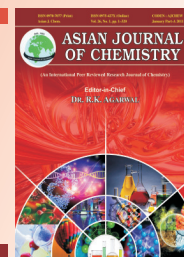




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Analysis On the Characteristic Aroma Components of *Chrysanthemum indicum* var. *aromaticum*

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Volatiles from all over *Chrysanthemum indicum* var. *aromaticum* had been analyzed by gas chromatography-mass spectrometry (GC/MS) combined with direct thermal desorption (DTD). The results showed that the volatile constituents of *Chrysanthemum indicum* var. *aromaticum* were distinct from the other wild related species. More floral scent constituents were detected in *Chrysanthemum indicum* var. *aromaticum* with higher amounts of fatty acid derivatives and benzenoids. Thujone was found to be the characteristic components of *Chrysanthemum indicum* var. *aromaticum*, which occupying the biggest percentage in the volatiles of flowers, leaves and stems.

Keywords: *Chrysanthemum indicum* var. *aromaticum*, Aroma component, GC/MS, Direct thermal desorption.

INTRODUCTION

Chrysanthemum species have always been important ornamental and medical plants in China. With the growing interests in the essential oil extraction and its application in the pharmaceutical and food industry¹, the need of developing wild fragrant medicinal plants has become necessary and urgent. Since *Chrysanthemum* wild species are enriched with terpenoids which were recognized as important materials of essential oil², studies on the volatile components of *Chrysanthemum* species have been paid more and more attention.

It is well known that most of chrysanthemum species smell herbaceous, camphoraceous and minty, while few of them emit floral scents. *Chrysanthemum indicum* var. *aromaticum*, which is more productive in the Shennongjia region of China, was found to release strong fragrance all over. This unique trait made *C. indicum* var. *aromaticum* significant in both garden application and economic field.

However, studies on the natural aroma scents of *C. indicum* var. *aromaticum* were not enough. The main essential oil components of the plant were detected to be α -thujone, β -thujone and camphor by Qi-hong Liu³. Wang *et al.*⁴ had tried to study the chemical components of dried *C. indicum* var. *aromaticum* in the use of GC/MS. They found that both dried flowers and leaves had bornylacetate, α -phellandrene and cymene as their principal volatile components, but the proportion was different respectively. In dried stems, *trans*- β -farnesene, germacrene, β -phellandrene, β -caryophyllene were

discovered to be the main ingredients⁴. Other studies on *C. indicum* var. *aromaticum* mostly focused on the essential oil extraction and its medical effects⁵⁻⁷.

The research was expected to lay foundation for further researches and practical application by studying the characteristic fragrance components of *Chrysanthemum indicum* var. *aromaticum* and the volatile compounds of its different organs using headspace adsorption and gas chromatography-mass spectrometry coupled to direct thermal desorption.

EXPERIMENTAL

Chrysanthemum indicum var. *aromaticum* and *Chrysanthemum indicum*, which were collected from the Shennongjia forestry region, have been cultivated in the greenhouses of University of Beijing Forestry since autumn 2012. *C. lavandulifolium*, *C. lavandulifolium* var. *seticuspe* and *C. charetii* were collected in the flowers nursery of University of Beijing Forestry. Flowers of these plants were sampled during the flowering period in autumn 2013. For specific sampling parts, eight to ten flowers in full bloom at the top of the inflorescence weighting 3 g were used for floral scents sampling.

Leaves, stems and roots of *C. indicum* var. *aromaticum* were collected in spring 2013. Detached leaf segments at the top of the stem weighting 3 g were collected for tests. Segments of stem weighting 3 g from one centimeter above the ground were prepared leafless for sampling. Detached roots weighting 3 g were sampled after they were rinsed and dried in the oven for 10 min.

