Asian Journal of Chemistry Vol. 21, No. 4 (2009), 2887-2892

Extraction and Analysis of Volatile Oil of *Mussaenda hainanensis* Merr. by GC/MS

WEN-TAO QIAO†, YAN-HUI LIU and KE YUAN*
Research and Development Center of Natural Medicine
Zhejiang Forestry University, Linan 311300, P.R. China
Tel: (86)(571)63743607; E-mail: yuan_ke001@163.com

In present study, the chemical constituents of essential oils and relative content of *Mussaenda hainanensis* Merr. are reported. The essential oils were extracted from *Mussaenda hainanensis* Merr. by Headspace-solid-phase microextraction (HS-SPME) technique and steam distillation (SD). The chemical constituents of essential oils separated were identified by GC/MS analysis, the relative content of the constituents from the essential oils were determined by area normalization method. Chromatographic condition were: Capillary column DB-5 (30 m × 0.25 mm, 0.25 μm); Program temperature: initial column temperature 40 ºC, maintained 2 min and increased to 270 ºC at the rate of 10 ºC min^{-1}, held 15 min. No split. Injector temperature 280 ºC. There were 37 constituents were separated and identified by Headspace-solid-phase microextraction, which accounted for over 97.45 % of total essential oil fraction. There were 42 constituents were separated and identified by steam distillation, which accounted for over 93.29 % of total essential oil fraction.

Key Words: *Mussaenda hainanensis* Merr., Essential oils, Headspace solid-phase microextraction, Steam distillation, Gas chromatography-mass spectrometry.

INTRODUCTION

Mussaenda hainanensis Merr. is a kind of climbing shrubs assigned to the family Rubiaceae. Its equal plant Mussaenda pubescens grow in slopes or in the bush. It distributes in Fujian, Guangdong, Guangxi, Yunnan, Sichuan, Guizhou and other places[1]. It has a diaphoresis, relieves summer heat, detoxifying and invigorating the circulation of blood[2]. But *Mussaenda hainanensis* Merr. produces in Hainan, for the Hainan unique plant. The chemical constituents of the volatile oil analysis is not yet reported in literature. Volatile oils were extracted respectively from the *Mussaenda hainanensis* Merr. using Headspace-solid-phase microextraction (HS-SPME) and steam distillation (SD), then identified the chemical compositions of the volatile oils by gas chromatography-mass spectrometry technique and simultaneously, the analyzed products have been compared under two kinds of extraction technology different test result, for the clear understanding of this plant.

†College of Pharmacy, Henan University of Traditional Chinese Medicine, Zhengzhou 450008, P.R. China.

EXPERIMENTAL

The fresh *Mussaenda hainanensis* Merr. was collected in Hainan, China. The species was identified by local botanists and a voucher specimen deposited at the HaiNan University of China.

GC-MS was performed with an GC1iA-QP5000 GC-MS; Capillary column DB-5 (30 m × 0.25 mm, 0.25 µm) quartz capillarity columniation. The following fibres (Supelco, Bellefonte, PA and USA) were used for the extraction procedures: 100 µm polydimethylsiloxane(PDMS).

Conditions of the GC-MS analysis: The analysis was carried out by a Finnigan Voyager GC-MS instrument. A 30 m 0.25 mm fused-silica capillary column coated with a 0.25 µm polyethylene glycol film was used. The temperature program used for analysis was as follows: initial temperature at 40 ºC for 2 min, then programmed at 10 ºC min^{-1} to 270 ºC. Helium (99.999 %) was the carrier gas at a flow-rate of 1.0 mL min^{-1}. No split.The injector temperature was set at 280 ºC and the desorption was performed at the injector for 3 min. The electron impact ionization conditions were as follows ion energy 70 eV and the mass range scanned was 30-500 a.m.u in the full-scan acquisition mode. The compounds were identified using the NIST Mass Spectral Search Program (National Institute of Standards and Technology, Washington, DC, USA) and the Wiley 6.0 (Wiley, New York, NY, USA) Mass Spectral Library.

