Journal of Biochemistry and Molecular Biology Research

Online Submissions: http://www.ghrnet.org/index./jbmbr/doi:10.17554/j.issn.2313-7177.2015.01.11

J Biochem Mol Biol Res 2015 June 1(2): 46-53 ISSN 2313-7177 (print)

ORIGINAL ARTICLE

Volatile Composition and Aroma Evaluation of Chrysanthemum Coronarium

Mitsuo Miyazawa, Hinako Otsuka, Satoshi Nakaya, Atsushi Usami

Mitsuo Miyazawa, Satoshi Nakaya, Atsushi Usami, Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University (Kindai University), 3-4-1 Kowakae, Higashiosaka-shi, Osaka 577-8502, Japan

Hinako Otsuka, Poduction Center I Science Program Division, Program Production Department, Japan Broadcasting Corporation [Nippon Hoso Kyokai (NHK)] 2-21, Jinnan, Shibuya-ku, Tokyo 150-8001, Japan

Correspondence to: Mitsuo Miyazawa, Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University, 3-4-1, Kowakae, Higashiosaka-shi, Osaka 577–8502, Japan.

Email: miyazawa@apch.kindai.ac.jp Telephone: +81-6721-2332

Received: March 29, 2015 Revised: May 22, 2015

Accepted: May 26, 2015 Published online: June 6, 2015

ABSTRACT

AIM: Chrysanthemum coronarium is native to Mediterranean and Asia and used as a food and ornament. In this study were to analyse the volatile compounds using two different extraction methods in order to compare the profiles obtained and to characterise the aromaactive compounds in the three parts (young leaves, mature leaves, and stems) by AEDA and odor activity value (OAV) methods.

MATERIAL AND METHODS: Using a hydrodistillation (HD) and a solvent-assisted flavour evaporation (SAFE) method to obtain the volatile oil from the three parts.

RESULTS: A total of seventy-three compounds were identified. The major compounds of three parts oils in both methods were β -myrcene and (E)- β -farnesene. Nineteen compounds were identified by GC-O analysis in two methods.

CONCLUSION: Two methods has its own advantages and disadvantages, and they are generally complementary to each other. On the basis of aroma evaluation, β -myrcene and (E)- β -farnesene are estimated as the main aroma-active compounds of young leaves,

mature leaves, and stems oils. As the other aroma-active compound, β -caryophyllene make a spicy odor of young and mature leaves oils. In stems oil, borneol contributes to camphor odor.

© 2015 ACT. All rights reserved.

Key words: *Chrysanthemum coronarium*; Volatile oil; Hydrodistillation (HD); Solvent-assisted flavor evaporation (SAFE); Aroma extract dilution analysis (AEDA)

Miyazawa M, Otsuka H, Nakaya S, Usami A. Volatile Composition and Aroma Evaluation of Chrysanthemum Coronarium. *Journal of Biochemistry and Molecular Biology Research* 2015; 1(2): 46-53 Available from: URL: http://www.ghrnet.org/index.php/jbmbr/article/view/1180

INTRODUCTION

The genus Chrysanthemum, golden flower in Greek, belongs to the Asteraceae family; it includes about 300 species^[1]. The chrysanthemum is distributed in two main centres, one in the Mediterranean area, the other in China and Japan^[2]. In Algeria, the genus includes 20 species with 8 endemic^[3]. The aerial parts of C. coronarium are used for food and for protection against several diseases in Oriental medicinal systems. Leaves and stems of the plant are a very popular food plant in Asia and Europe^[4]. In Japan the leaves are used for the suppression of the fishy odours in prepared foods^[5]. In France the young leaves are favored to eat raw. In addition, the plant has big capitula, usually bicolored white and yellow6 and cultivated for ornamental purposes in Mediterranean regions and some of Portugal^[7]. The essential oil from the aerial parts (flowerheads or/and leaf) of C. coronarium has been several reported^[4,6-13]. However, there are no reports on the aroma evaluation of volatile compounds in C. coronarium.

Volatile profiling, which aims to analyse several predefined volatile targets^[14], has been used as a tool to assess changes or differences

in predefined volatile compounds of several types in both foods and biological systems^[15-19]. There are several instrumental methods that can be used to obtain complete profiles of volatile oils. In addition, a number of different extraction techniques can be used in the analysis of volatiles. Hydrodistillation (HD) is a process traditionally used for the extraction of essential oils from aroma-active and medicinal plants on a laboratory scale. Indeed, in the case of HD, the possible hydrolysis and hydrosolubilisation of certain compounds is a serious obstacle in the reproduction of natural fragrances^[20]. Solvent-assisted flavour evaporation (SAFE) is a good technique for volatile extraction, and it can allow careful isolation of volatile compounds from complex matrices^[21]. High recovery has been achieved even for high-boiling-point compounds^[22].

The aims of the present study were to: (1) analyse the volatile compounds using two different extraction methods in order to compare the profiles obtained; and (2) to characterise the aroma-active compounds in the three parts (young leaves, mature leaves, and stems) of C. coronarium by AEDA and odor activity value (OAV) methods.

EXPERIMENTAL

1. Plant material

Sample of *Chrysanthemum coronarium* (aerial parts: 30-40cm) were obtained from Osaka prefecture of Japan in October 2014. The plant was identified of the performed, and a voucher specimen was deposited, at the biotechnology laboratory of Kinki University (Kindai University), Osaka, Japan.