Scent sampling: Direct thermal desorption (DTD) was applied for the scent sampling. Firstly, test materials were sealed in the polyethylene oven bags (Reynolds, Richmond, VA) and then air in the bags was extracted out to drive away ambient impurity. After wards, 1 L of air purified by activated carbons at a flow rate of 1L/min⁻¹ was bumped into the oven bags to capture the fragrance for 40 min placed in 23-25 °C incubator. Finally, volatiles were drawn out into a thermo desorption tube (CAMSCO, Houston, TX) filled with 200 mg adsorbent (Tenax GR, 60/80 mesh; Alltech, Deerfield, IL) by a battery-operated pump (GSP-300FT-2; Gastec Corp., Kanagawa, Japan) at a flow rate of 200 mL/min⁻¹. Two parallel experiments were conducted for each sample. Control tests were used by sampling without materials to distinguish flora scents from ambient air. For the sake of ambient impurity's potential effects, adsorption tubes were heated for 2 h by flowing helium^{8,9}.

Gas chromatography/mass spectrometry and data analysis: The adsorption tubes had their volatiles desorbed in an automatic thermal desorber through two stages. Firstly, the thermal desorption was conducted at 260 °C for 10 min. Then volatiles released from the tubes completed these condary cold desorption at a cold trap of -25 °C with a reverse flow of helium gas at a flow rate of 1.5 mL/min⁻¹ for preconcentration. Afterwards, the samples were held in a heat secondary trap, in which its temperature increased from -25 to 300 °C at a rate of 40 °C s⁻¹. Next, volatiles samples were injected into GC (Clarus 600; Perkin Elmer) for the analysis of the volatile compounds through a capillary transfer line at 250 °C. In the GC oven, heating program was set up at 40 °C for 2 min, increasing to 180 °C at 6 °C/min and then rising at 15 °C/min to 270 °C, in which temp samples were held for 3 min at last. The mass spectra (Clarus 600T; Perkin Elmer), combined with the GC, was adjusted to take in ionization at a voltage of 70 eV electron over a scan range of 30-500 *m/z*.

Turbo Mass 5.4.2 GC/MS software (Perkin Elmer) was applied for processing. Components were identified by the NIST 2008 mass spectral database and further verified by kovats retention index in pherobase database (<http://www.pherobase.com>). The peak areas of compounds were regarded as a standard to demonstrate its relative amounts in total aroma components.

RESULTS AND DISCUSSION

Volatile composition of different chrysanthemum species: By analyzing the peaks showed in the total ion chromatography of mass spectrometer, we found that the number of volatile compounds varied a lot in different species (Table-1). Seventy two aroma constituents were identified in the flora scents of *C. indicum* var. *aromaticum*, covering 98.03 % of all the volatile emission compounds, while there were much less components in other species (Table-2). Almost every compound could be retrieved in the pherobase database and mostly belonged to common floral chemical substances.

Concerning the synthesis pathways of volatile organic compounds, there were three groups of volatile compounds found in all *chrysanthemums* including terpenoids, fatty acid derivatives and benzenoids. Fig. 1 presented the volatile scents

of *C. indicum* var. *aromaticum* had similar composition with *C. indicum*, *C. chanelii* and *C. lavandulifolium* var. *seticuspe*, with terpenoids being the dominant compound class of volatiles accounting for 52.34 to 98.05 % of total emission amounts. The relative contents of fatty acid derivatives were significantly higher in *C. lavandulifolium* and *C. indicum* var. *aromaticum* than the others. Besides, *C. indicum* var. *aromaticum* and *C. lavandulifolium* var. *seticuspe* contained more amounts of benzenoids.

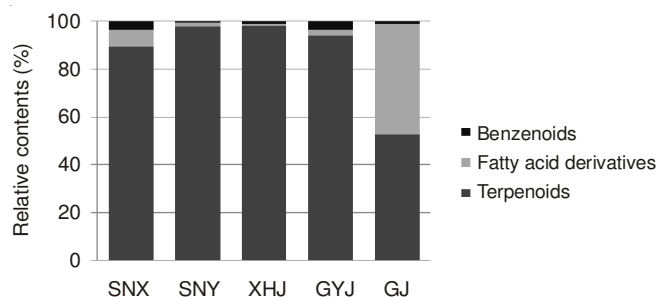


Fig. 1. Comparison of relative amounts of three major class of scent compounds in five species of *Chrysanthemum*. SNX = *Chrysanthemum indicum* var. *aromaticum*, SNY = *Chrysanthemum indicum*, XHJ = *Chrysanthemum. chanelii*, GJ = *Chrysanthemum. lavandulifolium*, GYJ = *Chrysanthemum. lavandulifolium* var. *seticuspe*

