Rapid determination of volatile composition from *Polygala furcata* Royle by MAE–HS-SPME followed by GC–MS

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Abstract Based on microwave-assisted extraction (MAE) and headspace solid-phase microextraction (HS-SPME) followed by gas chromatography mass spectrometry (GC–MS) was developed for the fast determination of volatile chemical compositions of *Polygala furcata* Royle, the experimental parameters of the MAE–HS-SPME were optimized by orthogonal experimental design. The results indicated that the optimum condition of the determination of the volatile compounds in *P. furcata* Royle, was achieved with the experimental parameters including microwave power of 400 W and irradiation time of 4 min, sample size 2.0 g. Under the optimal conditions, for the first time, 52 volatile compounds were separated and identified from the fresh plants of *P. furcata* Royle. The highest content component of the 52 compounds was 2-hydroxybenzoic acid methyl ester (21.65%). The relative standard deviation values <7% showed that the method of MAE–HS-SPME followed by GC–MS has good precision. The experimental results demonstrated that it is a simple, time-saving and solvent-free method, and it is a potential analytic tool for the determination of the volatile compounds of the vegetations materials and other materials.

Keywords *Polygala furcata* Royle · Volatile constituents · MAE–HS-SPME · 2-Hydroxybenzoic acid methyl ester

Introduction

Polygala furcata Royle, belongs to the family Polygalaceae and *Polygala*, the total amount of the Polygalaceae in the world is about 13–17 genera and about 1,000 species, widely distributed in worldwide, especially in tropical and subtropical regions of both hemispheres, and there are 5 genera and 53 species (24 endemic) in China. Chinese genera of economic importance include *Polygala* (medicinal), *Salomonina* (medicinal), *Securidaca* (medicinal), and *Xanthophyllum* (fine wood) [1], and the *Polygala* about 500 species, 44 species (21 endemic) in China. *P. furcata* Royle is one of the species, as a variation of *Polygala* is mainly wild and native to China mainly in Duyun of Guizhou province, Wenshan and Mengzi of Yunnan Province and Zhenkang of Guangxi Province. In addition, a small quantity of the plant distributed in India and Myanmar [2, 3]. Annual herbs, 5–15 cm tall, the flowering period of *P. furcata* Royle begins in late October to early November with primrose yellow color and four petals.

In recent years, studies on focused mainly on *P. tenuifolia* Willd, *P. tatarinowii* Regel, *P. sibirica* Linn and *P. japonica* Houtt, the literature reported that various types of activity constituents were separated from *P. tenuifolia* Willd, *P. tatarinowii* Regel, *P. sibirica* Linn and *P. japonica* Houtt, they are mainly saponins, ketones and sugar esters, and so on [4–9]. However, so far, the essential oils components in *P. furcata* Royle have not received much attention. Therefore, in the current work, for the first time, microwave-assisted extraction (MAE) [10–16] followed by HS-SPME [17–30] and GC–MS was developed for the fast

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determination of volatile chemical compositions in the fresh aerial parts of *P. furcata* Royle. The experimental parameters were studied by orthogonal array design, and the precision of the method was also investigated.

Experimental

Plant material, SPME fibers and microwave oven

Fresh aerial parts of *P. furcata* Royle were collected at full flowering in Duiyun City in Guizhou (Southern China) in October 2008. They were identified by associate professor Zhiyou Guo (Department of Life Sciences, Qiannan Normal University for Nationalities, city of Duiyun in Guizhou, China). After fresh aerial parts of the plants were cut in small pieces, the plants samples of *P. furcata* Royle were extracted using the MAE–HS–SPME. The fiber coatings 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) were purchased from Supelco (Bellefonte, USA). The microwave oven with a maximum delivered power of 700 W was purchased from Qingdao Haier Microwave Products Co., Ltd. (Qingdao, China).