2. Isolated of the essential oil

2.2.1. Hydrodistillation (HD) method: Young leaves (YL), mature leaves (ML), and stems (S) of C. coronarium (200 g) were hydrodistilled for 3 hours with a Likens–Nickerson-type apparatus, using diethyl ether, which was dried over anhydrous sodium sulphate. The oil was stored at 4°C in a refrigerator prior to analysis.

2.2.2. Solvent-assisted flavour evaporation (SAFE) method: Each parts of C. coronarium (200 g) were frozen in liquid nitrogen. The crushed frozen parts were added to dichloromethane (500 mL) as solvent, and the mixture was stirred and extracted. After standing for 2 days, the residual substances were removed by passing through filter paper. The volatile fraction was added to a dropping funnel of the SAFE apparatus (Kiriyama glass Co., Tokyo), which was heated to 25°C with a circulating H2O bath. The receiving flask for the distillate and the safety-cooling trap of the SAFE apparatus were cooled with liquid N2. During this procedure, the SAFE apparatus was connected to a high-vacuum pump (<13 Pa). After complete introduction of the filtrate into the SAFE system, distillation was carried out for 2 h at 10-4 torr. The volatile compounds were collected in a trap, which was submerged in liquid nitrogen. The volatile compounds were stored at 4°C in a refrigerator prior to analysis.

3. Gas chromatography-mass spectrometry (GC-MS)

The oil sample analysis was performed on an Agilent Technologies 6890 gas chromatograph coupled to an Agilent Technologies 5973 mass selective detector. GC conditions were: A HP-5MS column (5% phenyl 95% polydimethylsiloxane, 30 m \times 0.25 mm i.d., film thickness 0.25 mm) and a DB-WAX column (15 m \times 0.25 mm i.d., film thickness 0.25 mm); the column temperature was 40-260°C at a rate of 4°C/min and held at 260°C for 5 min. The carrier gas was He at a flow rate of 1.5 mL/min. The injector and detector temperatures

were 270 and 280°C, respectively. The ionization voltage was 70 eV and the split ratio was 1:40. One μL aliquot of oil was injected for each sample.

4. Sensory evaluation by GC-Olfactometry (GC-O)

A trained panel of sensory evaluation specialists measured the odor intensities of the main aroma-active constituents of C. coronarium. Eleven panellists, aged 21 to 26 years (8 males and 3 females, members of Kinki University, Japan), participated in this study. Sensory-analysis sessions were performed only after suitable training (> 30 h). The sniffing test by GC-O was carried out using an Agilent Technologies-6890N gas chromatograph equipped with an Agilent 5973 MSD mass spectrometer and sniffing port ODP 2 (Olfactory Detector Port 2, Gerstel). The GC instrument was equipped with a HP-5MS column. The sample was injected into the GC in splitless mode. The GC effluent from the capillary column was split in a 1:1 (v/v) ratio between the MS and the sniffing port. The oven conditions, injector and transfer line temperatures, the carrier gas, flow rate, and ionization mode were the same as those described above for the GC-MS

5. Aroma extract dilution analysis (AEDA)

The flavor dilution (FD)-factor of the odorants in the essential oil was determined by aroma extract dilution analysis (AEDA) of the following dilution series. The highest sample concentration (1 mg/mL) was assigned an FD-factor 1. The essential oil was stepwise diluted with diethyl ether (1+1, v/v), and aliquots of the dilutions (1 mL) were evaluated. Each aroma described was delivered, to the greatest extract possible. The process stopped when no aromas was detected by assessors. The result was expressed as the FD-factor, which is the ratio of concentration of the odorant in the initial volatile oil in which the odor is still detectable by GC-O.

6. Identification of compounds

Identification of the individual compounds was carried out as follows; (1) comparison of their GC-MS retention indices (RI) on apolar and polar columns, determined relative to the retention time of a series of n-alkanes (C5-C29), with those of authentic compounds; (2) computer matching with commercial mass spectral libraries and comparison of spectra with literature date^[23,24]; (3) The calculated RI were compared with the average RI from the literature, which were obtained from the Aroma Office database ver. 3.0 (Nishikawa keisoku Co. LTd., Tokyo, Japan). Aroma Office database var. 3.0 includes 72,120 entries of RI of aroma compounds and literature sources.

7. Quantification of aroma-active compounds

The odor components of the oils were quantitatively analyzed by internal standard addition method (alkanes C_{12} and C_{19}). The volatile oil was diluted 100 times with diethyl ether to a 1 mL volume, and 5 μL of a mixture of C_{12} and C_{19} (1 mg/mL) was added to the diluted oil. Then, the samples were subjected to GC-flame ionization detector (FID) analyses. The quantitative analysis was performed on the based on the calibration curves for (E)-3-hexen-1-ol (peak 2), (E)-2-hexen-1-ol (peak 3), camphene (peak 6), benzaldehyde (peak 7), β-myrcene (peak 8), limonene (peak 9), linalool (peak 17), borneol (peak 21), bornyl acetate (peak 27), (E,E)-2,4-decadienal (peak 29), β-caryophyllene (peak 34), and (E)-β-farnesene (peak 38), within the concentration range 0.5–1000 μg/mL. Because of the lack of a proper standard, (Z)-β-ocimene (peak 13), α -copaene (peak 33), β -cubebene (peak 39), germacrene D (peak 41), (E,E)- α -farnesene (peak 42), β -sesquiphellandrene (peak 45) and \square -muurolol (peak

48) were quantified by the calibration curves of β -caryophyllene and β -caryophyllene oxide, respectively.

