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Original Research Article

Headspace solid-phase microextraction and gas chromatography–mass spectrometry of volatile components of Chrysanthemum morifolium Ramat

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Abstract

Purpose: *To extract and analyze the volatile components of Chrysanthemum morifolium Ramat. 'huaiju' by headspace solid-phase microextraction (HS-SPME) and gas chromatography–mass spectrometry (GC–MS).*
Methods: *Volatile components were extracted by HS-SPME and identified by GC–MS. The relative contents of the components were determined by area normalization.*
Results: *The enhanced SPME conditions of C. morifolium involved sample extraction using a 65 μm polydimethylsiloxane/divinylbenzene extraction fiber after balancing for 40 min at 80 °C. A total of 48 components of the essential oil were identified. The major constituents are 2,6,6-trimethyl-bicyclo[3.1.1]hept-2-en-4-ol, acetate (15.90 %), 4,6,6-trimethyl-bicyclo[3.1.1]hept-3-en-2-one (14.86 %), 2,7,7-trimethyl-bicyclo[3.1.1]hept-2-en-6-one (13.08 %), and cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene (5.97 %).*
Conclusion: *HS-SPME and GC–MS are convenient, rapid, and reliable approaches for analyzing the volatile components of C. morifolium.*

Keywords: *Chrysanthemum morifolium Ramat., Headspace Solid-phase Microextraction, Gas Chromatography–Mass Spectrometry, Volatile component*

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INTRODUCTION

Chrysanthemum morifolium Ramat. is a traditional Chinese medicinal herb that belongs to the Compositae family. The plant has long been used as herbal medicine and tea for treating diseases, such as headache, influenza, and hepatic and eye diseases [1]. Many cultivars of *C. morifolium* flowers are available in herb or tea markets in China. Among these cultivars, 'Huaiju', 'Boju', 'Chuju', 'Gongju', and 'Hangbaiju' constitute the majority [2]. The plant's dried flowers contain alkanes, flavonoids, terpenoids, unsaturated fatty acids, polysaccharides, and essential oils [1–7]. Essential oils are blends of volatile secondary metabolites from plants; these oils feature a broad-spectrum activity because of the presence of several chemicals [5]. Currently, the main extraction methods for volatile oil from *C. morifolium* are steam distillation extraction (SDE) [4,5,7], ultrasonic extraction [8], and supercritical carbon dioxide extraction [3]. Different from the above-mentioned extraction methods, headspace solid-phase microextraction (HS-SPME) is simple and reproducible, does not involve solvent use, and includes a short extraction time [9]. Moreover, HS-SPME requires a small sample volume, and coupling with gas

chromatography (GC) and mass spectrometry (MS) affords the method with high sensitivity [10,11]. As such, the technique has gained wide acceptance in food, environmental, and clinical analyses. The present study aims to analyze volatile components from *C. morifolium* (Huaiju) by HS-SPME and GC–MS.

EXPERIMENTAL

Plant collection and identification

The tested samples were directly harvested from cultivated farms in Wuzhi County, Henan Province, PR China in September 2015. The samples were authenticated as *C. morifolium* Ramat. by Associate Professor Mingxing Zhi (Henan Institute of Science and Technology, China). Voucher specimens were stored in the reference herbarium of Henan Institute of Science and Technology.

Extraction of essential oils

Essential oils of *C. morifolium* Ramat. were obtained by HS-SPME. The sample (2 g) was introduced into a 20 mL HS vial. The fiber was coated with 65 μm polymethylsiloxane/divinylbenzene (PDMS/DVB), which is usually used for the absorption of volatile components. The sample was maintained at 80 °C for 40 min. During the sampling time, the sample was stirred at the constant speed of 250 rpm. Following HS extraction, SPME fibers were injected into the GC apparatus and then maintained in the GC inlet for 3 min.

