SUPPLEMENTARY MATERIAL

Application of HS-SPME-GC-MS combined with electronic nose technology in the odor recognition of Pseudostellariae Radix

Tehui Huang[b]*, Yan Sun[c]*, Yuanyuan Guo[a,b], Wei Wang[b], Ting He[a], Junling Cao[a]

[a]Department of Pharmacy, Dongfang Hospital of Beijing University of Chinese Medicine, Beijing, 100078, China

[b]College of traditional Chinese medicine, Beijing University of Chinese Medicine, Beijing, 100029, China

[c]Institute of Food Safety, Chinese Academy of Inspection& Quarantine, Beijing, 100176, China

* These authors contributed equally to this work.

Corresponding author. Junling Cao: caojunling72@163.com; Ting He: 109871713@qq.com.

ABSTRACT: Correct identification of the substance basis of Pseudostellariae Radix (PR) odor is important not only for the quality control of the products, but also for the safety of the consumers. PR is often described with a special smell, such as strange, moldy or earthy. Electronic nose-based technology coupled with headspace solid phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) was used to investigate the volatile components in PR from 47 germplasms cultivated in traditional fields. A total of 48 common compounds were identified based on HS-SPME-GC-MS technology, and 25 of them with aroma characteristics were found based on Alpha soft 13.4. The 1-Octen-3-ol, geosim, (E)-2-nonenal and 1-methylnaphthalene as contributing marker compounds of the 'specific smell' of PR were identified. The odor recognition mode, with demonstrated excellent accuracy in recognition abilities, enabled the correct identification of commercial samples including complex mixtures.

3. Experimental

3.1. Development of the HS-SPME method

3.1.1. Materials and instruments

47 germplasms cultivated of PR were collected, Anhui Province (10 batches), Fujian Province (11 batches), Guizhou Province (13 batches), Shandong Province (13 batches). These samples were sun-dried according to the primary processing method in Pharmacopoeia. GC-MS QP2010 Ultra gas-mass spectrometer (SHIMADZU Corporation, Japan), HS-2 Headspace Sampler (Beijing Zhonghuipu Company), Rtx-5ms quartz capillary column (30 m×0.25 mm×0.25 μm), 10 m headspace sampling bottle, Pharmacopoeia sieve, FW-135 pulveriser, BS-124S electronic balance, Manual sampling handle of SPME (Supelco Company, USA), Solid phase microextraction (SPME) 65 μm PDMS/DVB, 100 μm PDMS, and 85 μm PA (Supelco Company, USA), etc.

3.1.2. Selection of sample volume

The best conditions for measuring PR by electronic nose determination are as follows: weighing 0.5 g of sample powder (passed through a 50-mesh sieve), keeping the heating temperature at 40 °C for 360 s and injecting 1000 μL (Huang et al., 2020). After comparing the response values of each sensor, it was found that the value of electronic nose exhibited a downward trend with the increase of the sample volume over 0.5 g. In order to meet the best conditions in the experimental part of HS-SPME-GC-MS, the sample amount of 0.5 g was selected.

3.1.3. Selection of SPME

The SPME will be activated at 250 °C for 30 min before being used for the first time, and each sample will be activated for 5 min to remove impurities adsorbed on the SPME. The SPME heating temperature was 70 °C, and the extraction equilibrium for 30 min. Then taken out and immediately inserted into the gas chromatograph inlet (240 ℃) for 2 min desorption. The SPME types were investigated by using 65 μm PDMS/DVB, 100 μm PDMS and 85 μm PA respectively.

65 μm PDMS/DVB is suitable for volatile substances, amines and nitroaromatic compounds with molecular weights between 50 and 300, 100 μm PDMS is suitable for nonpolar volatile compounds with molecular weights between 60 and 275, and 85 μm PA is suitable for polar semi-volatile compounds with molecular weights between 80 and 300. The results show that more volatile components of PR can be extracted by 65 μm PDMS/DVB (Figure S1).

3.1.4. Optimization of extraction equilibrium temperature

The experimental conditions are the same as above, using 65 μm PDMS/DVB, then heating and balancing at 50 ℃, 60 ℃, 70 ℃, 80 ℃ and 90 ℃ for 30 minutes.

Increasing the extraction temperature of the sample can simultaneously increase diffusion coefficient, headspace efficiency and extraction rate, but at the same time it will also reduce the distribution coefficient after extraction equilibrium (Figure S2 and Figure S3). The best result was the 70 ℃ that allowed the chromatography without reducing efficiency and with full separation of other constituents from the matrices.

