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Volatile Aroma Composition of *Chrysanthemum indicum* L. Flower Oil

– Research Note –

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Abstract

The aroma constituents of *Chrysanthemum indicum* L. were separated by the hydro distillation extraction method using a Clevenger-type apparatus, and were analyzed by gas chromatography-mass spectrometry (GC/MS). The yield of *Chrysanthemum indicum* L. flower oil was 2.0% (w/w) and the color was light golden yellow. Sixty-three volatile flavor components, which make up 89.28% of the total aroma composition of the flower oil, were tentatively characterized. This essential oil contained 35 hydrocarbons (48.75%), 12 alcohols (19.92%), 6 ketones (15.31%), 3 esters (4.61%), 5 aldehydes (0.43%), 1 oxide (0.22%), and 1 miscellaneous component (0.04%). α-Pinene (14.63%), 1,8-cineol (10.71%) and chrysanthenone (10.01%) were the predominant volatile components in *Chrysanthemum indicum* L., an aromatic medicinal herbaceous plant.

Key words: *Chrysanthemum indicum* L., aroma constituent, hydro distillation, GC/MS

INTRODUCTION

Chrysanthemum indicum L., a perennial, aromatic and medicinal plant, belonging to the family Compositae, distributed in Asia including Korea, China, Taiwan and Japan (1,2). The Compositae family comprises approximately 1000 genera and 30,000 species, distributed more or less throughout out the globe (3). The flowers of *Chrysanthemum indicum* L. commonly known as *gamgug*, have yellow blossoms that bloom in September and October. *Chrysanthemum boreale* Makino called *sangug* is also distributed in China, Taiwan, Japan and Korea (4). *Chrysanthemum indicum* L. has been used in mixed spices, as a food additives for masking flavors, and used in teas and alcoholic beverages (combined with the flowers) in Japan, China and Korea from the ancient times (5,6). Moreover, the entire plant is used in traditional folk medicine in Asia and Europe especially Germany, against woman's diseases, nephritis, and the treatment of neurological problem and headache (7,8). Furthermore, recently its extracts has been reported to have central and peripheral analgesic properties, lowering blood pressure as well as anti-inflammatory activities (8,9). *Chrysanthemum indicum* L. has been studied by several researchers (9-14). However, studies on the volatile aroma flavor components from *Chrysanthemum indicum* L. flower produced in Korea has been limited (13,14). The plant essential oils are very rich in terpenoids which exert inhibitory action against microorganisms by disrupting their membranes (15). They are the complex mixture of volatile flavor compounds consisting of terpenes, and their oxygenated derivatives (15,16). There are several extraction methods of the plant essential oil, simultaneous steam distillation extraction (SDE), steam distillation extraction, hydro distillation extraction and head space method etc. In this study, a modified SDE method, the hydro distillation extraction method, was used since it does not use organic solvents capable of contaminating the plant aroma (17). The objectives of this study were to isolate and characterize the volatile aroma compounds of *Chrysanthemum indicum* L. flower oil, and to evaluate the main chemical material, functional groups, and search their physico-chemical profiles, as a basis for further research evaluate its bioactive components.

MATERIALS AND METHODS

Plant materials

Chrysanthemum indicum L. harvested in the fall of 2006 from Namwon province (Jeonrabukdo, western Korea), was purchased at Gyungdong Herbal Market (Seoul, Korea) in the spring of 2007. This sample was kept at -70°C in air-tight bags until the analysis was performed.

Isolation of the aroma

The dried *Chrysanthemum indicum* L. was crushed for 10 sec by a blender (NJ-8060SM, NUC Electronics, Seoul, Korea) and 1 kg samples were extracted by hydro distillation extraction for 3 hr from setting using a

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Clevenger-type apparatus (Hanil Lab Tech Ltd., Incheon, Korea). The essential oil obtained was dried over anhydrous sodium sulfate overnight, measured and stored in hermetically sealed dark-glass containers in a freezer at -4°C until it was tested and analyzed by GC/MS.

Gas chromatography-mass spectrometry (GC-MS) analysis

Analysis was performed on an Agilent 6890 gas chromatography/5973 mass selective detector (Palo Alto, CA, USA) equipped with an HP-5MS (5%-phenyl-methylpolysiloxane) capillary column (30 m length × 0.25mm I.d. × 0.25 μm film thickness; Agilent Co., Palo Alto, CA, USA) 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; and 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, scanning interval 0.5 sec and detector voltage 1.2 kV, and the mass scanning ranges were recorded at m/z 33～330.

