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Comparison of headspace solid-phase microextraction with conventional extraction for the analysis of the volatile components in *Melia azedarach*

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ABSTRACT

The volatile compositions of *Melia azedarach* were studied by headspace solid-phase microextraction (HS-SPME). The result was compared with that obtained by soxhlet extraction (SE) and ultrasonic extraction (UAE). 79 compounds were identified in this study, among which 64 compounds were first reported. The experimental parameters including fiber type (PDMS, PDMS-DVB and CAR-PDMS), desorption time, extraction temperature and time were investigated. 37 compounds were obtained by HS-SPME, including curcumene (33.25%), α-cadinol (11.16%), α-muurolene (8.72%), copaene (5.04%), β-bisabolene (3.41%), and α-selinene (2.97%). The result suggested that the HS-SPME method is a powerful analytic tool and complementary to traditional methods for the determination of the volatile compounds in Chinese herbs.

1. Introduction

Belonging to the family Meliaceae, *Melia azedarach* (China berry) is of a large tree naturalized in many tropical and subtropical regions of Asia and Africa [1,2]. In China, it is widely distributed in Sichuan, Guangxi, Yunnan province and so on. In the Chinese Pharmacopoeia, *M. azedarach* is described as an insect repellent for threadworm and as a therapeutic medicine for acariasis [3]. Biological studies have shown that it possesses an array of biological activity such as antifeedant [4], cytotoxic [5,6], antifungal [7], antibacterial [8], antineoplastic [9], anti-inflammatory and analgesic [10]. The volatile components of *M. azedarach* also have Fusarium monilifome resistance [11]. Various active constituents, mainly sterols [12], limonoids [13,14], degraded limonoid [15] have been isolated from it in recent years. Compared to the studies on soluble constitutions, the volatile components of *M. azedarach* have received little attention. Only several volatile components have been reported from an analysis on a steam distillation (SD) extraction of *M. azedarach* using gas chromatography–mass spectrometry (GC–MS) [16]. Steam distillation is a conventional, time-consuming and laborious extracting method for volatile components from medicinal plants, which requires large amounts of sample. In addition, high extraction temperature would result in loss of some volatile components [17]. Other conventional extracting methods, such as ultrasonic and soxhlet extraction, are not only with low extracting efficiency, but also leading to toxic organic solvent residue in the system [18,19].

Sample pretreatment procedure is very important for GC–MS technique. Most of the time, it deals with the organic solvent, which may cause environmental pollution and be hazardous to health. Therefore, developing environmentally benign pretreatment procedures are highly desirable. Headspace solid phase microextraction (HS-SPME) that is solvent-free, rapid and simple has been recognized as an effective extracting technique for analysis of the volatile components [20–23].

HS-SPME is performed by exposing a fiber coated with single or multiple polymers to the headspace of a sample matrix until equilibrium is reached between the sample matrix and a stationary phase coated on the fiber [20]. The amount of the analyte is affected by the fiber thickness and the distribution constant, and linearly proportional to its concentration in the sample matrix. With the development of fused silica fibers coated with different stationary phases, it has been widely used for extraction of volatile components in various matrices such as juices [24–28], fruits [29,30], and cosmetics [31].

In this study, we applied HS-SPME technique to collect the volatile components of *M. azedarach*. The experimental conditions,

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including fiber type (PDMS, PDMS-DVB and CAR-PDMS), desorption time, extracting temperature and time were optimized. The obtained volatile samples were analyzed by GC–MS. The total peak areas of all constitutions and peak areas of three major compounds—curcumene, α-selinene, and α-muurolene—were employed to estimate the efficiency of the HS-SPME extraction. The results were compared with those obtained by SE and UAE.

2. Experimental

2.1. Materials and reagents

The bark of *M. azedarach* was purchased from Changchun Xikang medical company in Changchun, China. Prior to use, the samples were grounded to certain particle size (60 mesh) and stored in tightly sealed plastic bags. The SPME manual holder and the fibers of polydimethylsiloxane (PDMS, 100 μm), polydimethylsiloxane-divinylbenzene (PDMS–DVB, 65 μm), and carboxen-polydimethylsiloxane (CAR-PDMS, 75 μm) were purchased from Supelco, USA. The n-alkane mixture, consisting of C_8–C_{30} straight-chain alkanes, was supplied by Supelco, USA.