SPME sample preparation was as follows: 2.0 g of *Mussaenda hainanensis* Merr. were ground to powder and instantly introduced into a 15 mL headspace vial. The sample vials were stored at -10 ºC until used. The oil of *Mussaenda hainanensis* Merr. was isolated by steam distillation according to Qiu's method; 100 g of *Mussaenda hainanensis* Merr. were weighted into a 1000 mL distillation flask, 700 mL of deionized water was added and the mixturewas distilled for 5 h. Oil was collected from the condenser. The components were analyzed by GC-MS.

Taken steam distillation method and Headspace solid phase micro-extraction of essentialoils extracted amount by GC-MS analysis identified. Using normalized method to confirm quantitative compounds and use of Hewlett-Packard software processing system to calculate peak areas, in order to test various components of the volatile oil content relative percentile.The total ion chromatogram of the sample by HS-SPME-GC-MS and by SD-GC-MS are shown in Fig. 1.

Total ion flow chart of the various MS-After scanning through Mass Spectrometry Data System (NIST and Willey 6.0)[3] retrieval control and access to the relevant information[4] MS combined with artificial analysis, identification markings of *Mussaenda hainanensis* Merr. in the volatile oils chemical composition, the results in Tables 1 and 2.

RESULTS AND DISCUSSION

There were 37 constituents were separated and identified by Headspace-solid-phase microextraction, which accounted for over 97.45 % of total essential oil fraction. Content tiptop is 2,3-diol-1,4-dioxane, which relative contents is 28.50 %; in the next place is 4-hydroxy-2-butanone with relative contents is 24.03 %.

TABLE-1
ANALYTICAL RESULTS OF CHEMICAL CONSTITUENTS OF ESSENTIAL OIL BY HEADSPACE SOLID-PHASE MICROEXTRACTION

No.	RT (min)	Name	m.f.	m.w.	Area (%)
1	2.73	Ethyl formate	$C_3H_6O_2$	74	2.58
2	2.93	4,4-dimethyl-3-Hexanol	$C_8H_{18}O$	130	0.22
3	3.49	2-Butanone	C_4H_8O	72	0.22
4	4.08	2-Ethoxyethylamine	$C_4H_{11}NO$	89	10.86
5	4.17	1-methylpropyl ester -Formic acid	$C_5H_{10}O_2$	102	0.33
