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Analysis of Phytochemical Constituent of the Volatile Oil in the *Wedelia biflora* by HS-SPME/GC-MS

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To analyze the chemical constituents of the volatile oil in the green peel of *Wedelia biflora*, the chemical constituents was extracted from the *Wedelia biflora* by solid-phase microextraction. The chemical constituents of the volatile oil separated and identified by GC-MS. The relative content of each component was determined by area normalization. Seventy-two kinds of components were separated. Among them, 68 components were identified, accounting *ca.* 98.51 % of the total chemical constituents, most of them belonged to sesquiterpenoids, the main chemical constituents of the *Wedelia biflora* are D-limonene (15.40 %); 4,11,11-trimethyl-8-methylene-[1R-(1R*,4Z,9S*)]-bicyclo[7.2.0]undec-4-ene (9.70 %); 1R-α-Pinene (8.94 %); 4-methyl-1-(1-methylethyl)-didehydro derive bicyclo[3.1.0]hexane (8.07 %); 1-methyl-3- (1-methylethyl)-benzene (6.71 %); 1,1,4,8-tetramethyl- *cis*, *cis*, *cis*-4,7,10- cycloundecatriene (5.39 %); germacrene D (4.65 %), *etc*. The result of the experiments can provide reference for further research on the chemical components of *Wedelia biflora* volatile oil as well as for the exploration and utilization of it.

Key Words: *Wedelia biflora*, Volatile oil, Solid-phase microextraction, GC-MS.

INTRODUCTION

Wedelia biflora (Linn.) DC., also called chrysanthemum sesarmid crab, chrysanthemum ink vegetables, chrysanthemum dragon tongue plant, Tianhuang Ju and Ludiju, *etc*. It is used as medicine entirely[1-3]. We usually harvest this herb in summer and fall and then wash it clean, keep it fresh and sun-dried. The *Wedelia biflora* (Linn.) DC. is distributed in the Oceania, Indo-china peninsula, the Philippines, India, Japan, Taiwan Island, Malaysia, Indonesia and in the regions of the Chinese Mainland, such as Guangdong, Hainan, Guangxi and Yunnan, *etc*. According to the literature, *Wedelia biflora* (Linn.) DC. has the pharmacologic functions[4-6] of liver-protection, antivirus, antitumors, antiinflammation and analgesia. In order to make full use of this resource, this work conducts a study on the chemical compositions of its volatile oil.

Solid-phase microextraction (SPME)[7-10] is a new technology put forward by the scientists like Pawliszyn in 1990. This method doesn't use organic solvent. It is easy and convenient, combining the following processes together: herbs-colleting, extracting, concentration and sample-feeding. And thus it has the advantages of fast analysis (generally it can reach absorbing balance within 2-30 min), high sensitivity and good repeatability. In view of its above advantages, this research uses the method of solid-phase microextraction for the first time to extract the chemical compositions in *Wedelia biflora* (Linn.) DC produced in Hainan Island. It adopts the coupling technique of gas chromatograph-mass spectrometer to analyze and determine its chemical compositions. Overall, it separated 72 components and determined 68 compounds. The determined components takes up 98.51 % of the total peak area. The relative contents of each peak can be calculated by the peak-area normalization method, which provides a reference for the further research of its active components in the volatile oil and its further exploitation of this natural resource.

EXPERIMENTAL

Trace MS Gas Chromatograph-tandem Mass Spectrometer (American Phenegon Company);Chromatographic Column; DB-WAX (30 m × 0.25 mm, 0.25 mm) Quarts Capillary Column; Manual Solid-phase Microextraction, SPME (American Supelco Company); the extracting fiber-head is 65 μm PDMS/DVB; the mixed alkane standard samples of C_8-C_{20} and C_{21}-C_{40}.

The *Wedelia biflora* (Linn.) DC. was picked in Sanya, Hainan Island in August, 2011. It was dried and kept in the dry containers for future use after being cleansed, crushed and sieved from the 100-mesh sift.

Solid-phase microextraction conditions: Weight 1.5 g powder of the *Wedelia biflora* (Linn.) DC and put it in the special sample-taking bottle of 15 mL and then insert it into the manual sample-feeding machine with the fiber-head of 65 μm PDMS/DVB. Its magnetic stirring speed is 1100 rpm; take away after 0.5 h of extraction at 90 ºC and immediately insert it into the sample-feeding mouth of the chromatograph (the temperature is 250 ºC) with 2.5 min of desorption.

