farnesene, δ-cadinene, germacrene B, eremophillene, α-gurjunene, and chamazulene (21.05%)] were identified in *C. zawadskii* var. *latilobum* K. Among them, α-pinene was described as the pine tree aroma and flavor and is a very important starting material for the perfume industry (26). α-Pinene was also found at high concentrations in *Juniperus oxycedrus ssp. oxycedrus* (27.40% of the total peak area) berry and wood oils from Cedar of Lebanon, and reportedly has antioxidant and hypoglycemic activities (32). β-Caryophyllene is a common sesquiterpene that is widely distributed plants and possesses both anti-inflammatory and anti-carcinogenic characteristics. A previous study suggests that caryophyllene derivatives could also play an important role in plant defense responses (33). Monoterpene compounds, ucalyoptol, camphor, borneol, thymol, α-pinene, β-pinene, bornyl acetate, and menthol inhibit bone resorption when added to the food of rats. Among them, borneol, thymol, and camphor directly inhibit osteoclast resorption (34).

In this study, we determined the volatile constituents of *C. zawadskii* var. *latilobum* K. FEOs by HDE-GC/MS. The common characteristics of FEOs from *C. zawadskii* var. *latilobum* K. tentatively identified were high contents of terpene alcohols and ketones and low contents of other functional groups (Table 1). Fifty-five volatile chemical compounds were characterized from the MS data, and contained 27 hydrocarbons, 12 alcohols, 7 ketones, 4 esters, 1 aldehyde, 1 amine, and 3 miscellaneous components. Borneol, (±)-7-epi-amiteol, and camphor were the major volatile flavor compounds of *C. zawadskii* var. *latilobum* K, an aromatic medicinal herbaceous plant. We envision a possible use of *C. zawadskii* var. *latilobum* K. in the food and pharmaceutical industries because of their several active bio-functional properties.

