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Headspace solid-phase microextraction gas chromatography–mass spectrometry analysis of *Eupatorium odoratum* extract as an oviposition repellent

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ABSTRACT

Headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC–MS) analysis was used to study volatile and semi-volatile compounds emitted by the *Eupatorium odoratum* (*E. odoratum*) extract. Variables of HS-SPME such as the type of SPME fiber, extraction time and temperature, incubation time, desorption time and temperature have been optimized. Optimized conditions were obtained by the use of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber, 5 min/20 min incubation/extraction time at 65 °C, 5 min desorption time at 260 °C. Using three different polar chromatographic columns to get retention index and mass spectrometry data, 99 volatile and semi-volatile compounds were tentatively identified in the *E. odoratum* extract. This study has identified the promising source of *E. odoratum* oviposition repellent.

1. Introduction

Eupatorium odoratum, native to the neotropics from the eastern USA to northern Argentina, had become a major invasive plant to crops, plantations, savannas and natural forests in many parts of the world. In China, *E. odoratum* is called Feiji Cao, which is considered a serious menace to the ecosystem due to rapid propagation. *E. odoratum* was first recorded in the southern part of Yunnan Province in 1934. Since then, it spread extremely rapidly and can now be found in Yunnan, Guangdong, Guangxi, Hainan, Guizhou, Taiwan provinces, and Macao and Hong Kong cities, posing a threat to local diversity and economics.

The well-known drawbacks of conventional insecticides have aroused interest in the development of alternative strategies such as plant-derived compounds. Recently, people have given much attention to the repellent effect of bio-insecticides [1–8]. *E. odoratum* is ubiquitous, grows abundantly and is considered to be a serious menace to the ecosystem due to very rapid multiplication and hence the exploitation of the plant as bio-insecticide is a variable proposition. Bhattacharyya et al. found that steroidal allelochemics identified from *E. odoratum* can act as attractant/repellent of Aphis spiraecola Patch [9]. Ling et al. found the volatile oil from *E. odoratum* had a significant oviposition deterrent effect on the striped flea beetle (*Phyllotreta striolata*) and the diamondback moth (DBM) (*Plutella xylostella*) [10]. Our group's work showed that chloroform extract from *E. odoratum* had oviposition repellent effect against *Conopomorpha sinensis* Bradley and an 18% (v/v) microemulsion of the extract was made and patented [11].

Studies on the chemical identification and detection of extract of *E. odoratum*, however, are few. To the best of our knowledge, only some researchers have studied the essential oil of *E. odoratum* [10,12–17]. Chowdhury studied the essential oils obtained from the leaves of *E. odoratum* collected from Shillong Meghalaya, North-East India and found that the oil contained mainly caryophyllene oxide (18.34%) [12]. Ling et al. used GC–MS to analyze the volatile oil from *E. odoratum* and 33 components were identified [10]. Bamba et al. reported that 38 compounds were isolated from *E. odoratum* oil of which 36 were identified. Terpenes amounted to 88% of the oil, which was characterized by the presence of pregeijerene (14%) and geijerene (5%) [13]. Lamaty et al. studied the chemical composition of the essential oils that were obtained from the leaves of *E. odoratum* collected in Cameroon and Congo [14]. Inya-Agha et al. found some components including tannins, phenols, pinene, cadinene, camphor and limonene in the essential oil of *E. odoratum*, which had an antibacterial effect [15]. Yuan et al. identified 68 compounds from the essential oil of *E. odoratum* by carbon dioxide supercritical fluid extraction and GC–MS method [16].

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SPME is a rapid sampling technique that is well-adapted to GC analysis [18–20]. SPME has been applied to the analysis of volatile and nonvolatile compounds in gaseous, liquid and solid samples. SPME can eliminate the need for solvents or a complicated apparatus for concentrating volatile or nonvolatile compounds in different samples. SPME can extract analytes from a variety of matrices by partitioning from the sample into an immobilized stationary phase. HS-SPME is based on the equilibrium of analytes among three phases of the system including the fiber coating, the headspace, and the sample. HS-SPME has been successfully used as a technique for screening complex volatile mixtures [21–26].