8 Determination of odor activity value (OAV)

OAV were determined by dividing concentration of a component to its odor threshold. The odor threshold data was obtained from reported literature data^[24-27].

RESULTS AND DISCUSSION

1 Chemical constituents of C. coronarium

Table 1 lists the identified volatile compounds, their concentrations, and the RIs of the compounds on two columns from the HD and SAFE oils. The HD oils were 0.033% (w/w) for YL, 0.007% (w/w) for ML, and 0.007% (w/w) for S, respectively. While the YL and ML oils had a spicy-woody odor, the S oil had a spicy-camphor odor. On the contrary, the SAFE oils were 0.032% (w/w) for YL, 0.007% (w/w) for ML, and 0.007% (w/w) for S. While the YL and ML oils had a spicy-woody odor, the S oil had a spicy-camphor odor. A total of seventy-three compounds were identified (thirty-six compounds were newly identified). The major compounds of three parts oils in both methods were β-myrcene (peak 10, 6.0-32.9%) and (E)-β-farnesene (peak 47, 10.5-22.9%). These compounds belong to different chemical classes of organic compounds (mono- and sesquiterpene). Monoterpenes, have shown sound effects on mevalonate metabolism, linked to the maintenance of cell membrane, which could add to terpene tumor suppressive action. Thus, the presence of monoterpenes in the selected active fractions explains their antiproliferative actions against some tumor cell lines. Moreover, sesquiterpene biosynthesis seems to be complex since the formation via either pathway (mevalonic or methylerythrytol) or a combination of both has been reported^[28]. Nevertheless, these appear to be ubiquitous in plant taxa and some insects, and are associated to the cytosolmitochondria. Sesquiterpenes were reported to have antihyperlipidemic activity. Studies indicated that the activity of the essential oil may be due to the synergistic effects of the active compounds^[29]. Among acetylenic compounds in C. coronarium oils, four compounds were identified. Note that (Z)-tibetin spiroether (peak 58) and (E)-tibetin spiroether (peak 59) were characteristic compounds. It had been reported that volatile compounds of Mataricaria chamomilla L. and Artemisia roxburghiana Besser (Asteraceae)[30-32]. However, this is the first report of these compounds in Chrysanthemum species.

The six oils showed mainly quantitative differences, but in the essential oil obtained from YL fewer compounds were identified. This may be attributed to the fact that different parts of a plant normally stored different chemical compounds^[33]. Comparing each part, yield of YL oil was the 5 times than the other parts on the both method. To the best of our knowledge, this is the first report that YL oil is contained so many volatile compounds as compared to other parts. The classification of the oils on the basis of functional group is summarized in Table 1. The compounds were separated into hydrocarbons, alcohols, aldehydes, ketones, esters, ethers, and acids. The most representative compounds were hydrocarbons, ethers, alcohols, and esters in HD oils. SAFE method efficiently furnished hydrocarbons, alcohols aldehydes, and esters, which are the four classes with relatively high contents. The results of both methods indicated that the major classes were hydrocarbons. Comparing the oils from the two methods, that the levels of hydrocarbons and aldehydes in SAFE oil were more than

those in HD oil. It can be observed that fatty acids extracted by HD method are not present in SAFE method, possibly because of thermal degradation of these compounds due to the high temperature required for HD. Moreover, the SAFE method utilises vaporisation of volatiles from non-volatile materials at relatively low temperature ranges ($40 \pm 20^{\circ}$ C) under ultrahigh vacuum. Thus, the formation of thermal artefacts can be avoided during the SAFE extraction procedure and it has a high extraction efficiency and recovery^[21,34].

2 GC-O, AEDA, and OAV

To clarify the most potent odorants contributing to the characteristic odor, the AEDA method was performed combined with GC-O analysis. The aroma-active compounds of both oils (HD and SAFE methods) were assessed using GC-O and AEDA. In both oils, nineteen compounds were identified; eleven hydrocarbons, five alcohols, two aldehydes, and one ester. All of the aroma-active compounds were satisfactorily identified based on their retention indices (RIs) and their mass spectra. In Table 2, the aroma-active compounds identified for the oils in both methods are presented. A comparison of the gas chromatogram and the FD factors (RIs vs. FD-factor) of their respective peaks are shown in Figs. 1 and 2, respectively. As seen in Figure 1, β-myrcene (peak 8) showed the highest FD-factor of 6-8, and (E)-β-farnesene (peak 47) showed a high FD-factor of 5-6. The following compounds had FD-factors greater than 4: β-caryophyllene (peak 34 in YL and ML oils) and boreol (peak 21 in S oil), respectively. The AEDA results revealed that β-myrcene and β-caryophyllene emits a spicy odor, and (E)-β-farnesene contributed to the odor in the YL and ML HD oil. β-Myrcene, β-caryophyllene and borneol were produced to the odor in the S HD oil. In SAFE oil, β-myrcene had the highest FDfactors of 6-8, followed by (E)-β-farnesene with an FD-factor of 5-6 in Figure 2. β-Caryophyllene (the YL and ML oils) and borneol (the S oil) had FD-factors greater than 4. These compounds were estimated as characteristic aroma-active compounds in three parts SAFE oils. In order to determine the relative contribution of each of the aroma-active compounds to the characteristic odor, the OAV method was used. The OAV was obtained by taking into account the concentration and the odor threshold of each compound. The OAVs of aroma-active compounds in the oils are shown in Table 2. In the six oils, β-myrcene had the highest OAV (280.0-7018.7), followed by β-caryophyllene (14.9-105.6 in the YL and ML oils) and borneol (46.7-70.0 in the S oil). These compounds showed particularly high FD-factors, indicating these compounds determined the key aroma-active compounds. Generally, the aroma-active compounds with a high FD-factor also had high OAVs, confirming the positive relationship previously found between the FD-factor and OAV.35

