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Analysis of the Volatile Constituents of *Rabdosia rubescens* by Gas Chromatography-Mass Spectrometry Using Headspace Solid-Phase Micro-Extraction

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The extraction of chemical constituents of volatile oil from *Rabdosia rubescens* by the technique of headspace solid-phase micro-extraction (HS-SPME) is reported. The chemical constituents of volatile oils separated were identified by gas chromatography-mass spectrometry analysis. The relative percentage content of chemical constituents of volatile oil were determined by area normalization method. Chromatographic conditions: HP-5 elastic quartz capillary-tube chromatographic column (30 m × 0.25 mm, 0. 25 mm); temperature-increasing procedure: keep the initial temperature 45 ºC for 2 min and then increase the temperature to 250 ºC and keep it for 0.5 h at the rate of 10 ºC min^{-1}. The temperature at the sample-feeding entrance is 250 ºC and the flowing rate is 0.8 mL min^{-1} without any divided sample-feeding. By the method of solid-phase micro-extraction, we identified 60 kinds of constituents, the identified part occupies 98.27 % of the total volatile oil and its main chemical constituents are the compounds of hydrocarbon, alcoholate and ketone. Compared with the traditional steam distillation method, the technique of headspace solid-phase micro-extraction has the advantages of short extracting time, small sample amount and no need of extracting the solution. This experiment result enhances present understanding of chemical constituents of volatile oils in *Rabdosia rubescens* and provides further reference for the development and utilization of the natural medicinal resource.

Key Words: *Rabdosia rubescens*, Headspace solid-phase Micro-extraction, Gas chromatography-mass spectrometry, Volatile constituents.

INTRODUCTION

Nowadays, in the research area of volatile constituents, the method of solid phase micro-extraction (SPME)[1] is becoming more and more important. Solid-phase micro-extraction is a non-solvent extraction method. This is a new kind of sample pre-treating method. It enriched the volatile and half-volatile constituents in the sample by using adsorption and de-sorption technique and it can be used jointly with other instruments to determine and analyse the organic matter of the

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volatile and half-volatile. There is no need of organic solvent. It combines in one with the sample collection, extraction, concentration, feeding sample and can reach adsorption balance usually in 2-30 min. The solid-phase micro-extraction method has the advantages of simple device, convenient operation, fast analysis, high-sensitivity and good repetition. This method enables us to choose a fibre plug of proper polarity with the right electrode according to the different property of the extraction matter so that it can expel or reduce the disturbance of other experiment constituents, realizing the long desired selective extraction. The extracting sensitivity for many constituents of solid-phase micro-extraction method surpasses the traditional methods and it is now widely used in many study areas and with good effects.

Rabdosia rubescens (Hemsl.) Hara belongs to the plant of sweet tea genera, labial plant family. It is mainly distributed on the slopes of the hills, road-sides, in the forest and over the grasslands along the Huang He river and its southern part of China. The Chinese herbs are a mostly available in Henan, Anhui and Jiangsu Provinces, China. The whole plant of *Rabdosia rubescens* (Hemsl.) Hara can be used as medicines with good medical effects. The related literatures[2,3] show that *Rabdosia rubescens* (Hemsl.) Hara has the effects of relieving fever, detoxication, antibacteria, diminishing inflammation, detumescence, acesodyne, antitumour, *etc.* It is used clinically in treating the diseases like chronic and acute throat inflammation, tonsillitis, *etc.* *Rabdosia rubescens* (Hemsl.) has a series of complicated chemical constituents, from the constituents of single terpene, diterpene, to triterpene. Oridonin is a antitumour constituent extracted from *Rabdosia rubescens* (Hemsl.). The experiment shows[4,5] that the oridonin can be regarded as an antitumor drug. It is used to cure liver cancer, esophagus cancer and pancreas cancer by clinically and with good medical effects.

Rabdosia rubescens (Hemsl.) has become a widely-used herbal medicine in China with its vast resources, excellent pharmacodynamic activity and huge development prospect. As for the study on the volatile chemical constituents in *Rabdosia rubescens* (Hemsl.), to our best of knowledge, there is no reports on the use of solid-phase micro-extraction to extract its volatile constituents in *Rabdosia rubescens* (Hemsl.). This paper report solid-phase micro-extraction method to extract the chemical constituents of volatile oil from the whole plant of *Rabdosia rubescens* (Hemsl.) and uses gas chromatography-mass spectrometry to determine the chemical constituents in the volatile oil. The method of area normalization is used to determine the relative percentage content of chemical constituents of volatile oil. This experiment provides the reference for the use of solid-phase micro-extraction method in this study area and the further development of the volatile constituents in *Rabdosia rubescens* (Hemsl.).