The detection and identification of volatile and semi-volatile compounds emitted by chloroform extract of *E. odoratum* is of key importance and the first step to find the compound base for this kind of bio-repellent product. The aim of this study is to develop a simple and feasible method to capture and analyze the volatile and semi-volatile compounds in the extract of *E. odoratum* by using optimized HS-SPME-GC–MS. Moreover, the authentication and validation of the compounds were performed in three different polar columns to get the retention index. Then, using the developed HS-SPME-GC–MS method combined with retention index and pure standard comparison, 99 compounds were tentatively identified.

2. Experimental

2.1. Chemicals and supplies

The alkane mixtures containing C_8 to C_{20} and C_{21} to C_{40} straight-chain alkanes of 40 mg/L in hexane and toluene, respectively, were purchased from Fluka (Buchs, Switzerland). Standard compounds of α-pinene, α-humulene, trans-caryophyllene, caryophyllene oxide, dibutyl phthalate, palmitic acid ethyl ester, ethyl linoleate, phytol (97% mixture of isomers, 2/1: *trans*/*cis*) for identification were all purchased from Sigma–Aldrich (Oakville, Canada). Helium of purity 5.0 (Mississauga, Canada) was utilized as the GC carrier gas. The SPME fiber optimization step was carried out by testing commercially available silica SPME fibers obtained from Supelco (Bellefonte, PA, USA) and coated with the following seven polymers: polydimethylsiloxane (PDMS 100 μm, PDMS 30 μm and PDMS 7 μm), polydimethylsiloxane/divinylbenzene (PDMS/DVB 65 μm), carboxen/polydimethylsiloxane (CAR/PDMS 75 μm and CAR/PDMS 85 μm) and DVB/CAR/PDMS 50 μm/30 μm. All fibers were conditioned according to the manufacturer's recommendations prior to their first use. Clear glass crimp cap 10 mL SPME vials (22 mm × 46 mm) and caps equipped with polytetrafluoroethylene (PTFE)/silicone septa (20 mm) were purchased from MicroLiter Analytical Supplies Inc. (Suwanee, GA, USA) and Canadian Life Science (Peterborough, Ontario, Canada), respectively.

2.2. Headspace SPME GC–MS analysis

About 0.1 g of the chloroform extract of *E. odoratum* was placed in a clear glass crimp cap 10 mL SPME vial sealed with a screw-capped top containing a PTFE silicone septum and an aluminium foil. Then, the HS-SPME was performed by a CTC Combi PAL autosampler (Zwingen, Switzerland). The CombiPAL SPME autosampler with an agitator and SPME fiber conditioning station (needle heater) was utilized in conjunction with the GC–MS. The CombiPAL autosampler with SPME option was operated via the PAL Cycle Composer with Macro Editor software, version 1.4.0 using the associated Cycle Composer, Firmware 2.2.4, with Macro Editor software, PAL Cycle Composer Version 1.4.0. For the loading of retention index probes onto the SPME fiber, the 0.1 μL of the alkane retention index probe was manually sprayed into the coating of the fiber then the autosampler did the desorption (injection) step as sample. Unless specified otherwise, all of the optimization experiments were performed in triplicate. After each particular experiment, the fiber was placed in the needle heater for 20 min at the desorption temperature to avoid the carry-over effect.

A Saturn 3800 GC/2000 ITMS system (Varian, Sunnyvale, CA, USA) was used for the analyses. The GC–MS was equipped with a 1079 Programmable Temperature Vaporizing Injector and to obtain better sample transfer efficiency, an SPI liner (2.4 mm I.D. × 4.6 mm O.D. × 54 mm) was used. Three columns namely, Restek Corp. 30 m × 0.25 mm × 0.25 μm with non-polar dimethylpolysiloxane stationary phases (RTX-1MS), ULBON 30 m × 0.25 mm × 0.25 μm with mid-polar 7% cyanopropyl:7% phenylmethyl silicone (HR-1701) stationary phases and ULBON 30 m × 0.32 mm × 0.25 μm with polar polyethyleneglycol (HR-20m) stationary phases were used for the analysis. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The 1079 injector was set at 260 °C. The temperature program for the RTX-1MS column was from 80 °C to 260 °C at a rate of 2 °C/min; for the HR-1701 column from 60 °C (2 min) to 260 °C at a rate of 3 °C/min and for the HR-20m column from 40 °C (2 min) to 230 °C at a rate of 3 °C/min. MS detection was performed under electron impact (EI) ionization conditions at 70 eV by operating in the full-scan acquisition mode in the 35–450 m/z range and the instrumental parameters were: emission current of 10 μA; scan time of 0.39 s; automatic gain control of 25,000; trap temperature was 170 °C; manifold and transfer line temperatures were 50 °C and 260 °C, respectively. Signal acquisition and data processing were performed using the Saturn Workstation v. 5.51 (Varian).