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REFERENCES

1. Yuk CS. 2006. *Original Colors pictorial book of the Korean medicinal use*. Academy Books, Seoul, Korea. p

537.
2. Kim YY, Lee SY, Yim DS. 2001. Biological activities of linarin from *Chrysanthemum zawadskii* var. *latilobum* K. *Yakhak Heoji* 45: 604-610.
3. Burt S. 2004. Essential oils: their antibacterial properties and potential applications in food－a review. *Int J Food Microbiol* 94: 223-253.
4. Draushon FA. 2004. Use of botanicals as bio-preservatives in foods. *Food Technol* 58: 20-28.
5. Kil YC, Kim SW, Kim JD, Lee SY. 1996. Antioxidant effect of wild plants extracts. *Food Ind Nutr* 1: 76-77.
6. Matsuda H, Morikawa T, Toguchida I, Harima S, Yoshikawa M. 2002. Absolute stereo structures of two new flavanone glycosides and a phenylbutanoid glycoside from the flowers of *Chrysanthemum indicum* L.: their inhibitory activities for rat lens aldose reductase medicinal flowers. VI. *Chem Pharm Bull* 50: 972-975.
7. Andriani B, Milisa P, Mria C, Orga T. 2007. Essential oil composition of the flower heads of *Chrysanthemum coronaium* L. from Greece. *Flavour Fragr J* 22: 197-200.
8. Yang DS, Park KH, Yang MS. 1998. Germacrenlides from flowers of *Chrysanthemum boreale* MAKINO. *Korean J Pharmacogn* 29: 67-70.
9. Shin YJ, Jeon JR, Park GS. 2004. Physiochemical properties of *Gamgug* (*Chrysanthemum indicum* L.). *J Korean Soc Food Sci Nutr* 33: 146-151.
10. Choi SH, Im SI, Bae JE. 2006. Analysis of aroma components flower tea of German chamomile and *Chrysanthemum boreale* Makino. *Korean J Soc Food Sci* 22: 768-773.
11. Shin SH, Choi YI. 1992. Analysis of essential oil from *Chrysanthemum sibiricum* and comparison with essential oil from Seoul *Chrysanthemum* spp. *Korean J Pharmacogn* 13: 153-156.
12. Kim YS, Lee JH, Kim MN, Lee WG, Kim JO. 1994. Volatile flavor compounds from raw mugwort leaves and parched mugwort tea. *J Korean Soc Food Nutr* 23: 261-267.
13. Stoianova-Ivanova B, Tsoutsoulova A, Mladenova K, Brauner A. 1993. Essential oil of *Chrysanthemum indicum*. *Planta Med* 49: 236-239.
14. Kang HS, Park MJ, Jin KS, Kim YH, Jun M, Lim HJ, Jo WK, Kim JS, Jeong WS. 2008. Regulatory roles of *Chrysanthemum zawadskii* roots in nuclear factor E-2-related factor 2/antioxidant response element pathway. *Food Sci Biotechnol* 17: 362-372.
15. Kim JR, Kim JH, Lim HA. 2005. Induction of quinon reductase and anti carcinogenic marker enzyme by extracts from *Chrysanthemum zawadskii* var. *latilobum* K. *J Food Sci Nutr* 10: 340-343.
16. Schulz TH, Flayh RA, Mon TR, Eggling SB, Teranishi R. 1997. Isolation of volatile components from a model system. *J Agric Food Chem* 25: 446-452.
17. Clevenger JI. 1928. Apparatus for the determination of volatile oil. *J Am Pharm Assoc* 17: 345-349.
18. van den Dool H, Kratz PD. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J Chromatogr* 11: 463-471.
19. Kondjoyan N, Berdague JL. 1996. A compilation of relative retention for the he analysis of aromatic compounds. Laboratorie flaveur, de recherches sur la Viande, Clemont-Ferrand, France. p 15-138.
20. Acree T, Arn H. Flavornet and human odor space Available from: http://www.flavornet.org. Accessed Mar. 29, 2007.
21. Kundakovik T, Fokialakis N, Kovacevic N, Chinou I. 2007. Essential oil composition of *Achillealingulata. Flavour Fragr J* 22: 184-187.
22. Farid BA, Aoumeur B, Brahim YM. 2007. Chemical composition of seed essential oils from *Algerian Nigella*. *Flavour Fragr J* 22: 148-153.
23. Wijaya CH, Hadiprodjo IT, Apriyantono A. 2002. Identification of volatile compounds and key aroma compounds of *Andaliman*. *Food Sci Biotech* 11: 680-683.
24. Kawakami M. 2000. *Aroma study of tea*. Koseikan, Tokyo, Japan. p 214.
25. Available from: http://en.wikipedia.org/wiki/Borneol. Accessed Feb. 23, 2012.
26. Arctander S. 1969. *Perfume and Chemicals*. Montclaire, Newark, NJ, USA. p 2510-2659.
27. Chang KM, Kim GH. 2008. Comparative chemical composition of domestic and imported *Chrysanthemum indicum* L. flower oils. *Food Sci Biotechnol* 18: 1288-1292.
28. Francis MJ. 1971. Monoterpene biosynthesis. In *Aspects of Terpenoids Chemistry and Biochemistry.* Goodwin TW, ed. Academic Press, London, UK. p 20-56.
29. Whittaker D. 1972. The monterpenes. In *Chemistry of Terpenes and terpenoids.* Newman AA, ed. Academic Press, London, UK. p 11-87.
30. Chang KM, Kim GH. 2009. Constotuents of the essential oil from *Eclipta prostrata* L. *J Food Sci Nutr* 14: 168-171.
31. Chang KM, Kim GH. 2010. Chemical components of *Atractylode japonica* rhizome oil. *J Food Sci Nutr* 15: 147-151.
32. Loizzo MR, Tundis R, Conforti F, Saab AM, Statti GA. 2007. Comparative chemical composition, antioxidant and hypoglycemic activities of *Juniperus oxycedrus ssp. oxycedrus* L. berry and wood oils from Lebanon. *Food Chem* 105: 572-578.
33. Shen HY, Li ZQ, Wang H, Ma LQ, Liu BY, Yan F, Li GF, Ye HC. 2007. Advances in sesquiterpene synthases (Cyclases) of *Artemisia annua*. *Chinese J Biotech* 23: 976-981.
34. Mühlbauer RC, Lozano A, Palacio S, Reinili A, Felix R. 2003. Common herbs, essential oils, and monoterpenes potently modulate bone metabolism. *Bone* 32: 372-380.

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