EXPERIMENTAL

Varian CP3800/1200L gas chromatography-mass spectrometry series instrument (American Varian Corporation); chromatographic column: HP-5 (30 m × 0.25 mm,

0.25 μm) elastic quartz capillary-tube column; manual solid-phase micro-extraction device (American Supelco Company), the extraction fibre plug is 75 μm CAR/PDMS, 100 μm PDMS, 50/30 μm CAR/DVB/PDMS and 65 μm PDMS/DVB (American Supelco Company). The whole plant of *Rabdosia rubescens* (Hemsl.) was collected in the TaiHang mountain area of JiYuan, Henan Province, China, in Oct., 2008 and was identified by professor of botany Lu-Huan Lou of Zhejiang Forestry University. The samples of this plant are mixed and washed clean and then made into powder for further use.

Extraction of the volatile oil: Weigh 1 g of the dried and powdered the whole plant of *Rabdosia rubescens* (Hemsl.) and put it in the 15 mL sample bottle for solid-phase micro-extraction only. Put the solid-phase micro-extraction fibre plug on the sample-feeding mouth of gas chromatography instrument for ageing and keep the ageing temperature at 270 ºC, the gas-loaded flow at 0.8 mL min^{-1} and the ageing time is 10 min. Put the 75 μm CAR/PDMS fibre plug into the sample bottle through the rubber washer of the bottle cap, keep the mixing speed at 1100 r min^{-1} and headspace extract for 0.5 h under the temperature of 70 ºC. Then take out the extraction plug from the sample bottle and put it immediately into sample-feeding mouth of gas chromatography instrument to get rid of sorption for 3 min under the temperature of 250 ºC. Meanwhile start up the instrument to collect the experiment data.

Analytical conditions of gas chromatography-mass spectrometry: Gas chromatographic conditions: HP-5 (30 m × 0.25 mm, 0. 25 mm) elastic quartz capillary-tube column (30 m × 0.25 mm, 0. 25 μm); temperature-increasing procedure: keep the initial temperature 45 ºC for 2 min and then increase the temperature to 250 ºC and keep it for 0.5 h at the rate of 10 ºC min^{-1}. The temperature at the sample-feeding entrance is 250 ºC and the temperature at the vaporizing room is 260 ºC. The loaded gas is high-purified helium and the flowing rate is 0.8 mL min^{-1} without any divided sample-feeding.

Mass-spectrometry conditions: The ion source of electron impact (EI): the ion energy is 70 eV, the temperature of the ion source is 200 ºC, the temperature of the transmission wire is 250 ºC, the voltage of the checking device is 350 V, the scanning range is m/z: 33-450, the scanning speed is 0.5 s, the retrieving data-base is Willey and NIST standard mass-spectrometry data-base.

RESULTS AND DISCUSSION

Using HP-5 elastic quartz capillary-tube column and taking out 0.2 mL volatile oil extracted by the method of headspace solid phase micro-extraction. we analyze and identify the volatile chemical constituents by gas chromatography-mass spectrometry instrument. The ration of the compounds is by the method of area normalization. The calculation of the individual peak area is by Hewlett-Packard software treatment system. The calculation of the relative percentage content in each volatile component is by the method of peak area normalization. We analyzed the volatile chemical component in the whole plant of *Rabdosia rubescens* (Hemsl.) according to the

above GC-MS conditions and achieved the total ion flow chart (Fig. 1). After scanning each peak value to the total ion flow chart by mass spectrometry and retrieving and contrasting[6] by mass spectrometry data system (NIST and Willey Standard Atlas Data Bank) and consult the relevant information[7] of MS combined with artificial analysis, we identified the volatile oil chemical constituents in the whole plant of *Rabdosia rubescens* (Hemsl.). The results are given in Table-1.