2.3. Preparation of the chloroform extract of E. odoratum

Fresh stems and leaves from *E. odoratum* were collected in the farm of Hainan University in July 2005. Air-dried plant material was crushed and then sieved through a stainless steel sieve of 0.3 mm

Table 1
Comparison of the relative extraction efficiencies of various SPME fibers for the extraction of volatile and semi-volatile compounds in *E. odoratum* extract.

No.	RT (min)	Compound	A_n using different SPME fiber						
			DVB/CAR/PDMS	PDMS 100 μm	PDMS 30 μm	PDMS 7 μm	PDMS/DVB 65 μm	CAR/PDMS 75 μm	CAR/PDMS 85 μm
1	9.97	Naphthalene,decahydro-1,5-dimethyl	0.81	1.00	0.35	0.04	0.48	0.60	0.44
2	10.45	Naphthalene,decahydro-2,3-dimethyl	0.88	1.00	0.35	0.05	0.49	0.59	0.76
3	10.88	Naphthalene,decahydro-1,6-dimethyl	0.75	1.00	0.36	0.04	0.47	0.41	0.56
4	14.61	Cyprotene	0.91	1.00	0.42	0.05	0.56	1.00	0.95
5	19.97	α-Copaene	1.00	0.97	0.64	0.07	0.69	0.73	0.74
6	21.29	8,9-Didehydrocycloiso-longifolene	1.00	0.99	0.71	0.08	0.74	0.43	0.46
7	22.07	Bicycloopposit-4(15)-ene	1.00	1.00	0.60	0.09	0.78	0.50	0.76
8	27.28	Germacrene D	1.00	0.91	0.87	0.20	0.93	0.16	0.22
9	27.66	Calacorene	1.00	0.79	0.77	0.17	0.85	0.10	0.15
10	35.10	Cadalene	1.00	0.45	0.82	0.23	0.45	0.05	0.45
11	48.77	Dibutyl phthalate	0.25	0.05	1.00	0.15	0.13	0.05	0.05
12	51.91	Hexadecanoic acid, ethyl ester	0.44	0.18	1.00	0.19	0.11	0.20	0.11

before extraction. Ultrasonic extraction was carried out by mixing 30 g of powdered sample and 120 mL of ethanol in a flask, which was then placed in an ultrasonic bath for 30 min. The extraction was repeated two additional times and the extracts were combined. The combined extract was concentrated under vacuum to get the crude extract using a rotary evaporator. The crude extract was then dissolved with small volume of ethanol and later 4 diploid distilled water (ethanol:water, v/v = 4/1). The solution was transferred into a separatory funnel and the same volume of petroleum ether was added into the separatory funnel. The crude extract was partitioned between petroleum ether and water for three times and later the aqueous components were partitioned again using the same volume of chloroform for three times. Lastly, the combined chloroform components were concentrated under vacuum to get the final extract.

2.4. Identification of components

The identification of the volatile and semi-volatile compounds was performed by the combination of several methods. The first method was co-injection with pure compound and a comparison with its retention data in one to three GC columns; the second method was comparison of the three different polar chromatographic column retention index data with literature LTPRI; the third method was comparison with mass spectra from the US National Institute of Standards and Technology (NIST) and Saturn (Varian) libraries.

3. Results and discussion

3.1. Optimization of HS-SPME procedure

The influence of parameters such as the type of fiber, incubation temperature, extraction time and temperature, and desorption time on the amount of volatile and semi-volatile compounds extracted was studied using the univariate method. Twelve compounds of different polarities and volatilities (listed in Table 1) were selected across the chromatogram for the purpose of the method development. The target peaks were assigned, automatic integration was inspected and manually re-integrated if necessary. Several blank experiments (column blank, fiber blank, blank of the fiber inserted to empty vial) were performed. The aim of the study was to find the optimal values providing uniformity in the extraction efficiency of the maximal number of compounds extracted and good reproducibility.