Identification of chemical compounds

The volatile flavor compounds were identified by comparison of the mass spectra with those in an on-line computer library (Wiley 275) (Agilent Co., Palo Alto, CA, USA). 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 the published data (19,20). Especially, several compounds were identified with those of the literature (21-24), and identification based on co-injection with authentic compounds (Acoros, Sigma-Aldrich, MO, USA). The quantification of each individual volatile flavor components was performed based on the ratio of the peaks obtained from the mass total ion chromatogram and also marked quality percentage of the volatile flavor compounds from the MS data.

RESULTS AND DISCUSSION

Aroma profiles of *Chrysanthemum indicum* L.

To identify the aroma components of *Chrysanthemum indicum* L. flower, hydro distillation method was initially performed to extract the essential oil, which were then separated and analyzed as described in materials and methods. *Chrysanthemum indicum* L. flower yielded 2.0% (w/w) essential oil, and the color of the oil was light golden yellow. The list of detected compounds in the hydro distilled oil of *Chrysanthemum indicum* L. flower with their retention time, relative percentages of peak area, retention indices, quality percentages and percentage amounts of compound classes are given in Tables 1 and 2. The data are mean values of triplicates.

Table 1. The volatile flavor compounds of *Chrysanthemum indicum* L.

Compounds	RT[1)]	RI[2)]	QA%[3)]	PA%[4)]	Method of ID[5)]
(E)-2-Hexanal	06.39	0826	95	0.05	RT, MS/RI
Tricyclene	09.32	0891	96	0.04	RT, MS/RI
α-Pinene	10.43	0940	96	14.63	RT, MS/RI
Camphene	10.75	0945	98	0.40	RT, MS/RI
Benzealdehyde	11.30	0955	93	0.04	RT, MS/RI
Sabinene	12.04	0967	97	1.24	RT, MS/RI
β-Pinene	12.07	0968	97	0.23	RT, MS/RI[a)]
1,2,4-Trimethyl benzene	12.96	0983	90	0.06	RT, MS
Myrcene	13.13	0986	94	1.17	RT, MS/RI
α-Phellandrene	13.56	0993	95	0.49	RT, MS/RI
α-Terpinene	14.21	1004	98	0.58	RT, MS/RI[b)]
p-Cymene	14.61	1010	95	0.01	RT, MS/RI
1,8-Cineol	15.22	1020	98	10.17	RT, MS/RI
cis-Ocimene	15.53	1026	97	0.04	RT, MS/RI
Phenylacetaldehyde	15.66	1028	91	0.05	RT, MS
(E)-β-Ocimene	16.02	1038	97	0.03	RT, MS/RI
γ-Terpinene	16.48	1046	97	0.87	RT, MS/RI[*]

Table 1. Continued

Compounds	RT[1)]	RI[2)]	QA%[3)]	PA%[4)]	Method of ID[5)]
(E)-Sabinene hydrate	17.00	1056	94	0.77	RT, MS/RI[c)]
α-Terpinolene	17.85	1097	98	0.30	RT, MS
α-Campholene aldehyde	18.01	1100	83	0.14	RT, MS
Filifolone	18.69	1113	90	2.24	RT, MS
(E)-Chrysanthenol	19.45	1129	78	1.17	RT, MS
Chrysanthenone	20.25	1140	93	10.01	RT, MS
Isopinocarveol	20.56	1150	78	1.55	RT, MS
Camphor	20.79	1152	98	2.64	RT, MS/RI
Menthone	21.12	1155	83	0.03	RT, MS/RI[d)]
Pinocarvone	21.57	1171	69	1.19	RT, MS
Borneol	22.34	1186	87	3.02	RT, MS/RI[a)]
Terpinen-4-ol	22.63	1192	97	2.41	RT, MS/RI[*]
Endoborneol	22.77	1195	63	0.07	RT, MS
γ-terpinolene	23.23	1204	78	2.04	RT, MS
Methylchavicol	23.34	1207	95	0.14	RT, MS/RI[*]
(E)-carveol	24.48	1229	98	0.29	RT, MS
Carvone	25.43	1248	97	0.07	RT, MS/RI
Bornyl acetate	27.49	1289	98	3.64	RT, MS/RI
(E)-Carvyl acetate	29.72	1295	94	0.20	RT, MS/RI
Eugenol	30.52	1350	97	0.14	RT, MS/RI
α-Copaene	31.19	1375	99	0.23	RT, MS/RI
β-Elemene	32.06	1382	99	3.18	RT, MS/RI
Safranal	32.21	1384	86	0.15	RT, MS
α-logipinene	32.57	1391	69	0.09	RT, MS
β-Caryophyllene	33.09	1440	99	0.81	RT, MS/RI
Calarene	33.41	1448	97	0.04	RT, MS
α-Farnesene	33.75	1455	89	0.06	RT, MS/RI
α-Humulene	34.35	1467	98	0.13	RT, MS/RI
allo-Aromadendrene	34.66	1473	99	0.06	RT, MS/RI
(E)-β-Farnesene	35.04	1480	98	1.87	RT, MS/RI
α-Muurolene	35.05	1481	91	0.28	RT, MS/RI
Germacrene D	35.70	1494	99	5.25	RT, MS/RI
α-Curcumene	35.82	1496	96	1.80	RT, MS/RI
Germacrene B	36.28	1505	98	0.80	RT, MS/RI
Zingiberene	36.53	1511	93	2.70	RT, MS
(-)-Sinularene	36.73	1514	69	3.95	RT, MS
β-Bisabolene	36.82	1516	98	3.95	RT, MS/RI
γ-Cadinene	36.96	1520	97	0.13	RT, MS/RI
β-Sesquiphellandrene	37.59	1532	98	1.19	RT, MS/RI[d)]
cis-α-Bisabolene	38.16	1543	98	0.08	RT, MS
Spathulenol	39.32	1567	95	0.68	RT, MS/RI
Caryophyllene oxide	39.48	1570	99	0.22	RT, MS/RI
α-Cedrol	40.82	1596	78	0.02	RT, MS
Eremophilene	41.15	1603	90	0.02	RT, MS
α-Bisabolol	43.54	1651	90	0.37	RT, MS/RI[c)]
Vulgarol B	45.98	1740	89	0.03	RT, MS/RI[a)]