Principal components differed greatly in each *chrysanthemum* (Fig. 2). β -Thujone, α -terpinene and camphor were detected to the major components in the floral scents of *C. indicum* var. *aromaticum*. Eucalyptol, bomyl acetate and camphor were main ingredients in the volatile emission of *C. indicum*. In *C. chanelii*'s flora scents, we found that β -myrcene, eucalyptol and α -pinene accounted for the majority. While in *C. lavandulifolium* var. *seticuspe* and *C. lavandulifolium*, the amount of *trans*-paranoid linalool oxide reached maximum in emission. There were 17 compounds identified in all chrysanthemum's flowers, with eucalyptol and camphor presenting higher concentration. Furthermore, α -terpinene, α -thujone, (s)-ethyl 2-methylbutanoate, propyl 2-methylbutanoate, germacrene were detected only in *C. indicum* var. *aromaticum*.

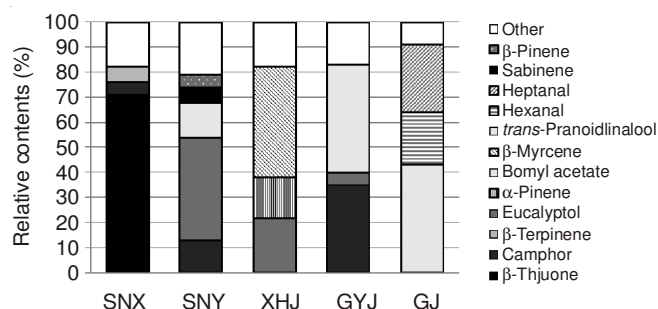


Fig. 2. Comparison of major volatile components in the five wild *chrysanthemum* species

Composition of different organ's flora scents: Experiments showed that 64 and 65 aroma constituents were found in the stems and leaves, while 72 compounds were identified in flowers. Besides, stems and leaves emitted less amounts of fatty acid derivatives and benzenoids than flowers, as well as the quantity of floral scents released.

