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

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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 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 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), (C₈-C₂₀, C₂₁-₄₀), Standard for GC, Fluka, USA], injected after the essential oil under the same chromatographic conditions (18). The RIs of the compounds, determined using C₈-C₂₂ 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), 9-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)-chavicol, 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-phellandrene-8-ol (p-Mentha-1,5-dien-8-ol), terpinen-4-ol, myrtenol, methyl chavicol, (E)-chavicol, thymol, nerolidol, spathulenol, and (±)-7-epi-amiteol. Borneol (C₁₀H₁₉O) has dry-camphoraceous and woody-peppy 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 Artemisia, Dip terocarpaceae, 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-terpinol) 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-Terpinepine              | 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-Terpinepine              | 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)-Chavicol               | 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/RI        |
| 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        |
| Germancrene 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        |
| Germancrene B              | 1528    | 97     | 0.28   | RT, MS/RI        |
| 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-Gurjinene                | 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           |
| Camerulene                 | 1696    | 91     | 0.74   | RT, MS           |
| Longifolendaldehyde        | 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 co-injection with authentic compounds (Acros, Sigma-Aldrich).
Table 2. Relative constitutions by functional groups of Chrysanthemum zawadskii var. latilobum K.

| Functional group | No. of peak | % of peak area |
|------------------|-------------|----------------|
| Hydrocarbon      | 27          | 23.62          |
| Aldehyde         | 1           | 1.79           |
| Ester            | 4           | 2.59           |
| Alcohol          | 12          | 37.57          |
| Ketone           | 7           | 18.63          |
| Amine            | 1           | 0.83           |
| Miscellaneous    | 3           | 3.35           |
| Total            | 55          | 88.38          |

1) Essential oil from the flowers of Chrysanthemum zawadskii var. latilobum 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)-chavel (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 fli-folone, camphor, pinocarvone, verbenone, piperitine, 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, verbenone, 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, eremophilene, α-gurjune, 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-amitool, 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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