6	4.90	2-(1-bromoethyl)-1,3-Dioxolane	$C_5H_9O_2Br$	181	0.26
7	5.43	(ñ)-2-Butanol	$C_4H_{10}O$	74	1.64
8	6.38	Hexanal	$C_6H_{12}O$	100	0.22
9	7.70	2-Ethoxy-3-chlorobutane	$C_6H_{13}ClO$	136	0.17
10	12.65	(E)-2-Octenal	$C_8H_{14}O$	126	0.24
11	13.72	4-hydroxy-2-butanone	$C_4H_8O_2$	88	24.03
12	12.91	Acetic acid	$C_2H_4O_2$	60	4.96
13	14.22	2,3-diol-1,4-dioxane	$C_4H_8O_4$	120	28.50
14	14.44	2-octyl-, methyl ester-Cyclopropanetetradecanoic acid	$C_{26}H_{50}O_2$	394	0.29
15	15.41	2,3-Butanediol	$C_4H_{10}O_2$	90	3.55
16	16.83	(E,Z)-5,6-bis(2,2-dimethylpropylidene)- Decane	$C_{20}H_{38}$	278	0.17
17	18.92	Hexanoic acid	$C_6H_{12}O_2$	116	0.27
18	19.83	2-hexadecen-1-ol -3,7,11,15-Tetramethyl	$C_{20}H_{40}O$	296	0.99
19	22.09	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	9.00
20	24.83	Triethylene glycol	$C_6H_{14}O_4$	150	0.17
21	25.45	3-(2-hydroxyphenyl)-, (E)-2-Propenoicacid	$C_9H_8O_3$	164	0.20
22	25.90	(Z)-(-)-1,9-heptadecadiene-4,6-diyne-3-ol -Falcarinol	$C_{17}H_{24}O$	244	1.15
23	26.18	(2S,2'S)-2,2'-Bis[1,4,7,10,13-pentaoxacyclopentadecane]	$C_{20}H_{38}O_{10}$	438	1.67
24	26.57	Heneicosane	$C_{21}H_{44}$	296	0.28
25	26.71	2'-hexyl-[1,1'-Bicyclopropyl]-2-octanoic acid, methyl ester	$C_{21}H_{38}O_2$	322	0.21
26	26.82	N-(benzoyloxy)-Cyclohexanamine	$C_{13}H_{17}N_2$	219	0.30
27	27.05	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308	0.38
28	27.63	15,15'-Bi-1,4,7,10,13-pentaoxacyclohexadecane	$C_{22}H_{42}O_{10}$	466	0.23
29	27.99	(Z,Z,Z)-9,12,15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292	0.23
30	28.20	Phytol	$C_{20}H_{40}O$	296	0.88
31	28.52	4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-2-cyclohexen-1-one	$C_{13}H1_8O_3$	222	0.33
32	28.82	(all-E)-2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-Tetracosahexaene	$C_{30}H_{50}$	410	1.31
33	29.03	(Z)-2-(9-octadecenyloxy)-Ethanol	$C_{20}H_{40}O_2$	312	0.17
34	29.88	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.26
35	30.06	2-[2-(2-butoxyethoxy)ethoxy]-Ethanol	$C_{10}H_{22}O_4$	206	0.22
36	32.22	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.54
37	32.62	Octadecanoic acid	$C_{18}H_{36}O_2$	284	0.42