The procedure of MAE–HS–SPME

The homemade MAE–HS–SPME apparatus is used in this study; 1.50 g of vegetation samples were cut into small pieces and followed was introduced into a 25 mL glass bottle. To absorb enough microwave energy, 1.50 g of water was used to moisten the vegetation samples. Then, the bottle was put into the microwave oven where the vegetation samples were heated at the power of 200, 400 and 700 W for 2–6 min, respectively, and a condenser with a continuous flow of freezing water was used to condense the vapors, so that the water could be used in the extraction process repeatedly. Then, in the process of heating, the volatile compounds were extracted from *P. furcata* Royle by fiber coating. All of the volatile compounds absorbed on the SPME fiber were desorbed at GC injector (250 °C for 3 min), and then analyzed by GC–MS, respectively.

GC–MS analysis

HP 6890N GC system, coupled with HP 5973 MSD quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) were used to analyze volatile compounds. The extracted compounds were separated on an HP-5MS capillary column (30 m length $\times$ 0.25 mm I.D., 0.25- μm film thickness). Splitless injection was employed. The column oven temperature was programmed to rise from an initial temperature of 50 °C (3 min) to 120 °C at 3 °C min^{-1} , then to 210 °C at 4 °C min^{-1} , at which the column was maintained

Table 1 The results of orthogonal tests $L_9(3^4)$

No.	Microwave power (W)	Irradiation time (min)	Samples size (g)	Sum of peak area $\times 10^7$
1	200	2.0	1.0	8.10
2	200	4.0	1.5	11.95
3	200	6.0	2.0	12.53
4	400	2.0	1.5	14.98
5	400	4.0	2.0	18.53
6	400	6.0	1.0	15.25
7	700	2.0	2.0	11.32
8	700	4.0	1.0	12.25
9	700	6.0	1.5	12.80
K1	32.5800	34.4000	35.6000	
K2	48.7600	42.7300	39.7300	
K3	36.3700	40.5800	42.3800	
R	16.1800	8.3300	6.7800	

Table 2 The results of variance analysis

Error sources	SS	F	S	F	P
A	47.7410	2	23.8705	173.6036	<0.01
B	12.4671	2	6.2335	45.3345	<0.05
C	7.7831	2	3.8915	28.3018	<0.05
Error	0.1375				

$$F_{0.01}(2,2) = 99.00, F_{0.05}(2,2) = 19.00$$

for 10 min. The injection temperature and ion-source temperature were 250 and 230 °C, respectively. Helium (99.999%) was used as the carrier gas, with a flow rate of 1 mL min^{-1} . The ionizing energy was 70 eV. A scan time of 0.5 s and a mass range of m/z 33–450. The percentages of compounds were calculated by the area normalization method without considering response factors. The components of the oil were identified by comparison of their mass spectra with those of the spectrometer database using the NIST147 mass spectral database. The identification was confirmed by comparison of Kovats indices (KI) [19, 20, 22, 31] with those reported in the literature [24, 31–36]. The KI was set-up a standard mixture of C_8 – C_{26} compounds under chromatographic conditions, consistent with those of the chromatographic conditions of the samples analyzed.

The precision of MAE–HS–SPME

The precision of the MAE–HS–SPME was studied with six replicate analyses of the essential oils in *P. furcata* Royle under the optimum conditions. The precision was expressed as the relative standard deviation (RSD%) of the peak areas. The peak areas of the volatile compounds in the vegetation obtained by replicate analyses were used for the calculation of their RSD values.