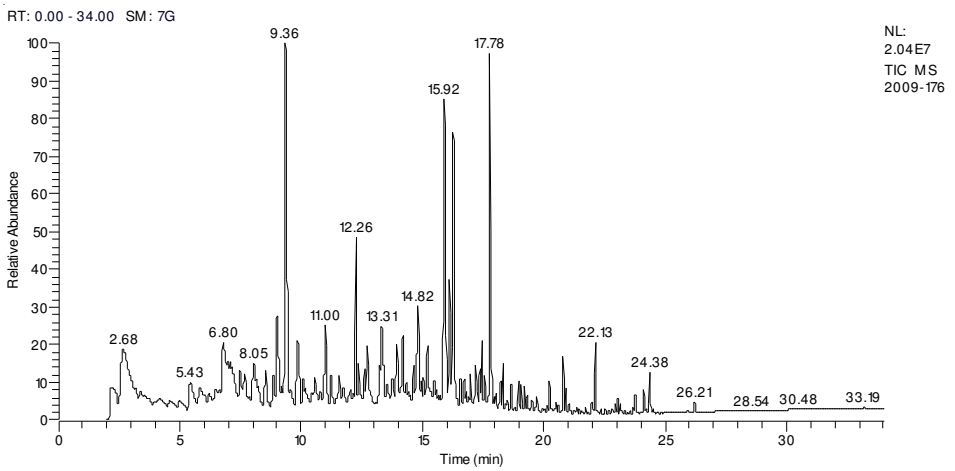

Fig. 1. GC-MS total ion current chromatogram of the volatile oil by solid-phase micro-extraction

TABLE-1
ANALYTICAL RESULTS OF CHEMICAL CONSTITUENTS OF VOLATILE OIL BY SPME

S. No.	RT (min)	Name of components	m.f.	m.w.	Area (%)
1	2.68	2-Hydroxy-2-methyl propanenitrile	C_4H_7ON	85.0	7.07
2	5.43	1R-α-Pinene	$C_{10}H_{16}$	136.0	2.14
3	6.80	Hexanal	$C_6H_{12}O$	100.0	3.01
4	6.98	*trans*-2-Methyl- cyclopentanol	$C_6H_{12}O$	100.0	0.80
5	7.07	Dodecane	$C_{12}H_{26}$	170.0	0.98
6	7.14	4-Ethyl octane	$C_{10}H_{22}$	142.0	0.78
7	7.48	3,6-Dimethyl decane	$C_{12}H_{26}$	170.0	0.94
8	7.67	Ethyl benzene	C_8H_{10}	106.0	0.94
9	8.05	1-Chloro-decane	$C_{10}H_{21}Cl$	176.5	1.61
10	8.54	(E)-Butanoic acid-3,7-dimethyl-2,6-octadienyl ester	$C_{14}H_{24}O_2$	224.0	1.33
11	8.86	1-Butanol	$C_4H_{10}O$	74.0	1.18
12	9.01	3-Benzoyl-3-(phenylmethyl)-2,4-(1*H*,3*H*)-quinolinedione	$C_{23}H_{17}O_3$	355.0	3.54
13	9.36	D-limonene	C_9H_{16}	124.0	11.92
14	9.85	2-Methyl-5-(1-methylethenyl)- (1α,2α,5α)-cyclohexanol	$C_{10}H_{18}O$	150.0	2.86
15	10.09	2-Hexenal	$C_6H_{10}O$	98.0	0.83
16	10.55	1,2-dipentylcyclopropene	$C_{13}H_{24}$	180.0	1.24
17	11.00	1,7,7-Trimethyl bicyclo[2.2.1]heptan-2-one	$C_{10}H_{16}O$	152.0	2.87
18	11.23	1,4-Diethyl benzene	$C_{10}H_{14}$	134.0	0.84
19	11.56	Hexadecane	$C_{16}H_{34}$	226.0	0.80