3.1.5. Optimization of extraction equilibrium time

Five different extraction equilibrium times of 10 min, 20 min, 30 min, 40 min and 50 min were compared. Although the peak in the chromatogram would be increased with the prolongation of extraction equilibrium time, the column loss at the extraction head would become more severe. Therefore, 30 min was chosen as the extraction equilibrium time (Figure S4 and Figure S5).

3.2. HS-SPME-GC-MS determination conditions

The conditions of HS-SPME were determined as follows: the sample injection volume was 0.5 g, 65 μm PDMS/DVB was the SPME, the heating temperature was 70 ℃, the desorption temperature was 240 ℃ and the desorption time was 2 min after the extraction equilibrium for 30 min.

GC condition: Rtx-5ms quartz capillary column (30 m×0.25 mm×0.25 μm). Temperature program: the initial temperature was 40 ℃, the temperature was kept for 3 min, and then raised to 240 ℃ at 5 ℃/min, the temperature was kept for 2 min. The carrier gas was high-purity helium (99.999%), the column flow rate was 1 mL/min, the inlet temperature was 240℃, no split injection, the solvent delay time was 2 min, and the high-pressure injection was 150 kPa.

MS condition: EI source; Ion source temperature: 250 ℃; Ionization voltage: 70eV; Temperature of quadrupole mass spectrometry: 150 ℃; The mass scan was over the range of 35~500 m/z; Scanning speed: 1666 u/sec.

3.3. Aroma characteristics recognition

The GC-MS total ion chromatography (TIC) shows that 48 common chromatographic peaks were identified in 47 batches of PR samples after comparison (Figure S6). Then 48 compound names were identified using the NIST 11.0 spectral database and those aroma characteristics were retrieved using Alpha 13.4 software. A total of 25 common compounds with aromatic characteristics were obtained.

Tables

Figures

Table S1 Cultivated Fields of Pseudostellariae Radix

Number	Field	Date of Collection
ah01	Shanguan Village, Taozhou Town, Xuancheng City, Anhui Province	2019/7/2
ah02	Shaba Village, Dongting Town, Xuancheng City, Anhui Province	2019/7/2
ah03	Fujia Village, Taozhou Town, Xuancheng City, Anhui Province	2019/7/2
ah04	Lvlin Village, Shijie Town, Xuancheng City, Anhui Province	2019/7/2
ah05	Baiqiao Village, Taozhou Town, Xuancheng City, Anhui Province	2019/7/2
ah06	Wubian Village, Huangdu Town, Xuancheng City, Anhui Province	2019/7/2
ah07	Tang Village, Yishan Town, Xuancheng City, Anhui Province	2019/7/2
ah08	Yangdaishan Village, Dongting Town, Xuancheng City, Anhui Province	2019/7/2
ah09	Guangde County, Xuancheng City, Anhui Province	2019/7/2
ah10	Huagu Village, Shijie Town, Xuancheng City, Anhui Province	2019/7/2
fj01	Houlong Village, Zhaizhong Town, Zherong County, Fujian Province	2019/8/8
fj02	Jitou Village, Chengjiao Town, Zherong County, Fujian Province	2019/8/8
fj03	Qianzhai Village, Fuxi Town, Zherong County, Fujian Province	2019/8/8
fj04	Chouling Village, Dongyuan Town, Zherong County, Fujian Province	2019/8/8
fj05	Baojianding Village, Zhayang Town, Zherong County, Fujian Province	2019/8/8
fj06	Huaping Village, Fuxi Town, Zherong County, Fujian Province	2019/8/8
fj07	Zhaizhong Village, Zhaizhong Town, Zherong County, Fujian Province	2019/8/8
fj08	Houlou Village, Junping Town, Zherong County, Fujian Province	2019/8/8
fj09	Puyang Village, Huangbai Town, Zherong County, Fujian Province	2019/8/8
fj10	Huacuo Village, Yingshan Town, Zherong County, Fujian Province	2019/8/8
fj11	Houping Village, Yingshan Town, Zherong County, Fujian Province	2019/8/8
sd01	Dalong Xingwang Village, Daxing Town, Linyi City, Shandong Province	2019/8/14
sd02	Shangshihe Village, Yushan Town, Linyi City, Shandong Province	2019/8/14
sd03	Chenxunhui Village, Diantou Town, Linyi City, Shandong Province	2019/8/14
sd04	Yuanlingdong Village, Yushan Town, Linyi City, Shandong Province	2019/8/14
sd05	Langlin Village, Diantou Town, Linyi City, Shandong Province	2019/8/14
sd06	Beiquan Village, Yushan Town, Linyi City, Shandong Province	2019/8/14
sd07	Dongzhu Cangyi Village, Yushan Town, Linyi City, Shandong Province	2019/8/14
sd08	Zhucang Weili Village, Yushan Town, Linyi City, Shandong Province	2019/8/14
sd09	Xiaopo Village, Daxing Town, Linyi City, Shandong Province	2019/8/14
sd10	Gucheng Village, Xianggou Town, Linyi City, Shandong Province	2019/8/14
sd11	Qichahe Village, Yushan Town, Linyi City, Shandong Province	2019/8/14
sd12	Hewan Village, Yushan Town, Linyi City, Shandong Province	2019/8/14
gz01	Gaochangba Village, Shibing County, Guizhou Province	2019/8/1
gz02	Xiawengshao Village, Shibing County, Guizhou Province	2019/8/1
gz03	Maxi Township, Shibing County, Guizhou Province	2019/8/1
gz04	Huashan Village, Shuangjing Town, Guizhou Province	2019/8/1
gz05	Guantianba Village, Shuangjing Town, Guizhou Province	2019/8/1
gz06	Zoumaping Village, Shibing County, Guizhou Province	2019/8/1
gz07	Wengtang Village, Yangliutang Town, Guizhou Province	2019/8/1