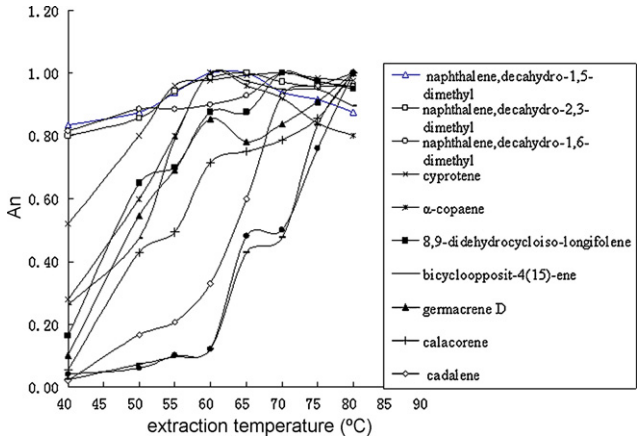

Fig. 1. Comparison of the relative extraction efficiencies of different extract temperature.

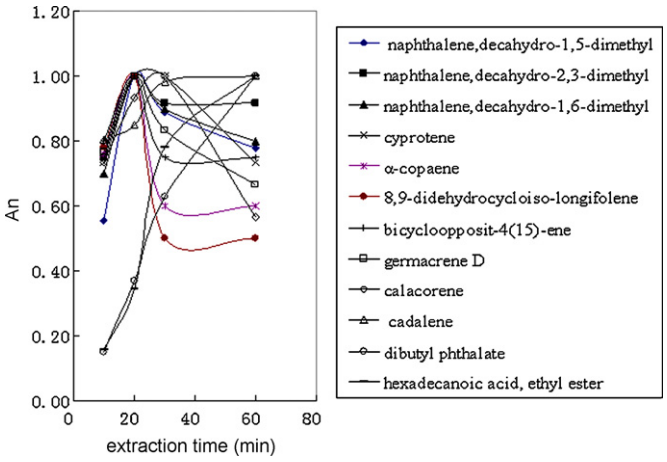

Fig. 2. Comparison of the relative extraction efficiencies of different extract time.

To assist and simplify the evaluation of the chromatographic profiles obtained from different HS-SPME parameter optimization, the peak areas were normalized according to the following equation:

$$A_n = \frac{A_x}{A_{\max}}$$

where A_n is the normalized area, A_x is the area of any chromatographic peak, and $A_{\max}$ is the maximum area obtained in the specific parameter condition.

3.1.1. Type of fiber

Silica fibers with seven commercially available SPME coating types were examined for the method optimization. The incubation/extraction temperature was 50 °C and the incubation/extraction time was 5 min/10 min; and desorption was operated at 260 °C for 5 min.

The relative extraction efficiencies of the tested fibers are summarized in Table 1. Some of the fibers other than DVB/CAR/PDMS exhibited similar sensitivities with this assembly. Especially the PDMS 100 μm fiber, which had a good selectivity for less polar analytes and the performance characteristics were almost identical to the DVB/CAR/PDMS fiber. However, the DVB/CAR/PDMS fiber proved to be the most universal assembly for sufficient isolation of compounds with different physico-chemical properties; this fiber was subsequently used in all further experiments. Other fiber coating types extracted only a portion of the compounds and, for some fibers, apparent complications during the thermal desorption of the analytes were observed. Some previously published studies reported the DVB/CAR/PDMS fiber to be the most appropriate for obtaining the widest volatile profile of plant product [26–29].

Table 2
Repeatability of the HS-SPME-GC–MS method for *E. odoratum* extract.

No.	Compound	RSD of peak area (%, $n = 5$)
1	Naphthalene,decahydro-1,5-dimethyl	3.5
2	Naphthalene,decahydro-2,3-dimethyl	3.7
3	Naphthalene,decahydro-1,6-dimethyl	4.8
4	Cyprotene	3.9
5	α-Copaene	4.5
6	8,9-Didehydrocycloiso-longifolene	6.9
7	Bicycloopposit-4(15)-ene	4.2
8	Germacrene D	7.2
9	Calacorene	7.5
10	Cadalene	8.0
11	Dibutyl phthalate	8.9
12	Hexadecanoic acid, ethyl ester	9.2

Table 3
Retention indices of *E. odoratum* extract volatiles on columns of different polarities.