CONCLUSION

To the best of our knowledge, this is the first report on the aromaactive compounds of essential oils of three parts of *C. coronarium*. The result reveals that the existence of various bioactive compounds and a total of nineteen aroma-active compounds were determined by the AEDA and OAV methods. The chemical compositions of the volatile oil were also described in detail. Further studies are needed to evaluate the bioactivity by which each component. We hope that these results will be used in the future investigations in to the utilization of food and medicinal plants.

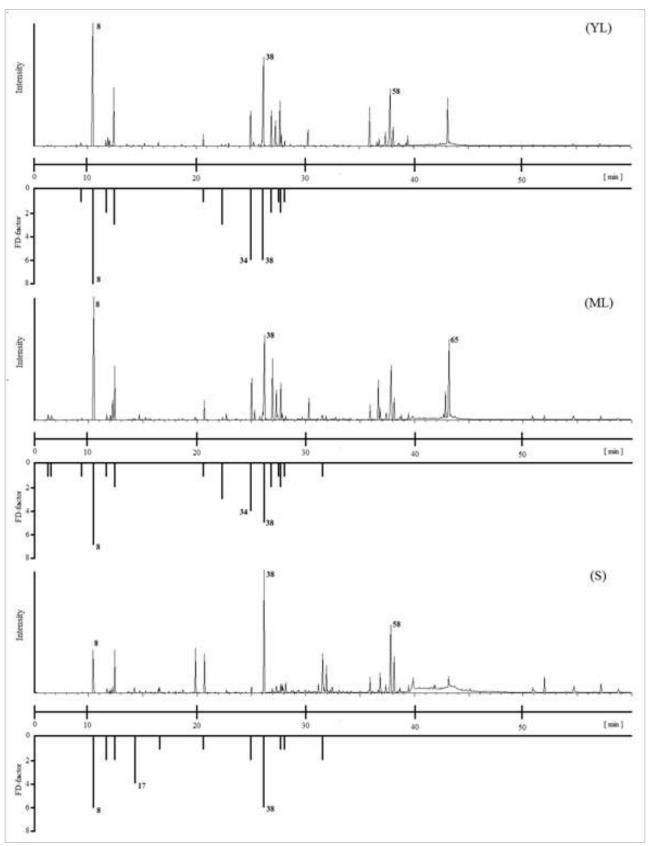


Figure 1 Gas chromatogram and aromagram (FD-factor) of essential oils by HD: 8, β -myrcene; 17, linalool; 34, β -caryophyllene; 38, (E)- β -farnesene; 58, (Z)-tibetin spiroether; 65, phytol.

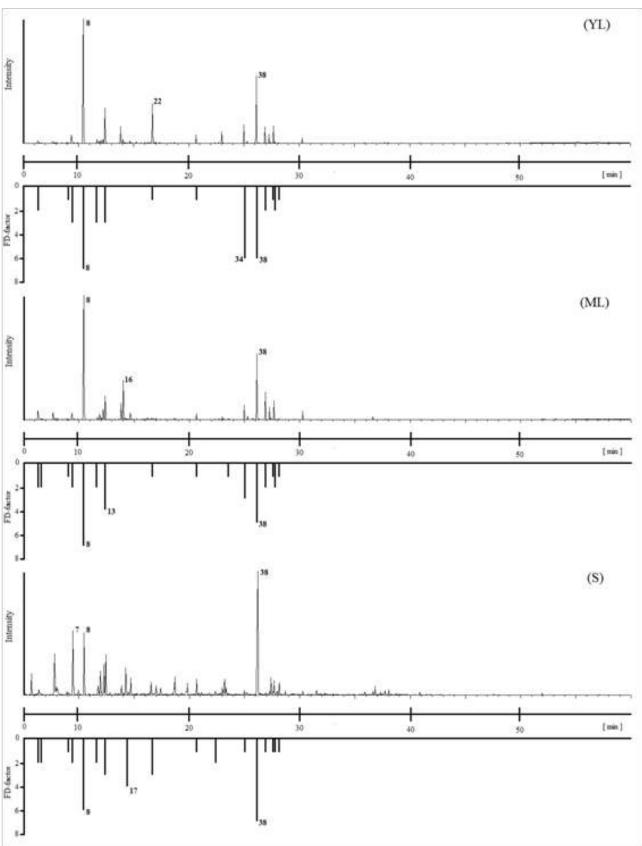


Figure 2 Gas chromatogram and aromagram (FD-factor) of essential oils by SAFE: 7, benzaldehyde; 8, β -myrcene; 13, (Z)- β -ocimene; 16, methyl benzoate; 17, linalool; 34, β -caryophyllene; 38, (E)- β -farnesene.