gz08	Maoerdong Village, Guzhen Town, Guizhou Province	2019/8/1
gz09	Maoli Fishing Village, Zhong 'an Town, Pinghuang, Guizhou Province	2019/8/1
gz10	Luna Village, Yuanyangchang Town, Guizhou Province	2019/8/1
gz11	Zijingguan Village, Dachang Town,Shibing County, Guizhou Province	2019/8/1
gz12	Tongmuxiang Village, Dachang Town,Shibing County,Guizhou Province	2019/8/1
gz13	Zhongteng Village, Yongxi Town, Zhenyuan City, Guizhou Province	2019/8/1

Table S2 Normality Test of Maximum Response Value of Electronic Nose Sensor

	Shpiro-Wilk	
Sensor	Statistics	Significance
LY2/LG	0.900	0.132
LY2/G	0.889	0.094
LY2/AA	0.884	0.081
LY2/Gh	0.880	0.070
LY2/gCTI	0.874	0.059
LY2/gCT	0.896	0.119
T30/1	0.922	0.269
P10/1	0.931	0.351
P10/2	0.933	0.370
P40/1	0.932	0.357
T70/2	0.915	0.214
PA/2	0.930	0.342

Table S3 25 of the 48 Common Compounds Contained Aroma Characteristics

Retention Time	Peak Number	Compound Name	Aroma Characteristics
4.46	1	1-Pentanol	Grass odor, Balsam odor, Fruity odor
5.12	2	Hexaldehyde	Fish odor, Fruity odor, Grass odor
7.70	3	1-Hexanol	Grass odor, Slight sawdust odor, Resin odor

8.31	4	Furfuryl alcohol	Burnt odor, Caramel odor, Fermentation odor
11.44	5	1-Octen-3-ol	Earthy odor, Dust odor, Mushroom odor
11.67	6	2-Pentylfuran	Mung bean odor, Butter odor, Fruity odor
11.91	7	Ethyl hexoate	Fruity odor, Wine caramel odor, Fennel odor
14.03	9	(E)-2-octenal	Burnt odor, Mushroom odor
15.35	11	Nonyl aldehyde	Citrus odor, Fruity odor, Grass odor
17.195	13	(E)-2-nonenal	Earthy odor, Grass odor, Plastic odor
17.61	14	n-Decanol	Grease odor
18.16	15	Ethyl caprylate	Fruity odor, Grass odor, Menthol odor
18.45	16	Decyl aldehyde	Burnt odor, Grass odor, Citrus odor
20.76	21	Amyl caproate	Fruity odor
20.995	22	Ethyl decanoate	Fruity odor, Grape odor
21.08	23	n-Hexadecane	Fruity odor, Fuel odor
22.98	25	Eugenyl acetate	Clove odor, Balsam odor, Fruity odor
23.395	28	Hexyl hexanoate	Fruity odor, Grass odor
23.74	30	n-Tetradecane	Fuel odor, Slight vegetation odor
24.06	31	Geosmin	Earthy odor, Beetroot odor
26.26	36	n-Pentadecane	Grass odor
35.66	43	Methyl hexadecanoate	Grease odor, Wax odor
36.99	44	Ethyl palmitate	Slightly sweet odor, Wax odor
40.09	47	Linoleic acid	Citrus odor
40.185	48	Ethyl oleate	Floral odor