TABLE-2
ANALYTICAL RESULTS OF CHEMICAL CONSTITUENTS OF ESSENTIAL OIL BY STEAM DISTILLATION

No.	RT (min)	Compound	m.f.	m.w.	Area (%)
1	2.73	Formic acid, ethyl ester	$C_3H_6O_2$	74	2.81
2	2.93	1-ethoxy-1-methoxy-Ethane	$C_5H_{12}O_2$	104	0.61
3	3.35	4-hydroxy-2-Butanone	$C_4H_8O_2$	88	4.22
4	4.12	DL-Methyltartronic acid	$C_4H_6O_5$	134	8.98
5	4.19	Formic acid, 1-methylpropyl ester	$C_5H_{10}O_2$	102	0.51
6	4.32	Ethanol	C_2H_6O	46	0.18
7	4.59	2-Pentanone	$C_5H_{10}O$	86	0.15
8	4.77	Acetic acid, 1-methylpropyl ester	$C_6H_{12}O_2$	116	0.22
9	4.91	1-(1-ethoxyethoxy)-Propane	$C_7H_{16}O_2$	132	2.05
10	5.23	3-methyl-2-Pentanone	$C_6H_{12}O$	100	0.16
11	5.45	(ñ)-2-Butanol	$C_4H_{10}O$	74	2.49
12	5.74	1,3-*Bis*(2-ethoxypropyl)benzene	$C_{16}H_{26}O_2$	250	0.74
13	6.33	1,1-diethoxy-Ethane	$C_6H_{14}O_2$	118	1.47
14	7.14	(R)-(-)-2-Pentanol	$C_5H_{12}O$	88	0.21
15	7.46	Ethylbenzene	C_8H_{10}	106	0.15
16	7.70	1,2-dimethyl-Benzene	C_8H_{10}	106	0.27
17	8.81	2-Ethoxy-3-chlorobutane	$C_6H_{13}ClO$	136	0.51
18	9.60	(R)-4-(1-Ethoxyethoxy)-3-fluoro-4-methyl-1-pentanol acetate	$C_{12}H_{23}FO_4$	250	0.59
19	12.86	Propanedioic acid	$C_3H_4O_4$	104	24.26
20	14.24	1,4-Dioxane-2,3-diol	$C_4H_8O_4$	120	31.95
21	14.73	(3-Methyl-oxiran-2-yl)-methanol	$C_4H_8O_2$	88	0.31
22	15.09	2,3-Butanediol	$C_4H_{10}O_2$	90	3.60
23	15.41	3-Methyl-2-pentanol	$C_6H_{14}O$	102	0.25
24	17.99	Methyl Salicylate	$C_8H_8O_3$	152	0.21
25	23.21	Docosane	$C_{22}H_{46}$	310	0.25
26	23.49	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.16
27	23.82	2-methyl-1-Hexadecanol	$C_{17}H_{36}O$	256	0.18
28	24.24	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	1.48
29	24.35	Tricosane	$C_{23}H_{48}$	324	0.36
30	24.85	Triethylene glycol	$C_6H_{14}O_4$	150	0.53
31	25.45	2,5,8,11,14-Pentaoxahexadecan-16-ol	$C_{11}H_{24}O_6$	252	0.35
32	25.90	2-[2-(2-ethoxyethoxy)ethoxy]-Ethanol	$C_8H_{18}O_4$	178	0.23
33	26.01	1,4,7,10,13,16-Hexaoxacyclooctadecane	$C_{12}H_{24}O_6$	240	0.36
34	26.18	5-octyl-1,2,4-Trioxolane-2-octanoic acid, methyl ester	$C_{19}H_{36}O_5$	324	0.40
35	26.37	(3á,5Z,7E)-9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol	$C_{27}H_{44}O_3$	416	0.22
36	26.58	Octacosane	$C_{28}H_{58}$	394	0.64
37	26.83	15,15'-Bi-1,4,7,10,13-pentaoxacyclohexadecane	$C_{22}H_{42}O_{10}$	466	0.22
38	27.28	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278	0.21
39	27.60	15-Isobutyl-(13alphah)-isocopalane	$C_{24}H_{44}$	333	0.18
40	27.90	Hexacosane	$C_{26}H_{54}$	366	0.20
41	28.20	Phytol	$C_{20}H_{40}O$	296	0.19
42	30.10	Pentaethylene glycol	$C_{10}H_{22}O_6$	238	0.23