2.2. HS-SPME extraction

The fibers were conditioned by inserting them into the GC injector port according to the manufacture instructions before first use: 0.5 h at 250 °C for PDMS and PDMS-DVB, 1 h at 300 °C for CAR-PDMS.

Sample powders (2.0 g) were quickly introduced into 20 ml headspace vials sealed with caps from Elite. The needle coated with different fibers was inserted into the vials allowing the fibers exposed to the headspace above the samples. After a period of extracting time at setting temperatures, the needle was removed from the headspace vials and directly inserted into the injection port of GC–MS. After a period of thermal desorption, the injected needle was removed and the GC–MS analysis procedure was initiated. Effects of different fiber types (PDMS, PDMS-DVB and CAR-PDMS), extracting temperature (50–90 °C), extraction time (20–60 min) and desorption time (2–6 min) were investigated.

2.3. Ultrasonic extracting procedure

M. azedarach powder (25 g) was placed into 200 ml of petroleum ether in a 250 ml cone-shaped flask. The mixture was ultrasonicated with a working frequency of 80 KHz for 0.5 h. Then the extracts were filtered and concentrated using a rotary vacuum evaporator. The concentrated extracts were deep frozen until being analyzed.

2.4. Soxhlet extraction procedure

The *M. azedarach* powder (10 g) wrapped in filter paper was put into soxhlet thimble and 200 ml of ethyl ether was added from the top. After an extraction of 20 h, the extracts were concentrated using a rotary vacuum evaporator at 40 °C and refrigerated until use.

2.5. GC–MS analysis

GC–MS analysis was performed on a Shimadzu GC–2010 gas chromatography instrument coupled to a Shimadzu 2010 mass spectrometer with a DB-5 capillary column (30 m × 0.25 mm i.d.; film thickness 0.25 μm). The instrument was calibrated by perfluorotributylamine every 24 h. The GC oven temperature was programmed as follows: from 50 to 280 °C at a rate of 5 °C/min and then held for 4 min for HS-SPME, 54 min for SE and UAE. High-purity nitrogen was used as the carrier gas at a flow rate of 1.24 ml/min. The split ratio was 1:20.

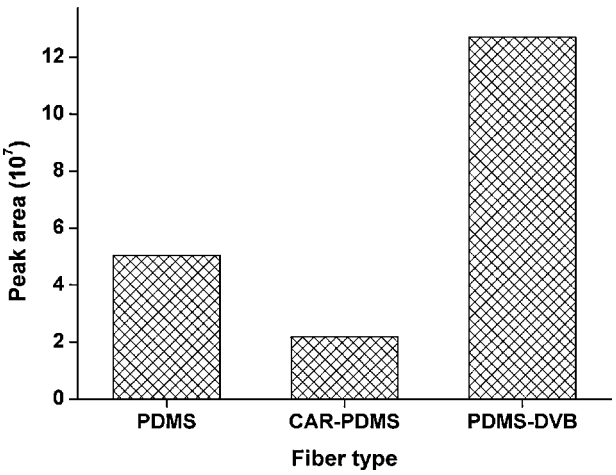

Fig. 1. The total peak areas of chromatography analysis for the samples extracted using different fibers.

The mass spectrometer was operated in electron-impact (EI) mode. The scan range was 30–500 amu. The scan rate was 0.5 s/scan. The temperature at ionization source and interface was 230 °C and 280 °C, respectively.

The compounds were identified by comparing the obtained spectra with standard ones from the National Institute of Standards and Technology (NIST05) and by the Kovàts retention indices calculated for each peak with reference to the normal alkanes C_8–C_{30} series. The relative amounts were calculated based on the peak areas.

3. Results and discussion

3.1. The optimization of SPME extraction

Our results showed that the samples obtained under different SPME extracting conditions had similar chromatograph spectra. The peak numbers were the same and only the relative peak areas varied with the extracting conditions. The peak area reflected the amounts of compounds which were introduced in the analysis. A more efficient extraction procedure is expected to provide a greater amount of samples for the following analysis. Therefore, the total peak area was used as a reference to evaluate the extracting efficiency [32,33].