Table 3 Results of the MAE–HS–SPME analysis of herbs of *Polygala furcata* Royle

No	Retention time (min)	Compounds	Kovats index	Relative content (%)	Group	RSD (%)
1	6.38	Phenol	980	0.36	PDC	4.8
2	6.47	6-Methyl-5-hepten-2-one	984	0.53	ACC	2.1
3	7.85	Eucalyptol	1,025	0.35	TODC	1.8
4	8.35	Benzeneacetaldehyde	1,038	0.42	PDC	4.9
5	10.75	Nonanal	1,102	0.53	ACC	5.6
6	10.99	Phenylethyl alcohol	1,107	0.55	PDC	3.2
7	14.14	Benzoic acid, 2-hydroxy-, methyl ester	1,182	21.65	FAE	2.7
8	14.47	Estragole	1,190	0.71	TODC	2.8
9	14.99	Decanal	1,202	0.37	ACC	2.6
10	19.19	2-Methoxy-4-vinylphenol	1,300	0.32	PDC	3.4
11	20.95	Eugenol	1,342	0.44	TODC	4.5
12	23.25	Tetradecane	1,400	0.69	AA	6.2
13	25.05	(E)-6,10-Dimethyl-5,9-undecadien-2-one	1,440	1.64	TODC	5.3
14	25.67	Acenaphthene	1,454	0.64	OC	2.6
15	26.09	(E)-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	1,464	0.35	TODC	4.3
16	27.21	Dibenzofuran	1,490	0.83	PDC	3.8
17	27.53	Pentadecane	1,500	2.23	AA	6.1
18	27.89	1-Methyl-3-[(2-methylpropyl)thio]-benzene	1,505	0.86	PDC	4.7
19	30.57	Fluorene	1,557	0.74	OC	5.4
20	30.96	2-Methyl-pentadecane	1,564	0.88	AA	3.5
21	31.27	8-Hexyl-pentadecane	1,571	0.59	AA	2.9
22	32.02	Cedrol	1,585	1.79	ACC	1.6
23	32.73	Hexadecane	1,600	5.27	AA	3.3
24	34.29	1,1'-Biphenyl, 2,2',5,5'-tetramethyl-	1,638	0.62	PDC	2.4
25	34.70	5-propyl-tridecane	1,649	4.78	AA	1.4
26	35.03	1,1-Bis (p-tolyl)ethane	1,657	2.82	PDC	2.7
27	35.33	2-Methyl-hexadecane	1,665	1.44	AA	3.2
28	36.25	(E)- 1,2,3-Trimethyl-4-propenyl-naphthalene	1,688	1.75	OC	4.8
29	36.40	Cyclopentadecane	1,692	0.58	AA	5.7
30	36.68	Heptadecane	1,700	4.53	AA	6.5
31	36.82	2,6,10,14-Tetramethyl-pentadecane	1,703	7.78	AA	2.3
32	37.58	(E)-2-Tetradecene	1,726	1.24	AA	1.5
33	38.16	Anthracene	1,744	3.17	OC	5.4
34	38.43	Octyl-cyclohexane	1,753	1.41	AA	4.4
35	38.56	4-Methyl-heptadecane	1,757	0.83	AA	3.5
36	38.78	2-Methyl-heptadecane	1,763	0.92	AA	2.8
37	38.99	3-Methyl-heptadecane	1,770	0.57	AA	4.6
38	39.28	1,2,3,4-Tetrahydro-7-methoxy-3-methyl-1-oxo-9H-carbazol	1,779	1.74	OC	6.5
39	39.92	Octadecane	1,800	1.20	AA	5.1
40	40.11	2,6,10,14-tetramethyl-Hexadecane	1,805	3.18	AA	3.2
41	41.05	6,10,14-trimethyl-2-Pentadecanone	1,838	1.00	ACC	4.5
42	41.38	Diisobutyl phthalate	1,849	4.07	FAE	6.3
43	42.17	Z-8-Hexadecene	1,877	0.46	AA	2.2
44	42.75	Nonadecane	1,900	1.11	AA	6.7
45	43.92	Di-n-butylphthalate	1,942	0.50	FAE	5.8
46	45.35	Eicosane	2,000	1.34	AA	6.9

Table 3 continued

No	Retention time (min)	Compounds	Kovats index	Relative content (%)	Group	RSD (%)
47	45.93	Isopropyl palmitate	2,021	0.40	FAE	3.7
48	47.31	E-15-Heptadecenal	2,078	0.28	ACC	5.2
49	47.75	Heneicosane	2,100	1.58	AA	4.3
50	50.01	Docosane	2,200	1.49	AA	2.1
51	52.16	2,6,10,14-tetramethyl-Heptadecane	2,295	2.75	AA	1.8
52	54.64	Tetracosane	2,400	1.01	AA	6.6
		Alkanes and alkenes (AA)		47.86		
		Fatty acids and esters (FAE)		26.62		
		Alcohols and carbonyl compounds (ACC)		4.50		
		Phenyl derivatives and related compounds (PDC)		6.80		
		Terpenes oxygen derivatives compounds (TODC)		3.49		
		Other compounds (OC)		8.04		
		Total identified (%)		97.29		