Analytical condition of the gas chromatograph-mass spectrometer: The gas chromatograph condition: the chromatographic column is the quarts capillary column of DB-WAX (30 m × 0.25 mm, 0.25 mm); temperature-raising process:the initial temperature is 45 ºC for 3 min and then raise the temperature to 100 ºC at the speed of 10 ºC min^{-1} and keep it for 15 min, then again raise it to 170 ºC at the speed of 5 ºC min^{-1}; raise it to 240 ºC for 7 min at the speed of 10 ºC min^{-1}; the temperature at the sample-feeding mouth is 250 ºC, the temperature at the evaporating room is 250 ºC; the carrier gas is helium (He) and its flow velocity is 0.8 mL min^{-1} and there is no bypass flow with its sample amount of 1.0 mL and its split ratio is 50:1.

The mass-spectrum condition:electron bombardment ion source (EI); ionizing energy is 70 eV, the temperature of the ion source is 200 ºC, the voltage of the detector is 350 V, the detection range is between 40-300 m z^{-1} and the detecting interval is 0.5 s. The scanning mass range is m/z: 333-500 AMU; the scanning speed is 0.5 s; the retrieval atlas databanks are the standard mass spectrum map depot of Willey and NIST.

Calculation of retention indices: This quasi-linear equation proposed by Van den Dool and Kratz[6] was used to calculate retention indices in this work:

$$IT = 100 \times n + 100 \times \frac{(tx - tn)}{(tn + 1 - tn)}$$

where IT is the temperature-programmed retention index of the interesting compound and tn, tn + 1 and tx are the retention times of the two standard *n*-alkanes containing n and n + 1 carbons and the compound of interest, respectively.

Determining results: Adopt the DB-WAX capillary column, take the suitable amount of the sample, analyze and determine by gas chromatograph-mass spectrometer and we get the total volatile oil ion flow chart of the *Wedelia biflora* (Linn.) DC. (Fig. 1). After the mass-spectrum scanning for each chromatographic peak, we get the mass-spectrum graph. We also consult the relevant mass-spectrum data through the mass-spectrum bank retrieval of NIST2008 standard. The compound quantity takes the area normalization method and we calculate each peak area by the Hewlett-Packard software system for the purpose of getting the relative percentage in each volatile oil component. Then calculate their RI values by choosing the possible matter with high matching degree with the mass-spectrum. And finally we determined the chemical composition of the volatile oil in the *Wedelia biflora* (Linn.) DC. by combining the artificial analysis (Table-1).

Fig. 1 showed that there are 68 kinds of chemical components in the volatile oil of the *Wedelia biflora* (Linn.) DC. The main contents are diterpene and aromatic components of micromolecules. Among them the high-content components are: Component **1**: D-Limonene (15.40 %); component **2**: 4,11,11-Trimethyl-8-methylene-[1R-(1R*,4Z,9S*)]-bicyclo[7.2.0]undec-4-ene (9.70 %); component **3**: 1R-α-Pinene (8.94 %); component **4**: 4-Methyl-1-(1-methylethyl)-didehydro derive bicyclo[3.1.0]hexane (8.07 %); component **5**: 1-methyl-3-(1-methylethyl)-benzene (6.71 %); component **6**: 1,1,4,8-tetramethyl-*cis*, *cis*, *cis*-4,7,10-cycloundecatriene (5.39 %); component **7**: Germacrene D (4.65 %); component **8**: copaene (2.90 %); component **9**: Octahydro-7-methyl-3-methylene-4-(1-methylethyl)- [3αS-(3αα,3αβ,4α,7α,7αS*)]-1*H*-cyclopenta[1,3]cyclopropa[1,2]benzene (2.46 %); component **10**: 1,2,4α,5,8,8α-hexahydro-4,7-dimethyl-1-(1-methylethyl)-[1S-(1α,4αα,8αα)]-naphthalene (2.20 %); component **11**: 2,3,4,7,8,8α-hexahydro-3,6,8,8-tetramethyl-[3R-(3α,3αα,7α,8αα)]-1*H*-3α,7-methanoazulene (2.10 %); component **12**: Octahydro-3,8,8-trimethyl-6-methylene-, [3R-(3α,3αα,7α,8αα)] -1*H*-3α,7-methanoazulene (1.96 %); component **13**: caryophyllene oxide (1.53 %), structure of components are given in Fig. 2.