Analysis of the essential oils

Volatile component analysis was performed on an Agilent 7890A gas chromatograph coupled with a 5977C mass selective detector (Agilent Technologies, USA). Compounds were then separated on a 30 m TG-WAXMS column with an internal diameter of 0.32 mm and a film thickness of 0.25 μm (Agilent, USA). The injector temperature was 230 °C, and the split ratio was 1:1. High-purity helium (99.999 %) was used as the carrier gas at a flow rate of 1 mL/min. The GC oven temperature was then programmed as follows: 40 °C for 2 min, 2 °C/min to 200 °C, 10 °C/min to 230 °C for 5 min. The interface temperature was 280 °C, and the quadrupole temperature was set to 150 °C. The mass spectrometer was fitted with an EI+ source operated at 70 eV with a source temperature of 230 °C, and mass spectra were recorded in the range of m/z 40–400 amu in full-scan acquisition mode. Oil components were identified on the basis of their retention indices and by comparison of their mass spectral fragmentation patterns with those reported in the literature and stored in the MS library.

RESULTS

Forty-eight compounds representing approximately 96.22 % of the oil were identified (Table 1). Significant differences between the main components of the essential oil were noted. The major constituents were 2,6,6-trimethyl-bicyclo[3.1.1]hept-2-en-4-ol acetate (15.90 %), 4,6,6-trimethyl-bicyclo[3.1.1]hept-3-en-2-one (14.86 %), 2,7,7-trimethyl-bicyclo[3.1.1]hept-2-en-6-one (13.08 %), and cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene (5.97 %).

DISCUSSION

HS-SPME is a common sample pretreatment technique that enhances the concentration of a target in biological samples. This procedure is performed by exposing a polymer-coated fiber to the HS of samples without any solvent. For example, a previous study compared SPME/GC–MS with the conventional SDE method followed by GC/MS to identify volatile compounds in *C. morifolium*. Thirty-two volatile compounds were identified using the newly developed SPME/GC–MS process, and relative standard deviation values of < 9.8 % demonstrate good repeatability. In comparison, 27 compounds were identified by traditional steam distillation–GC/MS [6].

In the present study, 26 compounds found in the essential oils of *C. morifolium* Ramat. were terpenes, representing approximately 69.67 % of the oil. Its chemical composition was fairly different from those reported in previous studies [6,12-15]. Sun *et al* [15] reported that 39, 20, 19, 33, 22, 18, 25, 29, 20, and 33 compounds were essential oils were identified the same plant materials from Bozhou Anhui, Wenxian Henan, Nanyang Henan, Mout Huangshan Anhui, Hangzhou1, Linying Henan, Lingbao Henan, Hangzhou2, Foshan Guangdong, and Wuxi Jiangsu, respectively. These essential oils contained 12 types of the same composition [15]. Significant differences were also noted among the components and contents of essential oils of Flos Chrysanthemi Indici from Guangxi, Guangdong, and Hubei [13]. The above-mentioned results suggest the varied chemical composition of essential oils extracted from different sites and at different collection times.