No.	RT (min)	Compound	RI (RTX-1MS)	RI (HR-1701)	RI (HR-20M)	*M*	Formula	CAS No.
1	6.94	Dehydro-para-cymene	1072	1149	1423	132	$C_{10}H_{12}$	1195-32-0
2	8.74	Para-cymene	1127	1250	1656	134	$C_{10}H_{14}$	25155-15-1
3	9.20	Artemesia ketone	1139	1165	1119	152	$C_{10}H_{16}O$	25155-15-1
4	9.97	Naphthalene,decahydro-1,5-dimethyl	1158		1222	166	$C_{12}H_{22}$	66552-62-3
5	10.45	Naphthalene,decahydro-2,3-dimethyl	1171		1240	166	$C_{12}H_{22}$	1008-80-6
6	10.88	Naphthalene,decahydro-1,6-dimethyl	1182		1245	166	$C_{12}H_{22}$	1750-51-2
7	11.20	Naphthalene,decahydro-2,6-dimethyl	1190		1252	166	$C_{12}H_{22}$	1618-22-0
8	11.30	Naphthalene,decahydro-1,2-dimethyl	1193		1263	166	$C_{12}H_{22}$	3604-14-6
9	11.50	Cycloundecene,1-methyl	1198		1268	166	$C_{12}H_{22}$	88828-82-4
10	11.60	Cyclodecene,1,2-methyl	1201		1273	166	$C_{12}H_{22}$	14113-67-8
11	11.75	Naphthalene,decahydro-2,3-dimethyl	1204		1291	166	$C_{12}H_{22}$	1008-80-6
12	11.88	Naphthalene,decahydro-2,2-dimethyl	1206			166	$C_{12}H_{22}$	31124-85-3
13	12.61	Naphthalene,decahydro-1,1-dimethyl	1221			166	$C_{12}H_{22}$	35431-04-0
14	14.11	Presilphiperfol-7-ene	1252	1333		204	$C_{15}H_{24}$	80931-09-5
15	14.61	Cyprotene	1262	1326	1504	192	$C_{14}H_{24}$	193695-14-6
16	14.84	Pacifigorgia-1,10-diene	1268	1320	1905	204	$C_{15}H_{24}$	
17[a]	16.42	Tridecane	1300	1300	1300	184	$C_{13}H_{28}$	629-50-5
18	17.93	1,4-Dimethyl azulene	1327	1420	1713	156	$C_{12}H_{12}$	
19	19.51	Petasitene	1357	1514	1931	204	$C_{15}H_{24}$	
20	19.76	3,10-Dihydro-1,4-dimethyl-azulene	1362	1479	1829	158	$C_{12}H_{14}$	
21[a]	19.97	α-Copaene	1366			204	$C_{15}H_{24}$	947-59-1
22	21.15	Naphthalene,1,7-dimethyl	1388	1523	1948	156	$C_{12}H_{12}$	575-37-1
23	21.29	8,9-Didehydrocycloiso-longifolene	1391	1509	2010	202	$C_{15}H_{22}$	74842-33-4
24	21.62	Aristolene	1397			204	$C_{15}H_{24}$	
25[a]	21.78	Tetradecane	1400	1400	1400	198	$C_{14}H_{30}$	629-59-4
26[a]	22.07	Trans-caryophyllene	1405	1710	1568	204	$C_{15}H_{24}$	87-44-5
27[a]	22.21	α-Cubebene	1408			204	$C_{15}H_{24}$	
28	22.46	α-Ylangene	1412			204	$C_{15}H_{24}$	14912-44-8
29[a]	22.82	α-Humulene	1419			204	$C_{15}H_{24}$	6753-98-6
30[a]	23.68	α-Amorphene	1434			204	$C_{15}H_{24}$	
31[a]	23.81	β-Elemene	1437	1297	1679	204	$C_{15}H_{24}$	11029-06-4
32	24.94	β-Cadinene	1457			204	$C_{15}H_{24}$	16509-53-8
33	25.10	γ-Muurolene	1460			204	$C_{15}H_{24}$	
34	25.30	drim-8(12)-ene	1472	1794	2260	206	$C_{15}H_{26}$	
35	25.78	α-Gurjunene	1481			204	$C_{15}H_{24}$	
36[a]	26.28	α-Muurolene	1484			204	$C_{15}H_{24}$	31983-22-9
37	26.42	g-Cadinene	1495			204	$C_{15}H_{24}$	38357-83-4
38	27.01	Calamenene	1499	1581	1804	202	$C_{15}H_{24}$	6617-49-8
39[a]	27.28	Germacrene D	1506	1557	1735	204	$C_{15}H_{24}$	105453-16-5