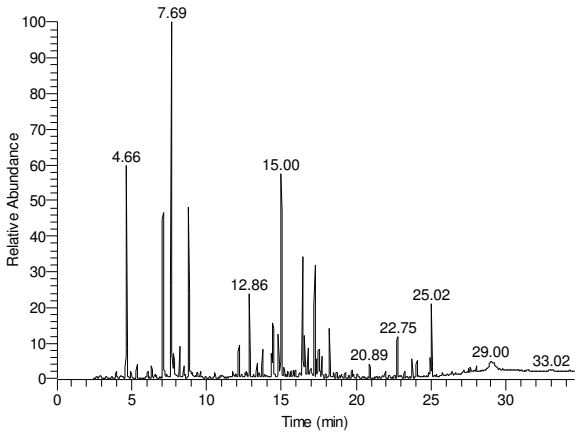


Fig. 1. GC-MS total ion current chromatogram of the volatile oil in the *Wedelia biflora*

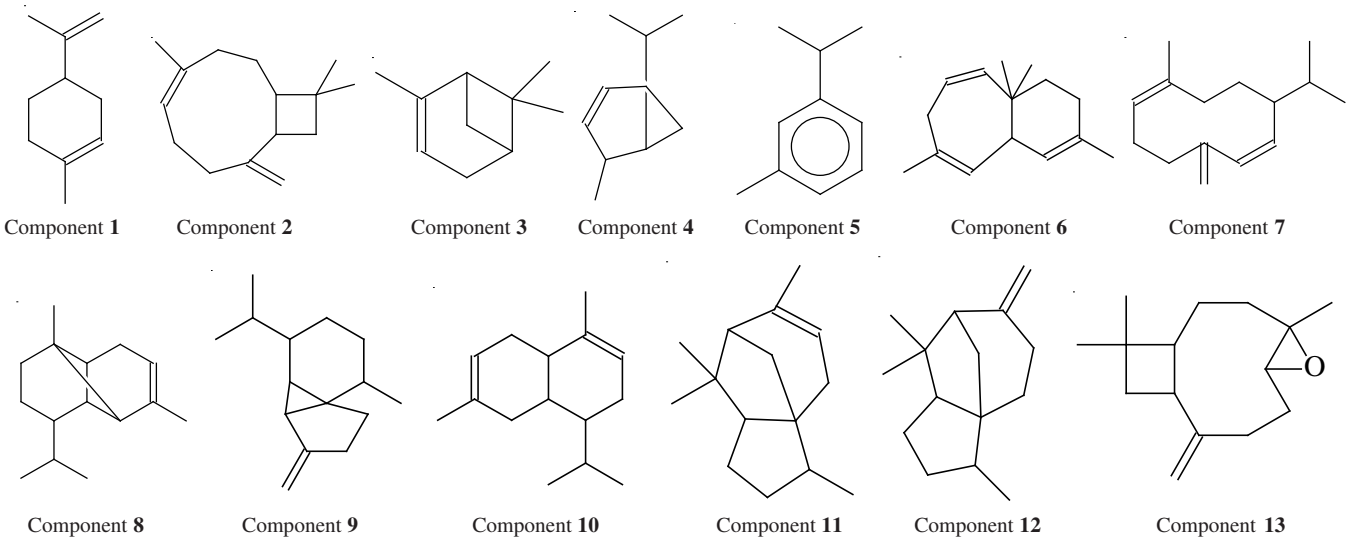
RESULTS AND DISCUSSION

It can also be seen in Table-1 that we can get 68 kinds of components by using solid-phase microextraction to extract the chemical compositions of the volatile oil in the *Wedelia biflora* (Linn.) DC. and by using gas chromatograph-tandem mass spectrometer to analyze and determine. The determined components take up 98.51 % of the total peak area. Its main components are: D-Limonene (15.40 %); 4,11,11-trimethyl-8-methylene-[1R-(1R*,4Z,9S*)]-bicyclo[7.2.0] undec-4-ene (9.70 %); 1R-α-pinene (8.94 %); 4-methyl-1- (1-methylethyl)-didehydro derive bicyclo[3.1.0]hexane (8.07 %); 1-methyl-3-(1-methylethyl)-benzene (6.71 %); 1,1,4,8-tetramethyl-*cis*,*cis*,*cis*-4,7,10-cycloundecatriene (5.39 %); germacrene D (4.65 %); copaene (2.90 %); octahydro-7-methyl-3-methylene-4-(1-methylethyl)-[3αS-(3αα,3αβ,4α,7α,7αS*)]-1*H*-cyclopenta[1,3]cyclopropa[1,2] benzene (2.46 %); 1,2,4α,5,8,8α-hexahydro-4,7-dimethyl-1-(1-methylethyl)-[1S-(1α,4αα,8αα)]-naphthalene (2.20 %); 2,3,4,7,8,8α-hexahydro-3,6,8,8-tetramethyl-[3R-(3α,3αα,7α,8αα)]-1*H*-3α,7-methanoazulene (2.10 %); octahydro-3,8,8-trimethyl-6-methylene-[3R-(3α,3αα,7α,8αα)]-1*H*-3α,7-methanoazulene (1.96 %); caryophyllene oxide (1.53 %).