Table 1: Composition of the essential oils of *C. morifolium* Ramat

No.	Component	Formula	Content (%)
1	α-Pinene	$C_{10}H_{16}$	0.203
2	2,7,7-Trimethyl-bicyclo[3.1.1]hept-2-en-6-one	$C_{10}H_{14}O$	13.082
3	4,6,6-Trimethyl-bicyclo[3.1.1]hept-3-en-2-one	$C_{10}H_{14}O$	14.859
4	Cyclohexen-1-one,3-methyl-6-(1-methylethenyl)	$C_{10}H_{14}O$	3.952
5	3,5-Heptadienal, 2-ethylidene-6-methyl	$C_{10}H_{14}O$	0.187
6	Eugenol	$C_{10}H_{12}O_2$	0.286
7	1,3,3-Trimethylcyclohex-1-ene-4-carboxaldehyde	$C_{10}H_{16}O$	2.017
8	6,6-dimethyl-2-methylene-Bicyclo[3.1.1]heptane	$C_{10}H_{16}$	0.188
9	(S)-cis-Verbenol	$C_{10}H_{16}O$	2.350
10	1,7,7-Trimethylbicyclo[2.2.1]-2-heptanone	$C_{10}H_{16}O$	0.374
11	Eucalyptol	$C_{10}H_{18}O$	0.226
12	Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene	$C_{10}H_{16}O$	5.968
13	2-(4-methylcyclohex-3-en-1-yl)propan-2-ol	$C_{10}H_{18}O$	0.312
14	2,6,6-trimethyl-2,4-Cycloheptadien-1-one,	$C_{10}H_{14}O$	0.984
15	2,6,6-trimethyl-bicyclo[3.1.1]hept-2-en-4-ol acetate	$C_{12}H_{18}O_2$	15.898
16	endo-1,7,7-Trimethylbicyclo[2.2.1]hept-2-yl acetate	$C_{12}H_{20}O_2$	0.507
17	Spathulenol	$C_{15}H_{24}O$	0.546
18	Longifolene	$C_{15}H_{24}$	0.201
19	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)	$C_{15}H_{24}$	0.520
20	Caryophyllene	$C_{15}H_{24}$	0.495
21	Farnesene	$C_{15}H_{24}$	2.641
22	β-Bisabolene	$C_{15}H_{24}$	0.228
23	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl	$C_{15}H_{24}$	0.396
24	3,7-Cycloundecadien-1-ol,1,5,5,8-tetramethyl	$C_{15}H_{26}O_2$	0.509
25	3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	$C_{15}H_{26}O$	1.312
26	Caryophyllene oxide	$C_{15}H_{24}O$	1.430
27	Butanoic acid,2-methyl-, propyl ester	$C_8H_{16}O_2$	0.146
28	Ethyl caproate	$C_8H_{16}O_2$	1.023
29	Hexanoic acid, 2-methyl-4-methylene-, methyl ester	$C_9H_{16}O_2$	0.635
30	Hexyl N-valerate	$C_{11}H_{22}O_2$	0.151
31	(Z)-Hexadecenoic acid, methyl ester,	$C_{17}H_{32}O_2$	0.263
32	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	0.504
33	Hexanoic acid, hexyl ester	$C_{12}H_{24}O_2$	0.219
34	Methyl salicylate	$C_8H_8O_3$	8.774
35	Pentanoic acid, phenylmethyl ester	$C_{12}H_{16}O_2$	0.207
36	Cis-3-Hexenol	$C_6H_{12}O$	0.142
37	2,6-Dimethyl-2,5-heptadiene-4-one	$C_9H_{14}O$	0.566
38	6-Methylhepta-3,5-diene-2-one	$C_8H_{12}O$	0.154
39	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	$C_9H_{12}O_2$	0.144
40	6-Methyl-5-hepten-2-one	$C_8H_{14}O$	2.027
41	1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-	$C_{10}H_{14}O$	1.839
42	1,1-Diethoxypentane	$C_9H_{20}O_2$	0.63
43	Acetal	$C_{12}H_{18}O_2$	0.01
44	1,3,5-Trimethylbenzene	C_9H_{12}	5.862
45	*Isopropenyltoluene*	$C_{10}H_{12}$	0.535
46	1,6-Dimethylhepta-1,3,5-triene	C_9H_{14}	0.798
47	Cyclopentene, 1-(3-methylbutyl)-	$C_{10}H_{18}$	0.22
48	1-isopropyl-2-methylbenzene	$C_{10}H_{14}$	1.703

The volatile components were affected by different temperatures, balance periods, and extraction fibers used under HS-SPME. Zhou *et al* [12] found that a favorable condition for the SPME of *C. morifolium* was achieved when the sample was extracted using a 100 μm PDMS extraction fiber after balancing for 6 h at 75 °C. In the present study, the essential oil in *C. morifolium* was extracted using 65 μm PDMS/DVB extraction fiber after balancing for 40 min at 80 °C. Overall, our results show that HS-SPME and GC–MS are convenient, rapid, and reliable methods for analyzing the volatile components of *C. morifolium*.

CONCLUSION

HS-SPME coupled with GC–MS is a rapid, eco-friendly method to analyze the volatile components of *C. morifolium* Ramat. The essential oil of *C. morifolium* Ramat. flower contains 48 compounds of varying concentrations.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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