Three different types of fibers (PDMS, PDMS–DVB, CAR-PDMS) were first tested for their extracting efficiency. The results showed that the PDMS-DVB fiber response was the most efficient (see Fig. 1). The total peak area of chromatography from PDMS and CAR-PDMS fibers was only 40% and 17% of that from PDMS-DVB, respectively. This suggested that the retention ability of the PDMS-DVB fiber for the volatile compounds in the medicinal plant is much stronger than that of the rest two. In other words, the PDMS-DVB fiber had higher extraction ability than the others. On the basis of the above results, the PDMS-DVB fiber was selected for the analysis of the volatile compounds in *M. azedarach*.

The extracting temperature and time were further optimized using the PDMS-DVB fiber. The temperature had contradictory effects on trapping the analytes. First, higher temperature could accelerate the diffusion of volatile analytes towards the fiber and shorten the equilibrium time. In contrary, as the absorption step was an exothermic process, the higher extraction temperature would lower the partition coefficients of the analytes on the fiber [31].

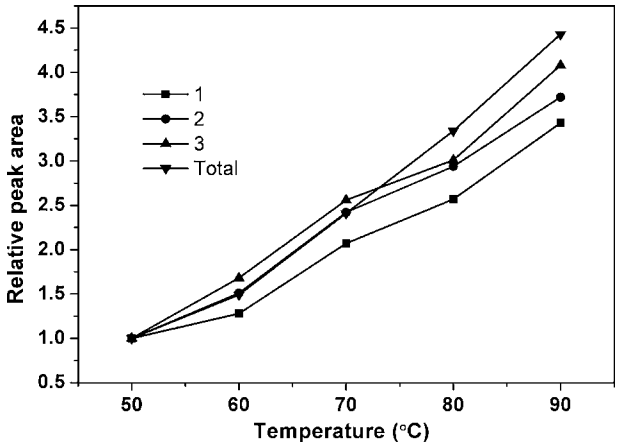

Fig. 2. The areas of the total (▼/19079300) and three major components—curcumene (■/4361536), α-selinene (●/685432), and α-muurolene (▲/1076433)—increased with the extracting temperatures. The values were normalized by setting the areas at 50 °C to be 1.

The extracting temperature (see Fig. 2) of 50, 60, 70, 80, and 90 °C was tested. As expected, the total peak area and the peak areas of the three compounds increased with rising of the temperature. In addition, all peaks observed at 50 °C still appeared at 90 °C, suggesting that these compounds were stable at this temperature. Further increase in temperature resulted in overlapping of some peaks. Considering the above results, the temperature of 90 °C was selected as the extracting temperature for the following experiments. The peak areas of three most abundant components, curcumene, α-selinene, and α-muurolene, had the same trend as the total peak area but with a relatively flat slope. In order to observe the temperature effect more clearly, Fig. 2 is plotted with revised data which are obtained from dividing the absolute peak areas by the initial peak areas (at 50 °C). The initial peak area of total area is much bigger than that of other three components, which makes some revised data of total area even smaller than those of others with some temperature values.

The extracting time (see Fig. 3) of 20, 30, 40, 50, and 60 min was examined for the extraction efficiency. In this study, we introduced the comparative values by setting the areas with 20 min extraction to be 1. The total peak area increased steadily with the extracting time. However, the peak areas for three main compounds reached the top at 50 min, which indicated that a longer extracting time

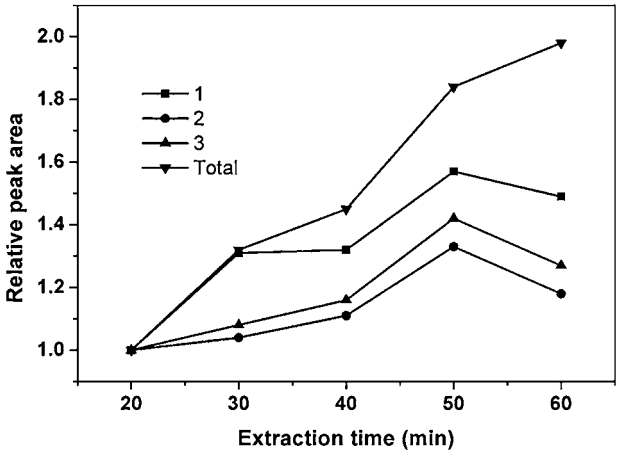

Fig. 3. The area changes of the total (▼/64249900) and three major components—curcumene (■/11385094), α-selinene (●/2093301), and α-muurolene (▲/3394347)—with the extracting times. The values were normalized by setting the areas with 20 min extraction to be 1.