[1)]RT is retention time. [2)]Retention indices were determined using n-alkanes (C_8-C_{22}) as external references. [3)]PA is peak area %; average of the relative percentage of the peak area in the MS total ion chromatogram (n=3) from *Chrysanthemum indicum* L. oil. [4)]QA means quality % of the MS data (n=3) of the essential oil from *Chrysanthemum indicum* L. [5)]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, USA). RI was consistent with that of the literature. [a)]Identification based on reference no.21. [b)]Identification based on reference no.22. [c)]Identification based on reference no.23. [d)]Identification based on reference no.24. [*]Identification based on co-injection with authentic compounds (Acros, Sigma-Aldrich, St. Louis, MO, USA).

As shown in Tables 1 and 2, sixty-three volatile flavor compounds were tentatively identified in the constituents of *Chrysanthemum indicum* L. flower oil. It contained 35 hydrocarbons with sesquiterpene predominating, 12

Table 2. Relative constitutions by functional group of *Chrysanthemum indicum* L.[1)]

Functional group	No. of peak	% of peak area[2)]
Hydrocarbon	35	48.75
Aldehyde	5	0.43
Ester	3	4.61
Alcohol	12	19.92
Ketone	6	15.31
Oxide	1	0.22
Miscelleneous	1	0.04
Total	63	89.28

[1)]Essential oil from the flowers of *Chrysanthemum indicum* L. by hydro-distillation-GC/MS.
[2)]Average (n=3) of the relative percentage of the peak area in the MS total ion chromatogram.

alcohols, 6 ketones, 3 esters, 5 aldehydes, 1 oxide and 1 miscellaneous component. The identified compounds of the essential oil from *Chrysanthemum indicum* L. constituted 89.28% of the total peak area. α-Pinene (14.63%), 1,8-cineol (10.71%) and chrysanthenone (10.01%) were the predominantly abundant components in *Chrysanthemum indicum* L. Germacrene D (4.40%), (-)-sinularene (3.95%), bornyl acetate (3.64%) and β-elemene (3.18%) were also detected as the main volatile aroma compounds. The major functional groups of the volatile flavor components from *Chrysanthemum indicum* L. were terpene hydrocarbon, alcohol and ketone.

Terpene Hydrocarbons

There were thirty-four terpene hydrocarbon compounds (48.69%) in the flower essential oil, fourteen monoterpenes [α-pinene, tricyclene, camphene, sabinene, β-pinene, myrcene, α-phellandrene, α-terpinene, *p*-cymene, *cis*-ocimene, γ-terpinene, *(E)*-β-ocimene, α-terpinolene and γ-terpinolene (22.07%)] and twenty sesquiterpenes [α-copaene, β-elemene, α-longipinene, α-humulene, calarene, β-caryophyllene, germacrene D, α-farnesene, *allo*-aromadendrene, zingiberene, γ-cadinene, α-muurolene, β-sesquiphellandrene, germacrene B, α-curcumene, β-bisabolene, (-)-sinularene, *(E)*-β-farnesene, *cis*-α-bisabolene and eremophillene (26.62%)]. Among them, α-pinene, the most abundant volatile flavor of *Chrysanthemum indicum* L. flower oil, was described as the pine tree aroma and flavor, and is a very important starting material for the perfume industry (25). This volatile flavor compound was also found 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 (26). It is so valuable and beneficial that the most abundant components of Korean *Chrysanthemum indicum* L. flower oil have physicochemical and bio-functional properties would be the useful and important materials for industrial and medicinal purposes. β-Elemene, a sesquiterpene identified in this study, was also detected in *Eugenia uniflora* L. leaf essential oil, and has been reported to inhibit mouse pancreatic cancer and to inhibit neoplastic metasis, has anti tumor effects (27). It could be used for chemotherapy and immunotherapy.