40[a]	27.66	Calacorene	1519	1610	1883	200	$C_{15}H_{20}$	21391-99-1
41	28.34	Carvacrol	1523	1309	1595	150	$C_{10}H_{14}O$	499-75-2
42	28.57	Silphiperfola-5,7(14)-diene	1527			202	$C_{15}H_{22}$	
43	28.81	α-Cadinene	1534	1808	2078	204	$C_{15}H_{24}$	483-75-0
44[a]	29.19	Fumaric acid, ethyl 2-(2-methylenecyclopropyl) propyl ester	1538	1914	2679	238	$C_{13}H_{18}O_4$	
45[a]	29.42	Spathulenol	1549	1698	2087	220	$C_{15}H_{24}O$	6750-60-3
46	30.00	Aromadendrene	1552	1361	1767	204	$C_{15}H_{24}$	6750-60-3
47	30.19	Africa-1,5-diene	1561	1550	2743	202	$C_{15}H_{22}$	
48	31.15	Epi-cedrol	1570	1713	2069	222	$C_{15}H_{26}O$	
49[a]	32.34	Caryophyllene oxide	1591	1704	2027	220	$C_{15}H_{24}O$	1139-30-6
50[a]	32.81	Hexadecane	1600	1600	1600	226	$C_{16}H_{34}$	544-76-3
51	33.31	Brasila-5(10),6-diene	1609	1816	2502	204	$C_{15}H_{24}$	
52	33.52	Pacifigorgia-1(6),10-diene	1613	1340	2194	204	$C_{15}H_{24}$	
53	33.67	Pacifigorgia-2,10-diene	1616	1773	2206	204	$C_{15}H_{24}$	
54[a]	34.09	δ-Cadinene	1624			204	$C_{15}H_{24}$	29350-73-0
55	35.10	Cadalene	1643	1778	2178	198	$C_{15}H_{28}$	483-78-3
56	35.35	Germacrene B	1648			204	$C_{15}H_{24}$	15423-57-1
57	37.74	Laurene	1693	1868	2366	200	$C_{13}H_{12}O_2$	20400-42-0
58	37.87	7-(4-Methoxylbenzylidene) bicyclo[4,1,0]heptane	1695		3008	214	$C_{15}H_{18}O$	82253-13-2
59[a]	38.11	heptadecane	1700	1700	1700	240	$C_{17}H_{36}$	629-78-7
60	38.90	Phenol,2-(1,2-dimethyl-2-cyclopenten-1-ny),acetate	1715			230	$C_{15}H_{18}O_2$	39877-95-7
61	39.14	4(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one	1720	1620	1969	190	$C_{13}H_{18}O$	1203-08-3
62	39.32	Aristolene epoxide	1724	2032	2668	220	$C_{15}H_{24}O$	
63	40.07	4(2-Isopropyl-5-methylphenyl)-3-methylbutyric acid	1738	2044	2848	234	$C_{15}H_{22}O_2$	22291-58-3
64	40.36	Benzofurane-2-carboxylic acid,5-cyclohexyl-3-methyl	1744	1643		258	$C_{16}H_{18}O_3$	
65	42.13	Tetradecanoic acid, ethyl ester	1779	1865		256	$C_{16}H_{32}O_2$	124-06-1
66	42.31	Verrucarol	1782	2081	2424	266	$C_{15}H_{22}O_4$	2198-92-7
67	42.85	2-Propenoic acid,2-methyl-oxybis(2,1-ethanediyl)ester	1793	1961	2399	330	$C_{16}H_{26}O_7$	109-17-1
68[a]	43.23	Octadecane	1800	1800	1800	254	$C_{18}H_{38}$	593-45-3
69	44.25	Diisobutyl phthalate	1821	2048	2506	278	$C_{16}H_{22}O_4$	84-74-2
70	44.57	Naphthalene,decahydro-2,6-dimethyl-3-octyl	1828	1945	2114	278	$C_{20}H_{38}$	54964-85-1
71	44.94	(2E)-3,7,11,15-tetramethyl-2-hexadecen-1-ol	1835	1860	1926	296	$C_{20}H_{40}O$	102608-53-7
72	46.07	Cis-phytol	1858	1885	1955	222	$C_{15}H_{26}O$	
73	46.23	1,1′-dianthrimide	1862	1652		429	$C_{28}H_{15}NO_4$	82-22-4
74	46.41	1,2-Benzenedicarboxylic acid,butyl octyl ester	1865			334	$C_{20}H_{30}O_4$	84-78-6
75[a]	46.93	Trans-phytol	1876	1907	1981	222	$C_{15}H_{26}O$	150-86-7