20	12.26	3,6-Dimethoxy-9-(2-phenylethynyl)- fluoren-9-ol	$C_{23}H_{18}O_3$	342.0	2.34
21	12.36	6-Methyl-5-hepten-2-one	$C_8H_{14}O$	126.0	0.74
22	12.62	2,6,10-Trimethyldodecane	$C_{15}H_{32}$	212.0	0.65
23	12.71	2,6,10,14-Tetramethyl heptadecane	$C_{21}H_{44}$	296.0	2.22
24	13.25	(Z)-3-Hexen-1-ol	$C_6H_{12}O$	100.0	0.96
25	13.31	Hexadecane	$C_{16}H_{34}$	226.0	1.26
26	13.37	Tetradecane	$C_{14}H_{30}$	198.0	1.46
27	13.96	4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-3-buten-2-one	$C_{13}H_{20}O$	192.0	1.55
28	14.17	1-(3-Methoxyphenyl)-ethanone	$C_9H_{10}O_2$	150.0	0.98
29	14.20	1-Octen-3-ol	$C_8H_{16}O$	128.0	1.25
30	14.63	4,5,6,7-tetrahydro-5-benzyl- imidazo[3,4-c]pyridin-6(3H)-on-1-propanoic acid ethyl ester	$C_{18}H_{21}O_3N_3$	327.0	0.46
31	14.82	1-Chlorohexadecane	$C_{16}H_{33}Cl$	260.5	2.16
32	15.16	4-(2,6,6-Trimethyl-2- cyclohexen-1-yl)-2-butanon	$C_{13}H_{20}O$	192.0	0.45
33	15.21	2,3,4-Trimethyl-2-cyclopenten-1-one	$C_8H_{12}O$	124.0	1.28
34	15.86	Di-epi-α-cedrene	$C_{15}H_{24}$	204.0	1.47
35	15.92	Cedrene	$C_{15}H_{24}$	204.0	4.37
36	15.96	1-(2,6,6-Trimethyl-1,3-cyclohexadi-1-yl)-2-buten-1-one	$C_{13}H_{18}O$	190.0	3.02
37	16.08	[1S-(1α,2α,4α)]-1-ethenyl-1-methyl-2,4-*bis*(1-methyl ethenyl) cyclohexane	$C_{15}H_{24}$	204.0	0.61
38	16.13	4,11,11-Trimethyl-8-methylyli-bicycld 7.2,0 undec-4-ene	$C_{15}H_{24}$	204.0	1.77
39	16.23	4-Ethenyl-4-methyl-3-(1-methylethenyl)-cyclohexene	$C_{15}H_{24}$	204.0	1.72
40	16.27	2,6-Dimethyl-6-(4-methyl-3-pentenyl)-bicycld 3,1,1-hept-2-ene	$C_{15}H_{24}$	204.0	3.97
41	16.32	2,4A,5,6,7,8-Hexahydro-3,5,5,9-tetramethyl-1*H*-benzocycloheptene	$C_{15}H_{24}$	204.0	1.61
42	16.57	1A,2,3,5,6,7,7A,7β-Octahydro-1,1,4,7-tetramethyl-1*H*-cycloprop E azulene	$C_{15}H_{24}$	204.0	0.39
43	16.72	1,3,5,7-Tetramethyltri cyclo[5.1.0.0(3,5)]octane-2,6-dione	$C_{12}H_{16}O_2$	192.0	0.56
44	16.97	2,6,10-Trimethylpentadecane	$C_{13}H_{28}$	184.0	0.44
45	17.19	*cis*, *cis*, *cis*-1,1,4,8-Tetramethyl-4,7,10-cycloundecatriene	$C_{15}H_{24}$	204.0	0.86
46	17.38	1,2,3,4,4A,5,6,8A-Octahydro-7-methyl-4- methylethenyl-1-(1-methyl)1-naphthalene	$C_{15}H_{24}$	204.0	0.72
47	17.46	(S)-α,α,4-Trimethyl-3-cyclohexene-1-methanol	$C_{10}H_{18}O$	154.0	1.02
48	17.56	5-Methyl-2-(1-methylethyl)-2-cyclohexen-1-one	$C_{10}H_{16}O$	152.0	0.52
49	17.78	(S)-1-Methyl-4-(5-methyl-1-methylene-4-hexenyl)-cyclohexene	$C_{15}H_{24}$	204.0	5.69
50	17.85	[2R-(2a,4aa,8aa)]-4a,8-Dimethyl-2-(1-methylethenyl)-1,2,3,4,4a,5,6, 8a-octahydronaphthalene	$C_{15}H_{24}$	204.0	0.70
51	18.18	5-Hexyl-2,3-dihydro-1*H*-indene	$C_{15}H_{22}$	202.0	0.41
52	18.24	5,6,7,7A-Tetrahydro-4,4,7A-trimethyl.(R)-benzofuranone	$C_{11}H_{16}O_2$	180.0	0.41
53	18.32	4-Methyl-1-(1,5-dimethyl-4-hexenyl) benzene	$C_{15}H_{24}$	204.0	0.60
54	18.66	6,6-Dimethyl- 2-methanol bicyclo[3.1.1]hept-2-ene	$C_{10}H_{16}O$	152.0	0.65
55	20.23	(E)- 4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	$C_{13}H_{20}O$	192.0	0.57
56	20.80	1,2,3,4,5,6,7,8-Octahydro-α,α,3,8-tetramethyl-5-azulenemethanol	$C_{15}H_{26}O$	222.0	0.81
57	22.13	Tricyclo-3,3,1,13,7-decane-2-carboxylic acid-4,10-dihydroxy methyl ester	$C_{12}H_{18}O_4$	226.0	1.13
58	24.38	1,1'-(1,1,2,2-tetramethyl-1,2-ethanediyl)bis-benzene	$C_{18}H_{22}$	238.0	0.81