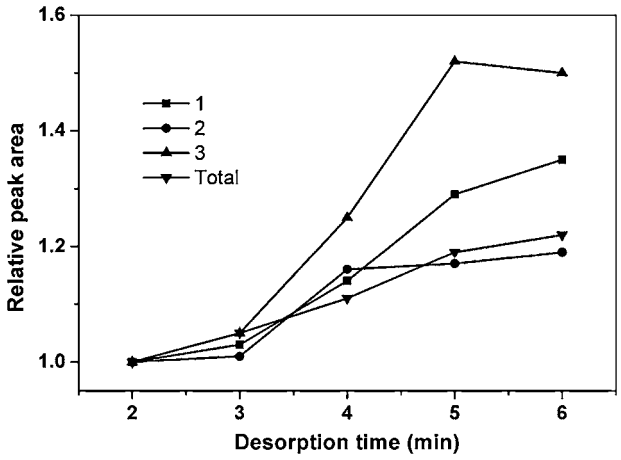

Fig. 4. The effect of desorption time on the peak areas of the chromatography analysis: the total (▼/106265300), curcumene (■/12560570), α-selinene (●/2161276), and α-muurolene (▲/2864074). The values were normalized by setting the areas with 2 min desorption to be 1.

was favorable for low volatile components but not necessary for the high volatile ones. In order to obtain more amounts of components, an extracting time of 60 min was selected in this study.

The thermal desorption is of an important factor for precision and sensitivity of gas chromatography. The repeatability of the measurements requires full desorption of the analytes. Fig. 4 showed that a 5 min desorption at 250 °C enabled to introduce the most analytes into the analysis column of gas chromatography.

Overall, among the factors, the extracting temperature had the most significant effect on the extracting efficiency. A rising of temperature from 50 to 90 °C resulted in the extractant amount increase more than 2 folds. Similarly, a longer extracting time (from 20 to 60 min) entrapped one-fold more analytes in total and about 20–50% more for the three most abundant compounds. The differences caused by the variation of desorption time were less than 60%.

3.2. Analysis of the volatile compounds

The total ion chromatograms of the samples using HS-SPME, SE, UAE methods were shown in Fig. 5 and the corresponding volatile compounds were listed in Table 1. The volatile compounds were identified by comparing the obtained mass spectra with standard ones from the National Institute of Standards and Technology (NIST05) mass spectral library and by the Kovàts retention indices calculated for each peak with reference to the normal alkanes C_8–C_{30} series. The relative amounts were calculated by the individual peak area relative to the total area.

There were 37, 22, 31 compounds identified by the HS-SPME, SE, UAE extracting methods, respectively. It was noteworthy that HS-SPME did not share compounds in common with the SE and UAE methods while SE shared 11 compounds in common with the UAE. Among the 79 compounds discovered in this study, 15 have been reported in previous literatures (Table 1). The other 64 compounds were first found in *M. azedarach*.

There are many functions among the 64 first found compounds. For example, octanoic acid is used as parenteral nutrition as well as in treating candidiasis (yeast infection) and bacterial infections. Pulegone which has a pleasant odor similar to pennyroyal, peppermint and camphor is used in flavoring agents and in aromatherapy. Caryophyllene oxide is well known as preservative in food, drugs and cosmetics. Stigmasterol may be useful in prevention of certain cancers, including ovarian, prostate, breast, and colon cancers.

Table 1
Volatile compounds in the bark of *M. azedarach* obtained by HS-SPME, SE and UAE.