TABLE-1
MAIN COMPOUNDS OF FLORAL SCENT EMITTED FROM FOUR SPECIES OF *Chrysanthemum*

KI ^a	Compounds	Relative contents (> 1 %)					Odor ^c
		SNXJ	SNYJ	XHJ	GYJ	GJ	
811	Hexanal	0.37 % ± 0.01 %	0.40 % ± 0.01 %	tr	tr	21.38 % ± 0.04 %	Fruity
857	(s)-Ethyl 2-methylbutanoate	2.31 % ± 0.02 %	-	-	-	-	
903	2-methylbutanoate	-	-	-	-	27.74 % ± 0.03 %	
929	α-Thujene	-	0.91 % ± 0.01 %	1.54 % ± 0.01 %	0.25 % ± 0.01 %	tr ^b	Woody, herbal, green
938	α-Pinene	0.71 % ± 0.01 %	1.09 % ± 0.02 %	16.22 % ± 0.05 %	0.66 % ± 0.01 %	0.29 % ± 0.01 %	
945	Propyl 2-methylbutanoate	1.44 % ± 0.01 %	-	-	-	-	
953	Camphene	0.96 % ± 0.01 %	1.67 % ± 0.01 %	0.12 % ± 0.01 %	1.86 % ± 0.02 %	tr	Fruity, floral, camphor
966	Benzaldehyde	0.96 % ± 0.03 %	-	-	-	-	
975	Sabinene	-	5.88 % ± 0.03 %	-	0.56 % ± 0.01 %	tr	
981	β-Pinene	0.18 % ± 0.01 %	4.87 % ± 0.05 %	0.78 % ± 0.01 %	0.26 % ± 0.01 %	0.08 % ± 0.01 %	Green, sweet, pine
993	β-Myrcene	0.14 % ± 0.01 %	0.71 % ± 0.01 %	43.85 % ± 0.07 %	1.79 % ± 0.03 %	tr	
1001	Carene	0.21 % ± 0.01 %	0.73 % ± 0.01 %	1.30 % ± 0.01 %	0.23 % ± 0.01 %	tr	
1012	α-Terpinene	5.64 % ± 0.04 %	-	-	-	-	Sweet, fruity, woody
1013	o-cymene	-	0.65 % ± 0.01 %	0.34 % ± 0.01 %	1.41 % ± 0.01 %	0.13 % ± 0.01 %	
1037	Eucalyptol	1.37 % ± 0.02 %	40.82 % ± 0.01 %	22.24 % ± 0.01 %	5.00 % ± 0.04 %	1.04 % ± 0.02 %	
1048	Ocimene	tr	0.21 %	0.89 % ± 0.01 %	0.16 % ± 0.01 %	0.05 %	Camphor, sweet, pineliquorices, men-thol, pine liquorices, menthol, pine liquorices, menthol, pine liquorices, menthol, pine liquorices, menthol, pine
1053	γ-Terpinene	0.39 % ± 0.01 %	1.12 %	1.71 % ± 0.01 %	0.34 % ± 0.01 %	tr	
1127	trans-Paranoidlinalool oxide	-	0.15 %	-	13.38 % ± 0.06 %	42.42 % ± 0.05 %	
1132	α-Thujone	2.54 % ± 0.03 %	-	-	-	-	Citrus-like, herbaceous
1146	β-Thujone	65.48 % ± 0.05 %	0.29 % ± 0.03 %	-	-	-	
1159	Camphor	5.11 % ± 0.02 %	12.36 % ± 0.04 %	tr	4.55 % ± 0.05 %	0.47 % ± 0.01 %	
1166	Borneol	tr	3.84 % ± 0.02 %	tr	tr	tr	Citrus-like, terpeny, herbaceous fruity, sweet herbaceous, fruity, sweet
1289	Bornyl acetate	0.74 % ± 0.01 %	13.24 % ± 0.01 %	-	0.62 % ± 0.01 %	0.11 % ± 0.01 %	
1301	Myrtenyl acetate	0.28 % ± 0.01 %	3.05 % ± 0.01 %	0.01 % ± 0.01 %	-	0.04 % ± 0.01 %	
1480	Copaene	1.02 % ± 0.02 %	0.11 % ± 0.01 %	0.78 % ± 0.01 %	0.05 % ± 0.01 %	tr	Sweet, floral, creamy
1524	β-Caryophyllene	1.12 % ± 0.01 %	0.37 % ± 0.01 %	2.24 % ± 0.03 %	0.35 % ± 0.01 %	tr	
1567	Germacrene	1.38 % ± 0.03 %	-	-	-	-	
1568	β-Cubebene	0.90 % ± 0.01 %	-	-	-	-	Minty
1618	δ-Isabolene	0.09 % ± 0.01 %	0.39 % ± 0.01 %	0.43 % ± 0.01 %	tr	tr	

^aKI^a is the kovats retention index, ^b'tr' is trace constituent, compounds holding less than 0.01 %, ^cOdor characteristics was achieved from <http://www.pherobase.com>; <http://www.flavormet.org/flavornet.html>

SNX = *Chrysanthemum indicum* var. *aromaticum*, SNY = *Chrysanthemum indicum*, XHJ = *Chrysanthemum chaneitii*, GJ = *Chrysanthemum. lavandulifolium*, GYJ = *Chrysanthemum. lavandulifolium* var. *seticuspe*

In the comparison of main components, it is concluded that thujone was the most important component in flowers, stems and leaves. Besides, leaves and stems also had their own particular ingredients. *cis*-3-Hexenol and germacrene presented to be the major volatile compounds in the leaves. While in the stems, main

ingredients were identified to be myrtenyl acetate and camphor. The emission constituents in roots varied a great deal from those in the above-ground organs. 1,3,6-Heptatriene, 2,5,5-trimethyl, β-farnesene, γ-selinene and thujopsene were essential components in the root's emission scents.