TABLE-1
ANALYTICAL RESULTS OF CHEMICAL CONSTITUENTS OF THE VOLATILE OIL IN THE *Wedelia biflora*

No.	Name of components	RT (min)	m.f.	RI	Relative concent (%)
1	3-Pentanone	3.98	$C_5H_{10}0$	979.433	0.37
2	1R-α-Pinene	4.66	$C_{10}H_{16}$	1022.543	8.94
3	Toluene	4.96	C_7H_8	1039.884	0.29
4	Camphene	5.35	$C_{10}H_{16}$	1062.428	0.39
5	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	6.09	$C_{10}H_{16}$	1105.263	0.29
6	α-Phellandrene	6.34	$C_{10}H_{16}$	1119.883	0.42
7	3-Hexen-2-one	6.56	$C_6H_{10}0$	1132.749	0.17
8	4-Methyl-1-(1-methylethyl)-didehydro derive bicyclo[3.1.0]hexane	7.10	$C_{10}H_{16}$	1164.327	8.07
9	1-Methyl-4-(1-methylethyl)-1,4-cyclohexadiene	7.34	$C_{10}H_{16}$	1178.363	0.23
10	D-Limonene	7.69	$C_{10}H_{16}$	1198.830	15.40
11	4-Methyl-1-(1-methylethyl)-bicyclo[3.1.0]hex-2-ene	7.82	$C_{10}H_{16}$	1206.707	0.96
12	3,6,6-Trimethyl-bicyclo[3.1.1]hept-2-ene	8.23	$C_{10}H_{16}$	1231.707	0.83
13	2-Methyl-5-(1-methylethyl)-(1α,2α,5α)-bicyclo[3.1.0]hexan-2-ol	8.41	$C_{10}H_{21}0_{18}$	1242.683	0.14
14	3,7-Dimethyl-(Z)-1,3,6-octatriene,	8.50	$C_{10}H_{16}$	1248.171	0.35
15	3-Octanone	8.56	C_8H_{16}	1251.829	0.15
16	1-Methyl-3-(1-methylethyl)-benzene	8.83	$C_{10}H_{14}$	1268.293	6.71
17	1,3,8-*p*-Menthatriene	9.03	$C_{10}H_{14}$	1280.488	0.30
18	2-Ethenyl-1,1-dimethyl-3-methylene-cyclohexane	9.40	$C_{11}H_{18}$	1302.793	0.14
19	3,6-Dimethoxy-9-(2-phenylethynyl)-fluoren-9-ol	9.61	$C_{23}H_{18}0_3$	1314.525	0.16
20	4,4,6,6-Tetramethyl-bicyclo[3.1.0]hex-2-ene	10.53	$C_{10}H_{16}$	1365.922	0.15
21	1-methyl-4-(1-methylethenyl)-benzene	11.74	$C_{10}H_{12}$	1430.928	0.34
22	1-Octen-3-ol	11.97	$C_8H_{16}0$	1442.784	0.16
23	α-Cubebene	12.17	$C_{15}H_{24}$	1453.093	1.05
24	1-Ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-cyclohexane	12.60	$C_{15}H_{24}$	1475.258	0.13
25	Ylangene	12.67	$C_{15}H_{24}$	1478.866	0.27
26	Copaene	12.86	$C_{15}H_{24}$	1488.660	2.90
27	Decahydro-3α-methyl-6-methylene-1-(1-methylethyl)-[1S-(1α,3αα,3αβ, 6αα,6αβ)]-cyclobuta[1,2:3,4]dicyclopentene	13.38	$C_{15}H_{24}$	1514.778	0.52
28	Di-epi-à-cedrene-(I)	13.49	$C_{15}H_{24}$	1520.197	0.16
29	Octahydro-7-methyl-3-methylene-4-(1-methylethyl)-[3αS-(3αβ,3αβ,4α,7α, 7αS*)]-1*H*-cyclopenta[1,3]cyclopropa[1,2]benzene	13.74	$C_{15}H_{24}$	1532.512	2.46