	RIª	500 SEC 2005 SEC 2005 SEC 2005		р	eak are	ea ^b (%))	identification		
No.	IP-5MS DI	compounds	YL	HD ML	S	YL	SAFE	S	method ^c	
1	824	- 3,3-dimethyl-1-butene	- 15	Dil.	-	-	IVIL	1.9	RI, MS	
*2	848	1362 (E)-3-hexen-1-ol		0.8	-	0.9	3.3	0.2	RI, MS	
3	860	1390 (E)-2-hexen-1-ol		0.8		0.0	0.4	0.4	RI, MS	
4	904	- 2-butoxy-ethanol		0.0		0.8	2.2	6.9	RI, MS	
5	913	- 1,2,3,4,5-pentamethyl-cyclopentane		920		0.4	0.7	2.4	RI, MS	
*6	944	1040 camphene	_			0.5	0.2	0.5	RI, MS	
*7	956	1516 benzaldehyde	0.6	0.3		3.0	2.3	8.6	RI, MS	
*8	991	1140 β-myrcene	20.3	19.8	6.0	32.9	31.0	8.1	RI, MS	
*9	1026	1146 limonene	0.7	0.6	0.8	0.9	0.7	1.8	RI, MS	
*10	1032	1779 benzyl alcohol	1.0	0.0	0.0	0.5	1.1	3.3	RI, MS	
*11	1036	1232 (E)-β-ocimene	0.7	0.4	0.5	0.7	0.5	0.6	RI, MS	
12	1042	1642 benzeneacetaldehyde		1.9	0.8	0.9	2.5	3.9	RI, MS	
*13	1042	1062 (Z)-β-ocimene	6.9	4.8	5.4	7.9	4.8	5.0	RI, MS	
14	1059	- 3-methyl-2-pentyl-cyclopentanone						0.5	RI, MS	
15	1074	- 5-decanone	-	-			-	0.3	RI, MS	
16	1094			-	-	1.1	7.9	0.4	RI, MS	
*17		1594 methyl benzoate			0.0					
	1099	1553 linalool		0.5	0.6	•	17	0.4	RI, MS	
*18	1111	1986 phenylethyl alcohol	-			0.2	1.7	2.0	RI, MS	
19	1156	- 1,4-octadiene	-	-	0.5		0.5	0.4	RI, MS	
20	1162	- 3,4-heptadiene	-	-	0.5	0.4	0.3	0.3	RI, MS	
*21	1165	1548 borneol	-	-	0.8	0.3	0.4	1.8	RI, MS	
22	1170	1640 ethyl benzoate		-	-	8.7	0.7		RI, MS	
23	1178	1527 6-undecanone	-		•	-	0.3	1.5	RI, MS	
24	1190	- p-menth-1-en-8-ol	0.0	-	0.5	0.4	0.0	1.0	RI, MS	
*25	1228	- neo- <i>allo</i> -ocimene	0.3	0.3	0.5	0.4	0.3	0.4	RI, MS	
*26	1261	- (Z)-verbenyl acetate		0.2	5.1			1.7	RI, MS	
*27	1286	1283 bornyl acetate	1.9	1.6	4.4	1.8	1.2	1.5	RI, MS	
28	1299	- 4-methoxymethoxy-oct-5-en-2-yne			- 3	-	- 3	0.3	RI, MS	
29	1337	1767 (E,E)-2,4-decadienal	0.2	0.2				0.5	RI, MS	
*30	1348	- lyratyl acetate	0.2	0.5	0.3	0.1	0.2		RI, MS	
31	1352	- 3,6-dimethyl-undecane	-	-	-	-	-	0.2	RI, MS	
*32	1357	2028 eugenol	0.3	-	-	2.5	0.7	0.7	RI, MS	
*33	1376	1517 α-copaene	-				0.2	- 7	RI, MS	
*34	1420	1579 β-caryophyllene	4.8	4.1	0.8	4.5	3.2	0.6	RI, MS	
35	1430	 epi-bicyclosesquiphellandrene 	0.5	0.8	0.5	0.4	0.7	0.4	RI, MS	
*36	1445	1448 α-cubebene	0.2	0.3			0.3	-	RI, MS	
*37	1454	1641 humulene	0.4	0.6		0107.00		-	RI, MS	
*38	1459	1670 (E)-β-farnesene	14.6	10.5	18.0	14.9	13.8	22.9	RI, MS	
39	1483	1683 β-cubebene	4.3	6.8	-	3.5		0.1	RI, MS	
*40	1495	1732 (Z,E)-α-farnesene	2.7	2.6	1.2	1.8	2.4	0.6	RI, MS	
*41	1498	1683 germacrene D	0.4	0.7	-	0.3	0.4	2.0	RI, MS	
*42	1509	1730 (E,E)-α-farnesene	5.2	3.2	1.1	3.8	3.6	1.6	RI, MS	
43	1517	- β-ionone	-		0.8	-	1.70	0.3	RI, MS	
44	1518	- dibenzyl	-	-		-	-	0.4	RI, MS	
*45	1524	1743 β-sesquiphellandrene	0.6	0.3	1.3	0.3	0.3	1.3	RI, MS	
46	1543	1567 α-bergamotene	0.2	-		-	-	0.4	RI, MS	
47	1598	1954 dendrolasin	1.8	1.8		1.1	1.6	0.5	RI, MS	
*48	1643	2168 τ-muurolol		0.5	6.0	_		0.5	RI, MS	
49	1648	- cedreanol		-	0.7	-	-	-	RI, MS	
*50	1655	- α-cadinol	-	0.4	3.4			0.2	RI, MS	
51	1670	 cadala-1(10),3,8-triene 			0.3	-			RI, MS	
*52	1676	2233 cadalene			0.9	-	-	-	RI, MS	
53	1805	2249 α-sinensal		-	0.4		-		RI, MS	
*54	1810	- (Z)-tonghaosu	5.9	1.4	2.0	-	-	0.4	RI, MS	
55	1838	- (E)-tonghaosu	0.5	3.7	0.4	-		0.4	RI, MS	
56	1845	2255 farnesyl acetone	0.9	1.1	2.6			0.9	RI, MS	
*57	1869	- 7-(2,4-hexadiynylidene)-1,6-	2.0	0.8	1.1		- 50	0.3	RI, MS	
*58	1886	- (Z)-tibetin spiroether	10.6	8.2	11.6			0.6	RI, MS	