TABLE-2
 MAIN COMPOUNDS IN THE *Chrysanthemum indicum* var. *aromaticum*

KI ^a	Compounds	Relative contents (> 1 %)				Odor
		Flower	Leaf	Stem	Root	
857	(s)-Ethyl 2-methylbutanoate	2.31 % ± 0.02 %	1.37 % ± 0.01 %	0.53 % ± 0.01 %	-	Fruity
945	Propyl 2-methylbutanoate	1.44 % ± 0.01 %	tr	tr	-	Sweet, fruity
953	Camphene	0.96 % ± 0.01 %	0.64 % ± 0.01 %	0.28 % ± 0.01 %	1.05 % ± 0.01 %	Fruity, floral, camphor
981	β-Pinene	0.18 % ± 0.01 %	tr	tr	1.49 % ± 0.01 %	Green, sweet, pine, resin
993	β-Myrcene	0.14 % ± 0.01 %	1.92 % ± 0.01 %	tr	1.04 % ± 0.01 %	Sweet, fruity, woody
1006	(z)-3-Hexen-1-ol	tr	5.01 % ± 0.03 %	tr	-	Fresh, grasslike, leafy
1012	α-Terpinene	5.64 % ± 0.04 %	tr	1.72 % ± 0.01 %	-	
1037	Eucalyptol	1.37 % ± 0.02 %	0.37 % ± 0.01 %	0.37 % ± 0.01 %	0.08 % ± 0.01 %	Camphor, sweet, pine, menthol
1132	α-Thujone	2.54 % ± 0.03 %	55.18 % ± 0.04 %	33.10 % ± 0.03 %	2.73 % ± 0.01 %	Minty
1146	β-Thujone	65.48 % ± 0.05 %	tr	37.02 % ± 0.03 %	tr	Minty
1159	Camphor	5.11 % ± 0.02 %	2.95 % ± 0.03 %	3.84 % ± 0.01 %	0.25 % ± 0.01 %	Camphor, green
1364	Myrtenyl acetate	0.28 % ± 0.01 %	0.04 % ± 0.01 %	4.83 % ± 0.01 %	-	
1377	(+)-α-Longipinene	-	-	-	3.27 % ± 0.01 %	
1480	Copaene	1.02 % ± 0.02 %	2.15 % ± 0.02 %	1.45 % ± 0.02 %	7.54 % ± 0.02 %	Wood, spice
1487	γ-Selinene	-	-	-	10.13 % ± 0.03 %	
1502	Thujopsene	-	-	-	7.62 % ± 0.01 %	
1524	β-Caryophyllene	1.12 % ± 0.01 %	3.96 % ± 0.03 %	2.39 % ± 0.01 %	-	Green, woody, fruity sweet
1530	γ-Cadinene	-	0.70 % ± 0.01 %	2.91 % ± 0.02 %	-	Wood
1534	α-trans-Bergamotene	tr	tr	0.27 % ± 0.01 %	3.73 % ± 0.01 %	Warm, tea-leaf-like
1552	β-Santalene	-	-	-	2.75 % ± 0.01 %	
1555	β-Farnesene	0.18 % ± 0.01 %	tr	0.65 % ± 0.01 %	21.29 % ± 0.02 %	Wood, citrus, sweet
1565	Alloaromadendrene	tr	tr	-	2.23 % ± 0.01 %	Wood
1567	Germacrene	1.38 % ± 0.03 %	5.59 % ± 0.04 %	tr	-	Oily, green, woody
1568	β-Cubebene	0.90 % ± 0.01 %	0.55 % ± 0.01 %	tr	4.85 % ± 0.01 %	Herbal, woody
1610	β-Isabolene	-	tr	-	1.48 % ± 0.01 %	Herbaceous

In this study, we find that the flowers of *C. indicum* var. *aromaticum* were detected to have the largest number of volatile compounds compared with the flowers of the other four species, which corresponded to the fact that *C. indicum* var. *aromaticum* emitted the strongest level of aroma scents among all.

From classification prospective, terpenoids were detected to be the dominate category in all the five species. Terpenoids, one of the largest groups of chemical compounds was reported to possess medicinal value and insect repellent function². By reason that most of the fatty acid derivatives and benzenoids were found to be aromatic, the differences in category ratio may have relationship with the fact that *C. indicum* var. *aromaticum*, *C. lavandulifolium* and *C. lavandulifolium* var. *seticuspe* emit fragrant, sweet and flowery scents but the other two smell fresh and herbaceous.