Alcohols

There were twelve terpenic alcohol compounds (19.92%) in *Chrysanthemum indicum* L. flower oil, consisting of 1,8-cineol, *(E)*-chrysanthenol, isopinocarveol, borneol, terpinen-4-ol, endoborneol, *(E)*-carveol, eugenol, spathulenol, cedrol, vulgarol B and α-bisabolol. Among them, eugenol was also characterized in the essential oil of *Epedra* (produced in Italy) as a main component making up 4.30% (28). The volatile flavor compound, 1,8-cineol, was also detected as a major constituent together with terpene hydrocarbons in this study, and was also found in *Chrysanthemum boreale* Makino but not German chamomile (13). This compound, known as eucalyptol or cajeputol, is a well-known important volatile flavor of mugwort (*Artemisia asictica* Nakai) of the Compositae family, and is also reported to have anti-cancer and anti-microbial effects (29-31). We also detected in this study, terpinen-4-ol (or 4-terpineol) by GC/MS, a volatile flavor compound that has been used for aroma therapy with lavender oil. It is also known as one of the major volatile aroma in tea tree oil, and its quality is dependent upon the concentration of this compound (32). α-Terpineol was detected in *Chrysanthemum indicum* L. produced in Korea, this volatile aroma chemical component is known as the fragrances of lilac blossom notes according to its purity, and has been used as an important ingredient in various pharmaceuticals (25).

Ketones, esters and aldehydes

There were six ketone compounds (15.31%) in *Chrysanthemum indicum* L., consisting of camphor, filifolone, chrysanthenone, menthone, pinocarvone and carvone. Three esters (4.61%), *(E)*-sabinene hydrate, bornyl acetate and *(E)*-carvyl acetate were found in the essential oil of *Chrysanthemum indicum* L. Aldehyde compounds constituted 0.43% of the distilled flower oil, with *(E)*-2-hexanal, safranal, campholen acetaldehyde, benzealdehyde and phenylacetaldehyde. Chrysanthenone was the main ketone component, and this aroma compound also detected in *Chrysanthemum boreale* Makino, but not in German chamomile tea (13). Chrysanthenone is the main volatile flavor compound of Compositae genus essential oil (13,33), however it has been reported that was not found in *Artemisia asictica* Nakai even though it belongs to the same Compositae family (34). *Chrysanthemum indicum* L. flower has some oriental volatile aroma flavor notes, it is known that this scent comes from chrysanthenone and bornyl acetate; chrysanthenone is well- known as the fresh volatile flavor component of pine tree aroma (25). This aroma compound was also found in the essential oil of *Z. piperitum* A.P. DC. (35). Camphor was also detected in this study making up 2.64%. This volatile flavor compound has a fresh sweet aroma note like mint, known as the one of the major volatile flavor compound of the Compositae family and the bio-functional aroma material (25). The essential oils of *Chrysanthemum* species in Korea have been studied by several researchers (10-14,33,36), the most predominant compounds of *Chrysanthemum indicum* L. and *Chrysanthemum boreale* Makino were docosane, santalol, chrysanthenone, borneol, benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methylbenzene and bornyl acetate. However, there were some differences from the experimental results in this study, the predominantly volatile flavor compounds of *Chrysanthemum indicum* L. were α-pinene (14.63%), 1,8-cineol (10.71%) and chrysanthenone (10.01%). There are also some variations in the volatile flavor compounds of different Korean *Chrysanthemum* species, due to differences in the region where it is grown, season, extraction method and storage conditions of the samples. The main volatile flavors were the terpene compounds and their oxygenated derivatives; their biological activites and physico chemical properties can be deduced by searching their chemical profiles. It is noteworthy that this terpenyl compounds (especially α-pinene, 1,8-cineol and chrysanthenone) would be useful and beneficial materials for the industrial and medicinal purposes. Thus, we envision that those biologically active components of *Chrysanthemum indicum* L. flower oil can provide lead compounds in the medicinal and pharmaceutical in the near future. Further studies are necessary to determine which components are responsible for antimicrobial activity.

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