30	2,3,4,7,8,8α-Hexahydro-3,6,8,8-tetramethyl-[3R-(3α,3αα,7α,8αα)]-1*H*-3α, 7-methanoazulene	14.39	$C_{15}H_{24}$	1564.532	2.10
31	Octahydro-3,8,8-trimethyl-6-methylene-, [3R-(3α,3αα,7α,8αα)]-1*H*-3α,7-methanoazulene	14.47	$C_{15}H_{24}$	1568.473	1.96
32	1-Ethenyl-1-methyl-2,4-*bis*(1-methylethenyl)-[1S-(1α,2α,4α)]-cyclohexane	14.79	$C_{15}H_{24}$	1584.236	1.60
33	4,11,11-Trimethyl-8-methylene-[1R-(1R*,4Z,9S*)]-bicyclo[7.2.0]undec-4-ene	15.00	$C_{15}H_{24}$	1594.581	9.70
34	2-Methyl-5-(1-methylethenyl)-cyclohexanone	15.17	$C_{10}H_{16}0$	1602.871	0.33
35	Thujopsene	15.46	$C_{15}H_{24}$	1616.746	0.20
36	α-Cubebene	15.65	$C_{15}H_{24}$	1625.837	0.24
37	Decahydro-1,1,7-trimethyl-4-methylene-[1αR-(1ααα,4ααα,7α,7ααα,7αβ)]-1*H*-cycloprop[e]azulene	15.94	$C_{15}H_{24}$	1639.713	0.29
38	1,1,4,8-Tetramethyl-*cis*, *cis*, *cis*-4,7,10-cycloundecatriene	16.44	$C_{15}H_{24}$	1663.636	5.39
39	2-Isopropenyl-4α,8-dimethyl-1,2,3,4,4α,5,6,7-octahydronaphthalene	16.54	$C_{15}H_{24}$	1668.421	1.65
40	4,6,6-Trimethyl-[1S-(1α,2α,5α)]-bicyclo[3.1.1]hept-3-en-2-ol	16.65	$C_{10}H_{16}0$	1673.684	0.29
41	(+)-Epi-bicyclosesquiphellandrene	16.73	$C_{15}H_{26}$	1677.512	0.21
42	1,2,3,4,4α,5,6,8α-Octahydro-7-methyl-4-methylene-1-(1-methylethyl)-(1α, 4αα,8αα)-naphthalene	16.80	$C_{15}H_{24}$	1680.861	1.34
43	α,α,4-Trimethyl-, acetate 3-cyclohexene-1-methanol	16.94	$C_{12}H_{20}0_2$	1687.56	0.21
44	α,α,4-Trimethyl-, (S)-3-cyclohexene-1-methanol	17.00	$C_{10}H_{18}0$	1690.431	0.27
45	Germacrene D	17.24	$C_{15}H_{24}$	1701.923	4.65
46	Decahydro-4α-methyl-1-methylene-7-(1-methylethenyl)-[4αR-(4ααα,7α, 8αα)]-naphthalene	17.45	$C_{15}H_{24}$	1712.019	1.13
47	α-Muurolene	17.52	$C_{15}H_{26}$	1715.385	1.30
48	1-Ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-cyclohexane	17.72	$C_{15}H_{24}$	1725.000	0.82
49	α-Farnesene	17.94	$C_{15}H_{24}$	1735.577	0.13
50	1,2,4α,5,8,8α-Hexahydro-4,7-dimethyl-1-(1-methylethyl)-[1S-(1α,4ααα, 8αα)]-naphthalene	18.20	$C_{15}H_{24}$	1748.077	2.20
51	1-(1,5-Dimethyl-4-hexenyl)-4-methyl-benzene	18.48	$C_{15}H_{22}$	1761.538	0.19
52	1,2,3,4,4α,7-Hexahydro-1,6-dimethyl-4-(1-methylethyl)-naphthalene	18.70	$C_{15}H_{24}$	1772.115	0.20