No.	Retention time (min)	KI[b]	Compounds	Similarity	Molecular weight	RA%		
						SPME	SE	UAE
1	5.290	902	4-Isopropenyl-1-methyl-1-cyclohexene	89	136	0.85	–	–
2	6.333	941	(E)-2-Octenal	86	142	0.15	–	–
3	8.002	1006	Nonanal[a]	94	142	0.65	–	–
4	9.866	1071	(E)-2-Nonenal[a]	92	140	0.41	–	–
5	10.085	1078	Octanoic Acid	85	144	0.15	–	–
6	10.655	1098	1-Methylene-1 H-indene	93	128	0.56	–	–
7	11.228	1117	6-Ethyl-2-methyldecane	90	184	0.13	–	–
8	11.398	1123	Decanal	95	156	0.83	–	–
9	12.400	1157	Pulegone	92	152	0.47	–	–
10	13.224	1184	2,6-Dimethoxytoluene	84	152	1.93	–	–
11	13.872	1206	Estragole	88	148	0.70	–	–
12	13.938	1208	5-[(1Z)-1-Propenyl]-1,3-benzodioxole	92	162	2.01	–	–
13	14.065	1213	2-Methylpentanal	88	100	0.20		
14	14.320	1222	Tridecane	92	184	0.39	–	–
15	15.699	1269	α-Cubebene[a]	88	204	0.45	–	–
16	16.168	1285	4-Methyl-2-propyl-1-pentanol	88	144	0.19	–	–
17	16.353	1291	Ylangene	94	204	2.53	–	–
18	16.538	1298	Copaene[a]	94	204	5.04	–	–
19	17.195	1321	Tetradecane	95	198	1.23	–	–
20	17.565	1335	Trans-α-Bergamotene	94	204	2.23	–	–
21	17.779	1342	4,11,11-Trimethyl-8-methylenebicyclo [7.2.0]undec-4-ene	92	204	0.69	–	–
22	19.249	1395	α-Amorphene[a]	93	204	2.59	–	–
23	19.393	1401	Curcumene	95	202	33.25	–	–
24	19.651	1410	α-Selinene	92	204	2.97	–	–
25	19.875	1419	α-Muurolene[a]	81	204	8.72	–	–
26	20.093	1427	β-Bisabolene	95	204	3.41	–	–
27	20.263	1433	γ-Muurolene[a]	95	204	2.80	–	–
28	20.376	1437	Cadina-1(10),4-diene	91	204	4.64	–	–
29	21.501	1480	3,8-Dimethylundecane	90	184	0.53	–	–
30	22.047	1501	Caryophyllene oxide	89	220	1.37	–	–
31	22.353	1513	4-Methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-cycloheptane	87	204	1.10	–	–
32	22.412	1516	Hexadecane[a]	96	226	2.06	–	–
33	23.665	1564	α-Cadinol	87	222	11.16	–	–
34	24.804	1611	Pentadecane[a]	95	212	1.23	–	–
35	24.874	1614	Sulfurous acid, 2-ethylhexyl nonyl ester	91	320	1.38		
36	27.211	1712	2,6,10,15-Tetramethylheptadecane	90	296	0.56	–	–
37	27.957	1745	Hexahydrofarnesyl acetone	89	268	0.44	–	–
38	32.001	1931	9-Hexadecenoic acid[a]	94	254	–	–	0.74
39	32.323	1947	Phthalic acid, diisobutyl ester	95	278	–	–	1.29
40	32.556	1958	n-Hexadecanoic acid[a]	94	256	–	14.78	6.75
41	33.147	1987	Hexadecanoic acid, ethyl ester[a]	95	284	–	–	3.80
42	35.947	2165	Telfairic acid	90	280	–	7.98	11.10
43	36.087	2181	Elaidic acid	94	282	–	13.62	–
44	36.124	2185	(Z)-6-Octadecenoic acid	92	282	–	–	9.24
45	36.402	2215	Linoleic acid ethyl ester	90	308	–	–	3.33
46	36.467	2221	Octadecanoic acid[a]	95	284	–	4.47	–
47	36.521	2227	Ethyl oleate	88	310	–	–	4.65
48	36.631	2237	(E)-9-Octadecenoic acid ethyl ester	92	310	–	–	0.54
49	37.015	2275	Ethyl octadecanoate	94	312	–	–	1.07
50	39.462	2311	Octadecanal	95	268	–	0.96	0.18
51	39.611	2315	Oleic Acid[a]	87	282	–	0.39	0.13
52	40.043	2328	Eicosanoic acid	94	312	–	0.53	–
53	41.559	2373	(Z,Z)-3,13-Octadecadien-1-ol[a]	88	266	–	–	0.53
54	42.233	2392	(Z)-9-Tricosene	95	322	–	–	1.68
55	42.262	2393	1-Heneicosanol	95	312	–	3.22	–
56	43.417	2458	Docosanoic acid	90	340	–	1.61	–
57	43.862	2498	Ethyl nonadecanoate	88	326	–	–	1.29
58	45.488	2545	1-Tetracosanol	96	354	–	4.70	2.39
59	46.580	2579	Tetracosanoic acid	93	368	–	1.63	–
60	46.983	2592	Ethyl tetracosanoate	89	396	–	–	2.05
61	47.285	2602	Squalene	96	410	–	–	0.50
62	48.925	2643	1-Triacontanol	94	438	–	2.58	1.77
63	50.891	2692	Ethyl docosanoate	84	368	–	–	1.42
64	51.104	2698	Gorgosterol	84	426	–	1.04	–
65	51.367	2702	Dihydrobrassicasterol	88	400	–	2.43	–
66	53.758	2733	1-Hexacosanol	96	382	–	1.29	2.01
67	56.935	2774	24-Ethylidenecholesterol	87	412	–	1.61	0.88
68	57.229	2779	22-Dihydrobrassicasterol	86	400	–	2.19	2.54
69	58.018	2790	Stigmasterol	86	412	–	2.57	1.45
70	60.148	2825	24-Methylenecycloartanol	84	440	–	4.44	–
71	60.551	2833	Clionasterol	85	414	–	11.55	11.39