Selection of adsorption temperature and time: Change of the adsorption temperature and time influence the peak area value, but they don't influence the amount of chromatographic peak. Therefore, we can optimize the solid-phase micro-extraction operating conditions by using the total chromatographic peak area as the parameter. Usually, increasing the temperature of the test sample can raise the concen-

tration of the headspace volatile compounds which is beneficial to the extraction. But when the temperature is raised to a certain degree, the adsorption and desorption reached to a dynamic balance and at this time, the adsorption amount of fibre plug is the largest. If the temperature is increased continually, the accelerating of the molecule thermal motion reduce the coefficient of the adsorption balance and thus possibly decrease the adsorption amount[8]. The experiment result shows that when the adsorption temperature maintained at 70 ºC, the adsorption amount has reached balance, so 70 ºC is the best extraction temperature. With the other conditions unchanged, we compare the different adsorption time of 20, 30 and 40 min and the result shows that the longer the adsorption time, the bigger the total peak area. When the adsorption time reached 30 min, the adsorption and desorption reached balance and its total peak area didn't change, so 30 min is the best adsorption time.

Selection of the desorption time: After the fibre plug adsorb the samples, put it into the GC sample-feeding mouth to deadsorb. If the desorption time is too short or the desorption temperature is too low, it will lead to incomplete desorption and the fibre coat will have memory effect, so the extraction error will result in next time. But the desorption time shouldn't be too long either, because the over-long desorption time will lead to the error in chromatographic peak amount and retaining time. It is concluded from testing that this experiment can achieve complete desorption under the temperature of 250 ºC for 3 min.

When carrying out solid-phase micro-extraction sample-taking, we chose the three extraction fibre plug by screening method, including the extraction fibre plug of 100 μm PDMS, 50/30 μm CAR/DVB /PDMS, 65 μm PDMS/DVB and 75 μm CAR/PDMS. The experiment shows that the 75 μm CAR/PDMS has the best extraction effect, which can effectively adsorb the volatile constituents in *Rabdosia rubescens* (Hemsl.) and can be suitable for the analysis and determining of its constituents. In this experiment, the extraction fibre plug of 75 μm CAR/PDMS is used.

It can be seen from Table-1 that by using solid-phase micro-extraction to extract the volatile constituents in the whole plant of *Rabdosia rubescens* (Hemsl.), 58 constituents were isolated, which takes up 98.27 % of the total peak area identified. Its main chemical constituents are hydrocarbon, alcoholate and ketone. The relatively high content in the whole plant of *Rabdosia rubescens* (Hemsl.) is D-limonene (11.92 %); 2-hydroxy-2-methyl propanenitrile (7.07 %); (S)-1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-cyclohexene (5.69 %); cedrene (4.37 %); 2,6-dimethyl-6-(4-methyl- 3-pentenyl)-bicycld 3,1,1-hept-2-ene (3.97 %); 3-benzoyl-3-(phenylmethyl)-2,4(1*H*,3*H*)-quinolinedione (3.54 %); 1-(2,6,6- trimethyl-1,3-cyclohexadi-1-yl)-2-buten-1-one (3.02 %) and hexanal (3.01 %).

It is already reported[7] that the using of steam distillation method to extract the volatile oil in *Rabdosia rubescens* (Hemsl.) and GC-MS method to separate and identify its chemical constituents of volatile oil. Comparing with the traditional steam distillation method and solid-phase micro-extraction method, it is noted that they nearly got the same type of chemical constituents of the volatile oil, but the

traditional steam distillation method needs a large amount of samples (100-1000 g) and it takes about 5-8 h; while the solid-phase micro-extraction method greatly reduces the pre-treatment procedure and thus increases the analytical speed and sensitivity. It has the advantages of short operating time, small sample amount and no need of extracting solution and it is suitable for the analysis of the volatile matter and has good reproducibility. At present, solid-phase micro-extraction method has been widely used in medicine, environment protection, biological and food analysis[10-12] and the area of volatile oil in traditional Chinese medicine[13,14].

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