53	1,7,7-Trimethyl-(1S-endo)-bicyclo[2.2.1]heptan-2-ol	19.00	$C_{10}H_{18}0$	1786.538	0.16
54	1,2,3,4-Tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-*cis*)-naphthalene	19.71	$C_{15}H_{22}$	1821.078	0.39
55	2-Methyl-5-(1-methylethenyl)-trans-2-cyclohexen-1-ol	19.83	$C_{10}H_{16}0$	1826.961	0.17
56	Benzocycloheptatriene	20.06	$C_{11}H_{10}$	1838.235	0.16
57	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	20.89	$C_{15}H_{26}0$	1878.922	0.74
58	Caryophyllene oxide	22.75	$C_{15}H_{24}0$	1974.093	1.53
64	1,2-Dimethoxy-4-(2-propenyl)-benzene	23.23	$C_{11}H_{14}0_2$	1998.964	0.32
59	1,5,5,8-Tetramethyl-[1R-(1R*,3E,7E,11R*)]-12-oxabicyclo[9.1.0]dodeca-3,7-diene	23.72	$C_{15}H_{24}0$	2029.936	0.69
60	Decahydro-4α-methyl-8-methylene-2-(1-methylethyl)-[1R-(1α,2α,4αα,8αα)]-1-naphthalenol	24.04	$C_{15}H_{26}0$	2050.318	0.62
61	Decahydro-4,8,8-trimethyl-[1S-(1α,3αα,4α,8αα,9R*)]-1,4-methanoazulene-9-methanol	24.86	$C_{15}H_{26}0$	2103.774	0.17
62	8αH-cedran-8-ol	24.93	$C_{15}H_{26}0$	2110.377	0.61
63	(-)-Spathulenol	25.02	$C_{15}H_{24}0$	2118.868	2.32
64	4,4-Dimethyl-tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol	27.18	$C_{15}H_{24}0$	2291.228	0.17
65	3-(1-Methyl-1*H*-pyrrol-2-yl)-pyridine	27.50	$C_{12}H_{10}N_2$	2321.569	0.13
66	Caryophyllene oxide	27.61	$C_{15}H_{24}0$	2332.353	0.17
67	Aromadendrene oxide-(2)	28.02	$C_{15}H_{24}0$	2372.549	0.22
68	1,2,3,4,4α,9,10,10α-octahydro-1,4α-dimethyl-7-(1-methylethyl)-[1S-(1α,4αα,10αα)]-1-phenanthrenemethanol	29.13	$C_{20}H_{30}0$	2484.553	1.09
Total					98.51



Component **1** Component **2** Component **3** Component **4** Component **5** Component **6** Component **7**

Component **8** Component **9** Component **10** Component **11** Component **12** Component **13**

Fig. 2. Representative volatile components of essential oils from *Wedelia biflora*

The determined 68 chemical components are retrieved through the computer bank. The similarities of 65 kinds of chemical composition are over 80 % (except that only 3 chemical components have the similarity of nearly 75 %), which shows that it has the relatively high reliability.

The main chemical components of the volatile oil in the *Wedelia biflora* (Linn.) DC. by the method of SPME-GC/MS are the terpenoids and the micro-molecular aromatic compounds like single terpene and diterpene, *etc*. Most of these diterpene compounds have strong fragrance and various bioactivities. For example, D-limonene has the strong citrus smell and the insecticidal action, which can be used as aromatic additives besides being used as botanical insecticides. All in all, through the method of solid-phase microextraction, SPME and the gas chromatograph-tandem mass spectrometer, it almost completely reflects the chemical components of the volatile oil in the *Wedelia biflora* (Linn.) DC. and thus it provides the reference data for its further development and utilization.

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