Table 3 (*Continued*)

No.	RT (min)	Compound	RI (RTX-1MS)	RI (HR-1701)	RI (HR-20M)	*M*	Formula	CAS No.
76	47.05	Tridecanoic acid 13-formyl-ethyl ester	1879	1966		270	$C_{16}H_{30}O_3$	101434-22-4
77[a]	48.10	Nonadecane	1900	1900	1900	268	$C_{19}H_{40}$	629-92-5
78	48.77	Dibutyl phthalate	1914	2153	2658	278	$C_{16}H_{22}O_4$	84-74-2
79	49.81	Isophytol	1937	2028	2287	296	$C_{20}H_{40}O$	505-32-8
80	50.55	3-Deoxyestradiol	1952	2091	2899	256	$C_{18}H_{24}O$	2529-64-8
81	51.33	Ethyl 9-hexadecenoate	1969	1561	2295	282	$C_{18}H_{34}O_2$	54546-22-4
82	51.91	Palmitic acid ethyl ester	1981	2066	2251	284	$C_{18}H_{36}O_2$	628-97-7
83	52.32	δ-Dodecalactone	1990	2010	2056	198	$C_{12}H_{22}O_2$	713-95-1
84[a]	52.77	Eicosane	2000	2000	2000	282	$C_{20}H_{42}$	112-95-8
85	56.33	Heptadecanoic acid ethyl ester	2080	2167		298	$C_{19}H_{38}O_2$	14010-23-2
86[a]	57.24	Heneicosane	2100	2100	2100	296	$C_{21}H_{44}$	629-94-7
87	57.72	δ-Nonyl-8-valeralactone	2111	2160		226	$C_{14}H_{26}O_2$	
88	58.03	N-(4-fluorophenyl)-3-[(veratryl carbonyl) hydrazono] butyramide	2118	2194	3096	387	$C_{20}H_{22}FNO_4$	
89	58.85	Ethyl linoleate	2137	2255	3190	308	$C_{20}H_{36}O_2$	2721-22-4
90	59.01	Ethyl linolenate	2141	2272	2576	306	$C_{20}H_{34}O_2$	1191-41-9
91	59.28	Ethyl oleate	2147	2251	2466	310	$C_{20}H_{38}O2$	544-35-4
92	60.67	Octadecanoic acid, ethyl ester	2180	2269		312	$C_{20}H_{40}O_2$	111-61-5
93[a]	61.54	Docosane	2200	2200	2200	282	$C_{20}H_{42}$	111-62-6
94	64.43	2-Ethylhexyl trans-4-methoxycinnamate	2270	2512	3106	290	$C_{18}H_{26}O3$	57274-46-1
95[a]	65.67	Tricosane	2300	2300	2300	324	$C_{23}H_{48}$	638-67-5
96	69.65	Tetracosane	2400	2400	2400	338	$C_{24}H_{50}$	83834-59-7
97	73.48	Diisooctyl phthalate	2499	2725	3135	390	$C_{24}H_{38}O_4$	131-20-4
98	79.68	Heptacosane	2700	2700	2700	380	$C_{27}H_{56}$	593-49-7
99	87.64	Nonacosane	2900	2900	2900	408	$C_{29}H_{60}$	27554-26-3

Note: CAS No.: Chemical Abstracts Service Number.

[a] Compound which has been reported from *E. odoratum*.

3.1.2. Incubation/extraction temperature

A summary of the incubation/extraction temperature experiment is shown in Fig. 1. Two opposite phenomena take place when the extraction temperature is increased. The rate of analyte transfer toward the fiber is increased. On the other hand, the distribution constant of the analyte between the headspace and fiber coating decreases, which might cause a significant decrease in the method sensitivity, depending on which phenomenon predominates [30]. Accordingly, the optimal temperature significantly differs for various compounds, as seen in Fig. 1. Temperatures as high as 80 °C were used for general method development purposes [26]. However, such high temperature might cause decomposition of some compounds. A temperature of 65 °C for both the incubation and extraction procedures was selected as a compromise to ensure the efficient extraction of both the volatile and semi-volatile analytes.