Miyazawa M et al. Composition and Aroma Evaluation of Chrysanthemum coronarium

Ma	RIª	2000000000		D D	eak are	ea ^b (%)	CAFE		identificatio	
No. HP-5MS DB-WA		compounds 3-WAX	YL	HD ML	S	YL	SAFE	S	method°	
*59	1897	- (E)-tibetin spiroether	2.4	2.4	5.3	-	-	0.6	RI, MS	
60	1947	1727 valencene	0.3	-	-	-	-		DI 110	
*61	1954	- methyl palmitate	1.2	0.8	0.9			-	DI 110	
*62	1969	2842 palmitic acid		0.6	4.2			12		
63	2092	- methyl linoleate		0.4			-	- 2		
*64	2105	- methyl linolenate	-	2.4	-			-		
65	2113	2646 phytol	6.1	9.6	1.5	-		- 0	ACC. 4 4 4 4	
66	2121	- 11,13-dimethyl-12-tetradecen-1-ol acetate	0.3	0.4		-		14		
67	2489	- cyclopentacosane		-	0.7				DI 110	
*68	2500	2500 pentacosane	0.2	0.4	1.2				DI 110	
69	2692	- heneicosyl formate	-	0.7				-	DI 110	
70	2700	2700 heptacosane			1.5			-	DI 110	
71	2823	3058 squalene	0.3	0.4	1.4	-			DI 110	
72	2895	- cyclononacosane	-	0.3	-	-	-	-	DI MC	
73	2900	2900 nonacosane	-	-	0.9	-	-		RI, MS	
		hydrocarbons	63.7	57.0	43.4	73.8	69.4	53.4		
		alcohols	7.4	12.5	13.4	5.1	9.7	17.4		
		aldehydes	0.8	2.3	1.2	3.9	4.8	12.9		
		ketones	0.9	1.1	3.4		-	2.0		
		esters	3.4	7.0	10.6	11.7	9.9	3.7		
		ethers	23.2	18.3	20.4	1.1	1.6	3.0		
		acids	-	0.6	4.2	-	-	-		
		total	99.4	98.8	96.6	95.7	95.4	92.4	RI, MS	

a) RI, retention indices determined on HP-5MS and DB-WAX culumns, using the homologous series of n-alkenes.

	odor description ^a	conc. (ppb) ^b						odor	-factor (2")c					OAV ^a						
No. compounds		YL	HD ML			SAFE		threshold		HD)	S	AF	E.		HD			SAFE	
yrac—a buntan-unvaria-				S	YL	ML	S	(ppb)	YL MI		SY		YL ML S		YL	ML	S	YL	ML	S
2 (E)-3-hexen-1-ol	green	4	560.0	-	2880.0	2310.0	140.0	1500°		1		2	2	2		>1		1.9	1.5	
3 (E)-2-hexen-1-ol	green, sweet	0.0	560.0			280.0	280.0	8000°		1		-	2	2	*	>1			>1	
6 camphene	camphor	1.0	1.0	1.0	1600.0	140.0	350.0	1500°				1	1	1				1.1	>1	
7 benzaldehyde	sweet	1980.0	210.0		9600.0	1610.0	6020.0	350°	1	1		3	2	2	5.7	>1		27.4	4.6	17
8 β-mycene	spicy	66990.0	13860.0	4200.0	*******	21700.0	5670.0	15 ^b	8	7	6	8	7	6	4460.0	924.0	280.0	7018.7	1466.7	378
9 limonene	lemon, orange	2310.0	420.0	560.0	2880.0	490.0	1260.0	200°	2	1	2	3	2	2	11.6	2.1	2.8	14.4	2.5	6
13 (Z)-β-ocimene	citrus	22770.0	3360.0	3780.0	25280.0	3360.0	3500.0	340°	3	2	2	3	3	3	67.0	9.9	11.1	74.4	9.9	10
17 linalool	floral	1.4	19	420.0		*	280.0	6 ^{tt}			4		*	4	+3	0.4	70.0	(9)		46
21 borneol	camphor	19		560.0	960.0	280.0	1260.0	140	٠		1	1	1	3	-		4.0	6.9	2.0	9
27 bornyl acetate	camphor	6270.0	1120.0	3080.0	5760.0	840.0	1050.0	1380	1	1	1	.1	1	1	4.5	>1	2.2	4.2	>1	
29 (E,E)-2,4-decadienal	fatty	660.0	140.0			20	350.0	10°	3	3		-		2	66.0	14.0	3.0			35
33 α-copaene	sweet, floral					140.0		N/A		+		-	1						N/D	
34 β-caryophyllene	spicy	15840.0	2870.0	560.0	14400.0	2240.0	420.0	150 ^f	6	4	2	5	4	1	105.6	19.1	3.7	96.0	14.9	- 2
38 (E)-β-famesene	woody	48180.0	7350.0	12600.0	47680.0	9660.0	16030.0	N/A	6	5	6	6	5	5	N/D	N/D	N/D	N/D	N/D	N
39 β-cubebene	citrus	14190.0	4760.0		11200.0	3780.0	70.0	N/A	2	2		2	2	1	N/D	N/D	100	N/D	N/D	N
41 germacrene D	woody, spicy	1320.0	490.0		960.0	280.0	1400.0	N/A	1	1		1	1	1	N/D	N/D	1	N/D	N/D	N
42 (E,E)-α-famesene	woody, sweet	17160.0	2240.0	770.0	12160.0	2520.0	1120.0	N/A	2	2	1	2	2	1	N/D	N/D	N/D	N/D	N/D	N
45 β-sesquiphellandrene	woody	1980.0	210.0	910.0	960.0	210.0	910.0	N/A	1	1	1	1	1	1	N/D	N/D	N/D	N/D	N/D	N
48 t-muurolol	herb, spicy		350.0	4200.0			350.0	N/A		1	2		-			N/D	N/D		-	N