Results and discussion

Optimization of the MAE–HS–SPME parameters

Optimization of the experimental conditions represents a critical step in the development of an MAE–HS–SPME method because various parameters will potentially affect the extraction process. In fact, the sample size, the microwave power and irradiation time are generally considered to be the most important factors. Optimization of the method can be carried out step-by-step or using an experimental design. Table 1 shows the results of MAE–HS–SPME extractions of *P. furcata* Royle carried out under different conditions according to the Taguchi experimental design [37]. The selected factors were examined using a three-level orthogonal array design with an L_9 (3^4) matrix (Table 1). The peak area sum of *P. furcata* Royle obtained under orthogonal conditions are also shown in Table 1, the sum of peak area was 8.10×10^7 – 18.53×10^7 .

The total peak area obtained at different sample size, microwave power and irradiation time are shown in Table 1, and the optimal conditions that the sum of the peak area achieves to the high level is at the microwave power of 400 W and irradiation time of 4 min, sample size of 2.0 g, respectively. Evidently, the best extraction efficiency was achieved at 400 W for 4 min and 2.0 g sample. The results of variance analysis showed that there are three significant factors in Table 2. Table 2 indicates that the three factors all have a significant impact on volatile compounds extracted in *P. furcata* Royle, and the most important is the microwave power, followed by irradiation time and sample size. Therefore, we should pay special attention to the control of microwave power in the

experiment that is why, based on the experimental results above, the optimal MAE–HS–SPME conditions are microwave power of 400 W and irradiation time of 4 min, sample size of 2.0 g.

Precision of MAE–HS–SPME

To obtain the precision of the method, six replicate analyses of the volatile compounds in *P. furcata* Royle were performed by MAE–HS–SPME at the optimum conditions. The RSD values were calculated by the peak areas obtained by replicate analyses. As shown in Table 3, the calculated RSD values <7% showed that MAE–HS–SPME followed by GC–MS had an accepted precision.

Analysis of the volatile compounds in *P. furcata* Royle

Under the optimal MAE–HS–SPME conditions, the volatile compounds in *P. furcata* Royle were extracted and concentrated by MAE–HS–SPME, followed by analyzing with GC–MS. The total-ion chromatogram of volatile compounds in *P. furcata* Royle was shown in Fig. 1. Fifty-two volatile compounds were identified by mass spectra library and KIs, and listed in Table 3. The total peak area of the 52 identified compounds was more than 97% of the total chromatographic area. As shown in Table 3, the 52 compounds were mainly ester and alkane compounds. They were all identified for the first time and mainly included 2-hydroxybenzoic acid methyl ester (21.65%), pentadecane (2.23%), hexadecane (5.27%), 5-propyl-tridecane (4.78%), 2-methyl-hexadecane (1.44%), heptadecane (4.53%), 2,6,10, 14-tetramethyl-pentadecane (7.78%), octadecane (1.20%), 2,6,10, 14-tetramethyl-hexadecane (3.18%), diisobutyl phthalate

Fig. 1 The total particle flow diagram of chemical constituents of volatile oil from *P. furcata* Royle

(4.07%), eicosane (1.34%), heneicosane (1.58%), docosane (1.49%), 2,6,10,14-tetramethyl-heptadecane (2.75%) and tetracosane (1.01%) etc. In addition, there were several kinds of low concentration terpenes oxygen derivatives, alcohols and carbonyl compounds in the volatile compounds. When compared with the conventional extraction and concentration methods, most of which involve several tedious and time-consuming sample preparation procedures, MAE–HS–SPME is indeed a simple, time-saving and solvent-free, and so on [20, 21, 38, 39].

Conclusions

In this work, MAE–HS–SPME followed by GC–MS was successfully performed for a fast determination of volatile compounds in *P. furcata* Royle. Using the proposed method, 52 compounds were identified for the first time. A time effort of only 4 min without solvent was needed in the experiment. The experimental results indicated that MAE–HS–SPME followed by GC–MS was a simple, time-saving, solvent-free and powerful method for the determination of volatile compounds in plant materials. Therefore, it can be concluded that this is a potential analytic tool for the determination of the volatile compounds of the vegetations materials and other materials.

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