3.1.3. Extraction time

The measurements when equilibrium is reached are more reproducible than non-equilibrium measurements. Therefore, the time the fiber was exposed to the headspace gas was optimized to determine the equilibrium time. The results are shown in Fig. 2. The extraction time needed to reach the distribution equilibrium depends on the compound. Thus, 10–20 min was sufficient for the volatile compounds, while the equilibrium was not reached in 60 min for semi-volatile compounds. An extraction time of 20 min was selected for further experiments as a compromise among sensitivity, reproducibility and analysis time.

3.1.4. Incubation time

The incubation time was not a significant variable, there was no tendency, and the signals were similar within the experimental error. Therefore, 5 min of incubation was chosen as a parameter for further experiments.

3.1.5. Desorption temperature and time

Desorption temperature is one of the main factors, which should be optimized during method development. Desorption temperatures of 260 and 270 °C were shown to be the most effective for the analytes tested. Since the latter temperature is the upper limit recommended by the fiber producer, a desorption temperature of 260 °C was selected to protect the assembly. A 5 min desorption was followed by a 10 min bakeout period to remove all potential interferences and to avoid the carry-over effect.

3.1.6. Repeatability of the analytical method

Five replicates of the *E. odoratum* extract sample were injected to evaluate the repeatability of the analytical method with the optimized experimental conditions. The repeatability of the optimized HS-SPME-GC–MS method for *E. odoratum* extract, expressed as relative standard deviation (RSD, %, $n = 5$), ranged from 3.5 to 9.2% for all the method optimization compounds (see Table 2). The significant errors in the repeatability resulted to the non-equilibrium conditions (the steep area of the extraction curve).

3.2. Identification of the volatile and semi-volatile compounds in *E. odoratum* extract

Based on the above results, optimized HS-SPME conditions for *E. odoratum* extract sample were as follows: A DVB/CAR/PDMS fiber was used to extract volatile and semi-volatile compounds from headspace (extraction temperature and time were 65 °C and 20 min, respectively); the analytes were desorbed into the GC injector at 260 °C (desorption time 5 min). The retention indices estimated for the volatile and semi-volatile compounds in *E. odoratum* extract on non-polar RTX-1MS, mid-polar HR-1701, and polar HR-20M stationary phases are listed in Table 3. Identification of the analytes was carried out by using the LTPRI method and the comparison between experimental and reference/library mass spectra. Ninety-nine compounds were temporarily identified as listed in Table 3.

Among the 99 compounds, α-copaene, β-elemene, germacrene D, caryophyllene, tetradecane and tridecane were also found by Bamba et al. from *E. odoratum*'s essential oil which was isolated by hydrodistillation [13]. Another research group Ling et al. [10] also used hydrodistillation method to get the essential oil from the leaves of *E. odoratum* then identified 33 compounds of the essential oil of *E. odoratum* by GC–MS and 18 of those compounds also existed in our result. And among the 18 components, 10 compounds (α-cubebene, α-copaene, β-elemene, germacrene D, trans-caryophyllene, humulene, α-amorphene, α-muurolene, δ-cadinene, α-calacorene and caryophyllene oxide) were sesquiter-

penoids and the other 8 compounds were alkanes [10]. Recently, Yuan et al. [16] identified 68 compounds from the essential oil of *E. odoratum* by CO_2 supercritical fluid extraction and GC–MS method and only 5 compounds α-cubebene, trans-caryophyllene, spathulenol, phytol and fumaric acid, ethyl 2-(2-methylenecyclopropyl) propyl ester were found in our result. All of the previous *E. odoratum* component analyses were about essential oil, perhaps this is the main reason about the component difference between our result and previous works.

4. Conclusion

HS-SPME showed to be a convenient tool to access chemical compounds that might be the repellent components of plant extract. HS-SPME eliminates the need for concentrating volatile compounds. In addition, SPME is solventless, simple to operate, and requires a low amount of sample, which highlights the potential of this technique as a research tool for repellent compound studies.

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