Table 1 (*Continued*)

No.	Retention time (min)	KI[b]	Compounds	Similarity	Molecular weight	RA%		
						SPME	SE	UAE
72	61.011	2842	24-Isopropyl-5,24-cholestadien-3β-ol	82	426	–	–	3.47
73	61.111	2844	Fucosterol	84	412	–	2.99	–
74	61.944	2861	Cholestenone	81	384	–	–	2.95
75	62.807	2878	22-Dihydrochondrillasterol	80	414	–	–	3.12
76	65.546	2926	24-Methylenecycloartan-3-one	85	438	–	–	5.31
77	66.200	2936	Stigmast-4-en-3-one	85	412	–	–	8.71
78	66.230	2937	Sitostenone	89	412	–	13.42	–
79	66.833	2946	Fucostenone	88	410	–	–	3.72

RA%: relative amount percent.

[a] Compounds previously reported.

[b] Experimentally determined Kovàts indices on the DB-5 column, relative to C_8–C_{30} hydrocarbons.

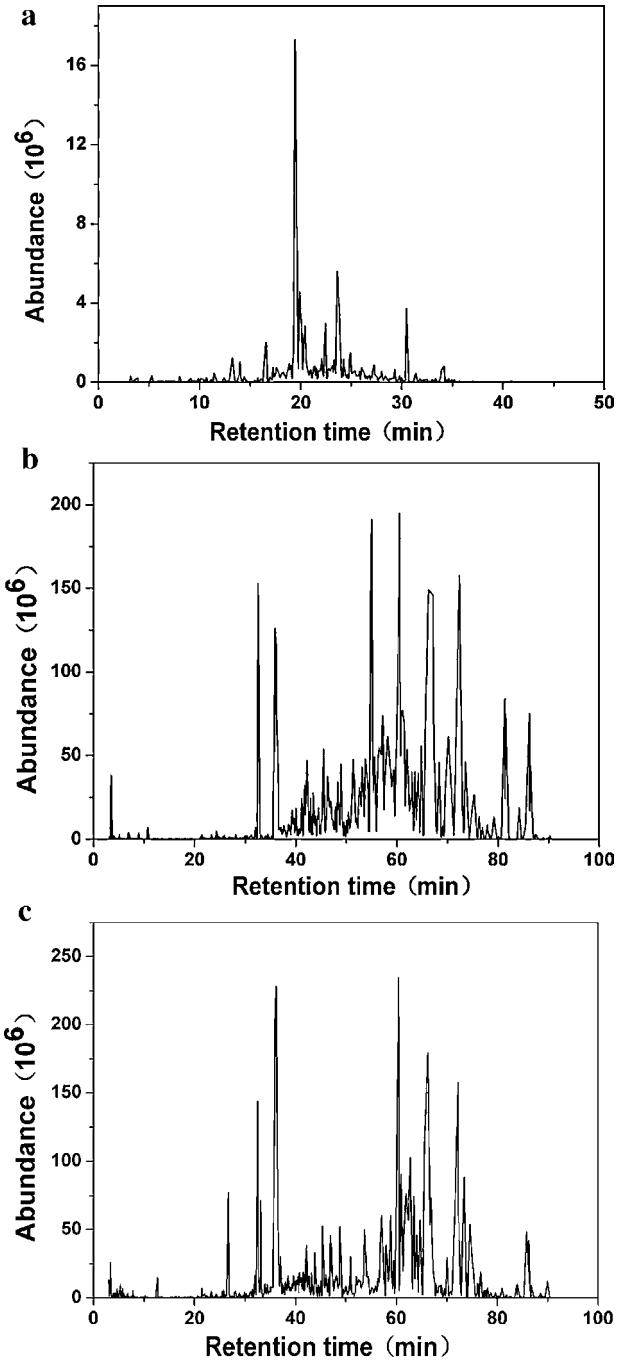

Fig. 5. Total ion chromatograms of volatile compounds of *M. azedarach* by (a) HS-SPME, (b) SE and (c) UAE.

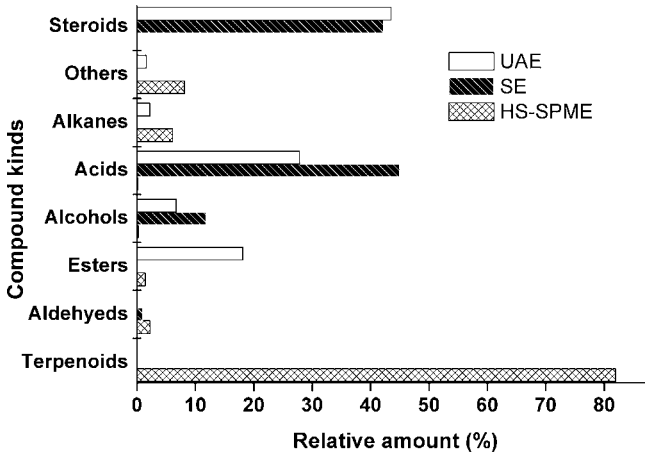

Fig. 6. Comparison of the chemical classes of compounds from *M. azedarach*.

Identification of 64 compounds should provide more information and expand the application of *M. azedarach*.

3.3. Comparison of extracting methods

Fig. 6 illustrated the chemical classes of compounds by the three methods. Terpenoids (81.95%) that did not appear in conventional methods were the dominant volatile chemicals obtained by HS-SPME. Alkanes (6.13%), aldehyeds (2.24%), and esters (1.38%) were the second to fourth most chemicals by HS-SPME. Others were less than 1%. The major compounds of the HS-SPME method included curcumene (33.25%), α-cadinol (11.16%), α-muurolene (8.72%), copaene (5.04%), cadina-1(10),4-diene (4.64%), β-bisabolene (3.41%), and α-selinene (2.97%).