Concerning the major ingredients, thujone occupied the overwhelming highest amount in all over *C. indicum* var. *aromaticum*, including flowers, leaves and stems, which was rarely found in others. Thujone, a terpene-like ketone, has an odor resembling menthol and it was also known as important flavor ingredients in making perfumes and extracting essential oil¹⁰ and also the major constitute in *Salvia officinalis* L.¹¹⁻¹⁴, and *Tanacetum vulgare* L.¹⁵. Recent researches showed that the biosynthesis of thujone required the monoterpene sabinene as a precursor¹⁶, which was detected to occupy 5.86 % in the *C. indicum*. Furthermore, sabinene was catalyzed by a series

of synthase to form thujone, embracing monoterpene synthase, acytochrome P450, a dehydrogenase and a reductants¹⁷. The lack of the relative enzymes may be the reason for *C. indicum*'s inability to product thujone even in the existence of substrate. Most of the volatile compounds of *C. indicum* var. *aromaticum* emitted at a relatively low level. Among this, α-terpinene and camphor in *C. indicum* var. *aromaticum* may explain its aromatic smell and faint scent, respectively.

The discrepancy in the composition of the volatile components may reflect taxonomic affinities in some degree¹⁸. *C. chaneltii*'s showed some resemblance in the main volatile compounds with *C. indicum*. Eucalyptol was identified to be the main volatile components of the two and also known to be the major volatile components of the Compositae family¹⁹⁻²¹. Eucalyptol, smelling fresh and minty²², was also reported in *C. boreale* Makino²³, *Coleostephus myconis*²⁴, *Artemisia asiatica* N²⁵, and *Chrysanthemum coronarium* L.²⁶. Nevertheless, *C. lavandulifolium* var. *seticuspe* and *C. lavandulifolium* appeared similar, with *trans*-paranoid linalool detected to hold more than 40 % of total. From the perspective of chemical synthesis, since all the tested *chrysanthemums* emitted high level softer penoids and geranyl pyrophosphate (orneryldi-phosphate) is the substrate precursor of terpenoids²⁷, the variation interpenoids products mainly depends on the responsible terpenoid synthase. In addition, aterpenoid synthase can form not only a kind of terpenoids, but also a number of minor products.

Volatile composition of different organs of *C. indicum* var. *aromaticum* showed that flowers were the main aromatic organs with more diverse fragrance components and more aroma quantity. Fig. 3 showed that the volatile constituents of leaves and stems reached some degree of consistency with that of flowers, probably for the reason that *C. indicum* var. *aromaticum* expires fragrance all over on the ground. The difference in the composition of volatile compounds between flowers, stems and leaves may have relationship with the regulation mechanism of gene space and temporal specificity. The high proportion of thujone in the whole plant may explain that *C. indicum* var. *aromaticum* smells stronger amora and has greater value in the essential oil industry and medical field. In addition, other main compounds in the leaves and stems should also require more attention. For instance, (z)-3-hexenol was considered to be one of major components of green odor²⁸, which was reported to exert influence in the defense related pathways of Arabidopsis²⁹ and maize³⁰. Myrtenyl acetate, known as essential material of perfume, was rich in *Lavandula stoechas* L.³¹, *Myrtus communis*³², strawberry³³. Compared with the previous researches, it is reasonable to find that aroma components of the same plant varied under different environments and measuring instruments owing to its quantitative characters.

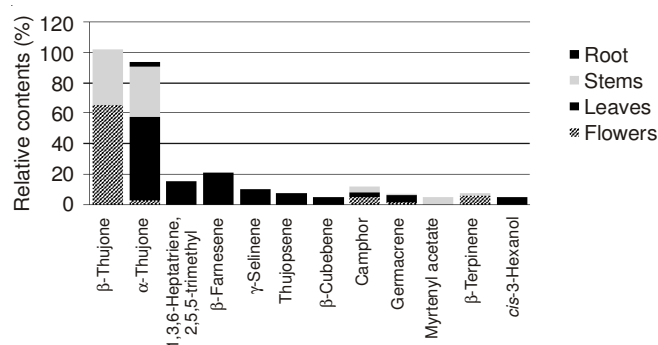


Fig. 3. Comparison of major volatile components in the flowers, stems, leaves and roots of *Chrysanthemum indicum* var. *aromaticum*

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