^{*} Odor quality perceived through the sniffing port.

b) peak area (%) was related to total detected compounds by GC-MS

c) identification methods: RI, retention index; MS, mass spectrum
*)previously identified
YL: young leaves, ML: mature leaves, S: stems

⁶ mg/kg given for 1 kg plant materials. These values were computed from the volatile oil yield and GC peak area.

^c The sample concentration (1 mg/mL) was assigned an FD factor of 1.

^d The OAV was obtained by dividing the concentrations of the odorants by their thresholds.

^{*} According to Aparicio & Morales, 1998.

According to Usami et al., 2014a.

⁹ According to Usami et al., 2014b.

According to Miyazawa et al., 2012.

CONFLICT OF INTERESTS

The Authors have no conflicts of interest to declare.

REFERENCES

- Kumar A, Singh SP, Bhakuni RS, Secondary metabolites of chrysanthemum genus and their biological activities. Current science 2005: 89: 1489-1501.
- 2 Dowrick GJ, The chromosomes of chrysanthemum I., The species 1952; 6: 365-375.
- 3 Quézel P, Santa S, Nouvelle Flore de l'Algérie et des régions désertiques méridionales. Ed CNRS Paris 1963, 1170p.
- 4 Chen W, Poon KF, Lam MHW, The application of solid phase microextraction in the analysis of organophosphorus pesticides in a food plant. Environ Sci Technol 1998; 32: 3816-3820.
- 5 Kasahara K, Nishibori K, The suppressing effect of garland chrysanthemum on the odor of Niboshi soup stock. Fish Sci 1995; 61: 672-674.
- 6 Alvarez-Castellanos PP, Pascual-Villalobos MJ, Effect of fertilizer on yield and composition of flowerhead essential oil of Chrysanthemum coronarium (Asteraceae) cultivated in Spain. Ind Crop Prod 2003; 17: 77-81.
- Marongiua B, Pirasa A, Porceddaa S, Tuveria E, Laconib S, Deiddaa D, Maxia A. Chemical and biological comparisons on supercritical extracts of Tanacetum cinerariifolium (Trevor) Sch. Bip. with three related species of chrysanthemums of Sardinia (Italy). Nat Prod Res 2009; 23: 190-199.
- 8 Wang H, Ye XY, Ng TB, Purification of chrysancorin, a novel antifungal protein with mitogenic activity from garland chrysanthemum seeds. Biol Chem 2001; 382: 947-951.
- 9 Hosni K, Hassen I, Sebei H, Casabianca H, Secondary metabolites from Chrysanthemum coronarium (garland) flowerheads: chemical composition and biological activities. Ind Crop Prod 2013; 44: 263-271.
- 10 Senatore F, Rigano D, Fusco RD, Bruno M, Composition of the essential oil from flowerheads of Chrysanthemum coronarium L. (Asteraceae) growing wild in southern Italy. Flavour Frag J 2004; 19:149-152.
- Basta A, Pavlovic M, Couladis M, Tzakou O. Essential oil composition of the flowerheads of Chrysanthemum coronarium L. from Greece. Flavour Frag J 2007; 22: 197-200.
- 12 Tawaha K, Hudaib M, Volatile oil profiles of the aerial parts of Jordanian garland, Chrysanthemum coronarium. Pharm Biol 2010; 48: 1108-1114.
- 13 Flamini G, Cioni PL, Morelli I, Differences in the fragrances of pollen, leaves, and floral parts of garland (Chrysanthemum coronarium) and composition of the essential oils from flowerheads and leaves. J Agric Food Chem 2003; 51: 2267-2271.
- 14 Fiehn O, Kristal B, Ommen B, Sumner LW, Sansone S-A, Taylor C, Hardy N, Kaddurah-Daouk R. Establishing reporting standards for metabolomic and metabonomic studies: a call for participation. OMICS 2006; 10: 158–163.
- 15 Cho IH, Choi H-K, Kim Y-S, Difference in the volatile composition of pine-mushrooms (Tricholoma matsutake Sing.) according to their grades. J Agric Food Chem 2006; 54: 4820–4825..
- Pongsuwan W, Fukusaki E, Bamba T, Yonetani T, Yamahara T, Kobayashi A, Prediction of Japanese green tea ranking by gas chromatography/mass spectrometry-based hydrophilic metabolite fingerprinting. J Agric Food Chem 2007; 55: 231–236.
- 17 Beleggia R, Platani C, Spano G, Monteleone M, Cattivelli L. Metabolic profiling and analysis of volatile composition of durum wheat semolina and pasta. J Cereal Sci 2009; 49: 301–309.
- 18 Ko B-K, Ahn H-J, Berg F, Lee C-H, Hong Y-S. Metabolomic insight into soy sauce through 1H NMR spectroscopy. J Agric Food Chem 2009; 57: 6862–6870.
- 19 Namgung H-J, Park H-J, Cho IH, Choi H-K, Kwon D-Y, Kim Y-S,