The chemical types obtained by SE were mainly steroids (42.24%), acids (45.01%), with less amounts of alcohols (11.79%) and aldehydes (0.96%). The most abundant compounds were elaidic acid (13.62%), clionasterol (11.55%), sitostenone (13.42%), 1-tetracosanol (4.70%) and 24-methylenecycloartanol (4.44%).

The chemical classes obtained by UAE were mainly made up of steroids (43.54%) and acids (27.83%), with relatively small amounts of esters (18.15%) and alcohols (6.7%). Clionasterol (11.39%), telfairic acid (11.10%), (Z)-6-octadecenoic acid (9.24%), stigmast-4-en-3-one (8.71%) and 24-methylenecycloartan-3-one (5.31%) were the main compounds.

4. Conclusions

HS-SPME, SE and UAE methods were used to study the volatile compositions of *M. azedarach*. 79 compounds were identified and among them, 64 were first reported. The experimental parameters of the HS-SPME method including fiber type (PDMS, PDMS-DVB

and CAR-PDMS), extracting temperature, adsorption and desorption time were investigated. The optimized extracting conditions were as follows: 65 μm PDMS-DVB fiber, an extracting temperature of 90 °C, an extracting time of 60 min, and a desorption time of 5 min. More compounds were obtained by HS-SPME method than the previously reported methods. Comparing with the traditional extracting methods, it is more efficient on the determination of volatile components. As a simple, fast and solvent-free method, it is promising for analysis of the volatile compounds in Chinese herbs.

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