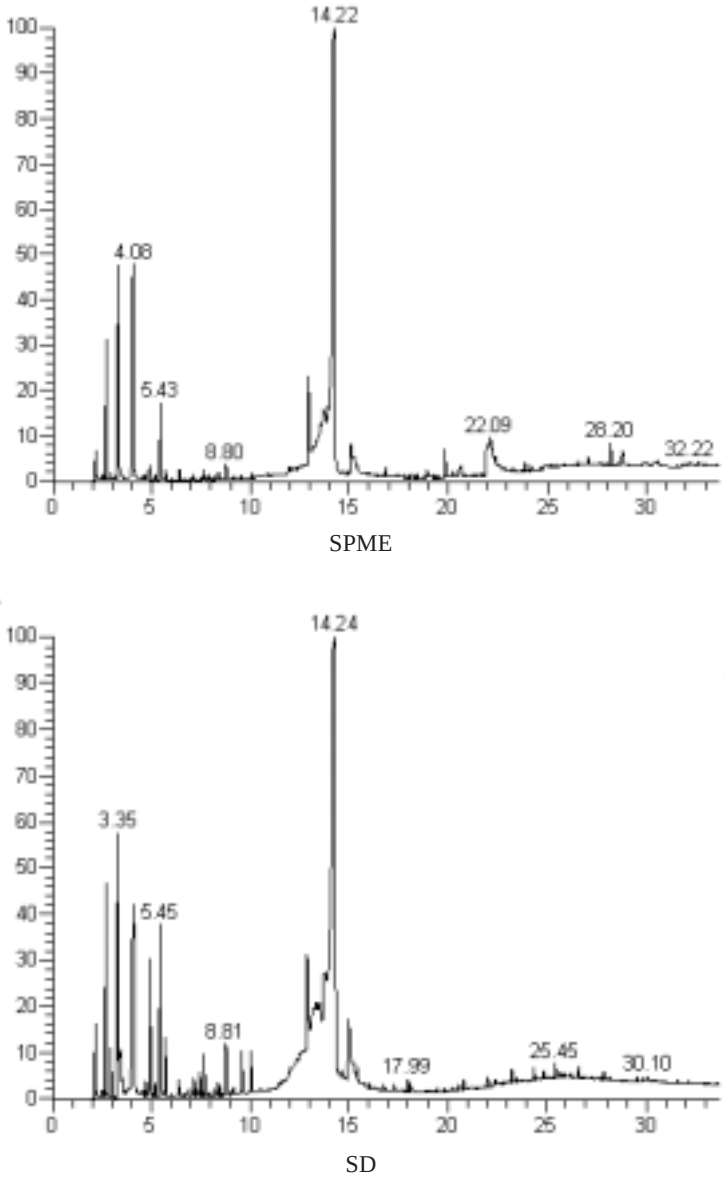

Fig. 1. GC-MS total ion current chromatogram of the essential oil by HS-SPME-GC-MS and SD-GC-MS

There were 42 constituents separated and identified by steam distillation accounted for over 93.29 % of total essential oil fraction. Content tiptop is 1,4-dioxane-2,3-diol, which relative contents is 31.95 %; in the next place is propanedioic acid which relative contents is 24.26 %. Both obtained volatile oil constituents are basic consistent.

The present method only needs simple sample preparation and the whole analysis time is less than 70 min. Compared with SD, HS-SPME is a simple, sensitive and rapid method suitable for the analysis of volatile constituents from Traditional Chinese Medicines. HS-SPME is a simple, sensitive and rapid method suitable for the analysis of volatile constituents from Traditional Chinese Medicines. At present solid phase micro-extraction has been widely used in environmental, biological and food analysis[5-7]. SPME with GC/MS analysis has also been applied to medicine in the field of volatile oil[8,9].

ACKNOWLEDGEMENTS

The author acknowledge the olfactory evaluations by the chief perfumers, Yu Ruipeng, Jian Nan University, China and the financial support by the Zhejiang Forestry University.

REFERENCES

1. Part of Fujian Institute of Chinese Medicine, Fujian Chinese herbs [M], Fujian Medical Institute Press (1970).
2. Guangxi Zhuang Autonomous Region Hygiene Government Department at the Provincial Level edits, Guangxi Chinese Medicinal Herbal [M], The Guangxi Zhuang Autonomous Region People Press (1959).
3. P.Z. Cong, The Application of Mass Spectrum in Organic Chemistry of Natural Products [M]. Beijing (Beijing): Science Press, p. 595 (1987).
4. Organic Committee of Chinese Mass Spectrometry Society, Flavor Mass Spectral Data Base (spice mass spectrum atlas), p. 179 (1992).
5. G.A. Mills and V. Walker, *J. Chromatogr. A*, **902**, 267 (2000).
6. S. Ulrich, *J. Chromatogr. A*, **902**, 167 (2000).
7. C. Chafer-Pericas, P. Campins-Falco and R. Herraez-Hernandez, *Anal. Biochem.*, **333**, 328 (2004).
8. C.H. Deng, G.X. Song, Y.M. Hu and X.M. Zhang, *Chromatographia*, **58**, 289 (2003).
9. C.H. Deng, G.X. Song, X.H. Zheng, Y.M. Hu and X.M. Zhang, *Chromatographia*, **57**, 805 (2003).

(*Received*: 17 May 2008; *Accepted*: 19 January 2009) AJC-7136