- Metabolite profiling of doenjang, fermented soybean paste, during fermentation. J Sci Food Agric 20010; 90: 1926–1935.
- Nejia H, Séverine C, Jalloul B, Mehrez R, Stéphane CJ, Extraction of essential oil from Cupressus sempervirens: comparison of global yields, chemical composition and antioxidant activity obtained by hydrodistillation and supercritical extraction. Nat Prod Res 2013; 27: 1795–1799.
- 21 Engel W, Bahr W, Schieberle P, Solvent assisted flavor evaporation - a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. Eur Food Res Technol 1999; 209: 237–241.
- 22 Mo X, Xu Y, Fan W, Characterization of aroma compounds in Chinese rice wine qu by solvent-assisted flavor evaporation and headspace solid-phase microextraction. J Agric Food Chem 2010; 58: 2462–2469.
- 23 Usami A, Ono T, Marumoto S, Miyazawa M, Comparison of volatile compounds with characteristic odor in flowers and leaves of nojigiku (Chrysanthemum japonense). J Oleo Sci 2013; 62: 631-636.
- 24 Usami A, Nakahashi H, Marumoto S, Miyazawa M, Aroma evaluation of setonojigiku (Chrysanthemum japonense var. debile) by hydrodistillation and solvent-assisted flavor evaporation. Phytochm anal 2014; 25: 561-566.
- 25 Aparicio R, Morales MT, 1998, Characterization of olive ripeness by green aroma compounds of virgin olive oil. J Agric Food Chem 1998; 46: 1116-1122.
- 26 Miyazawa M, Nagata T, Nakahashi H, Takahashi T, Characteristic odor components of essential oil from Caesalpinia decapetala. J Essent Oil Res 2010; 4: 441-446.
- 27 Usami A, Nakaya S, Nakahashi H, Miyazawa M, Chemical composition and aroma evaluation of volatile oils from edible mushrooms (Pleurotus salmoneostramineus and Pleurotus sajor-caju). J Oleo Sci 2014; 62: 1323-1332.
- Umlauf D, Zapp J, Becker H, Adam KP, Biosynthesis of the irregular monoterpene artemisia ketone, the sesquiterpene germacrene D and other isopreneoids in Tanacetum vulgare L. (Asteraceae). Phytochemistry 2014; 65: 2463-2470.
- 29 Prakasia PP, Nair AS, Chemical fingerprint of essential oil component from fresh leaves of Glycosmis pentaphylla (Retz.) Correa Pharm Inov J 2015; 3: 50-56.
- 30 Kobayashi Y, Takemoto H, Asaka Y, Fu Z, Takane T, Kitojo H, Chemical analysis of the volatile and polyphenolic components of the leaves and stems german chamomile, and the comparison with flower heads components. Aroma Res 2013; 14: 155-159.
- Rafieiolhossaini M, Adams A, Sodaeizadeh H, Van Damme P, De Kimpe N, Fast quality assessment of german chamomile (Matricaria chamomilla L.) by headspace solid-phase microextraction: influence of flower development stage. Nat Prod Comm 2012; 7: 97-100.
- 32 Bicchi C, Rubiolo P, Marschall H, Weyerstahl P, Laurent R, Constituents of Artemisia roxburghiana Besser essential oil. Flavour Fragr J 1998; 13: 40-46.
- 33 Lawal OA, Ogunwande IA, Opoku RA, Chemical composition of essential oils of Plumeria rubra L. grown in Nigeria. Eur J Med Pl 2015; 6: 55-61.
- 34 Werkhoff P, Brennecke S, Bretschneider W, Bertram H-J, Modern methods for isolating and quantifying volatile flavor and fragrance compounds. In Flavor, Fragrance, and Odor Analysis, Marsili R (ed.). New York: Marcel Dekker 2002; 139–204.
- 35 Grosch W, Determination of potent odorants in foods by aroma extract dilution analysis (AEDA) and calulation of odor activity values (OAV). Flavour Fragr J 1994; 9: 147–158.

Peer reviewers: Goutam Brahmachari, Professor, Laboratory of Natural Products & Organic Synthesis, Department of Chemistry, Siskha-Bhavana (Institute of Science), Visva-Bharati (a Central University), SANTINIKETAN-731235, WEST BENGAL, INDIA; Deia Abd El-Hady, King Abdulaziz University, Faculty of Science-North Jeddah, Chemistry Department, 80203 Jeddah, Saudi Arabia.