Table S4 Normality Test of HS-SPME-GC-MS Peak Area Percentage of Common Peaks

Peak Number	Shpiro-Wilk	
	Statistics	Significance
2	0.934	0.379
3	0.652	0.000
4	0.851	0.030
5	0.943	0.492
6	0.914	0.206

7	0.968	0.875
9	0.979	0.974
11	0.971	0.911
14	0.928	0.318
15	0.911	0.190
16	0.962	0.778
17	0.952	0.631
22	0.846	0.025
23	0.822	0.013
25	0.908	0.172
28	0.896	0.118
30	0.949	0.577
31	0.766	0.003
36	0.854	0.032
43	0.854	0.032
44	0.656	0.000
47	0.578	0.000
48	0.657	0.000

Table S5 Correlation Analysis between HS-SPME-GC-MS Data and Electronic Nose Data

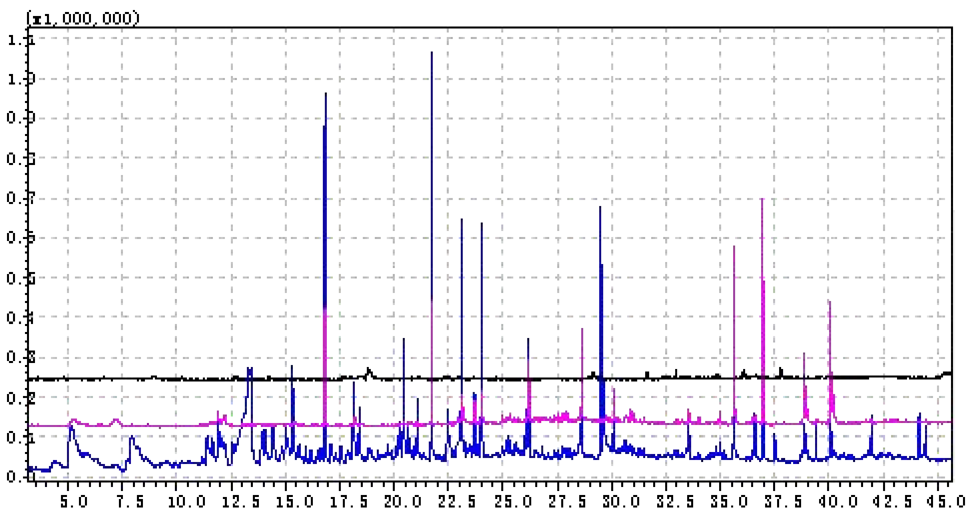
Sensor	Chromatographic Peak						
	5	16	22	25	28	44	47
LY2/LG	/	/	-0.601*	/	-0.576*	0.669*	0.694**
LY2/G	/	/	/	-0.599*	/	-0.58*	-0.653*
LY2/AA	/	/	/	-0.586*	/	-0.628*	-0.692**
LY2/Gh	/	/	/	-0.599*	/	-0.58*	-0.653*
LY2/gCTI	/	/	/	-0.599*	/	-0.58*	-0.653*
LY2/gCT	/	/	/	-0.599*	/	-0.58*	-0.653*
T30/1	0.621*	/	/	0.687**	/	0.597*	0.744**
P10/1	0.637*	-0.575*	-0.595*	0.799**	/	0.591*	0.749**
P10/2	0.637*	-0.575*	-0.595*	0.799**	/	0.591*	0.749**
P40/1	0.621*	/	/	0.687**	/	0.597*	0.744**

T70/2	0.637*	-0.575*	-0.595*	0.799**	/	0.591*	0.749**
PA/2	0.637*	-0.575*	-0.595*	0.799**	/	0.591*	0.749**

Note: * means P value < 0.05 has correlation, * * means P value < 0.01 has correlation, / means P value > 0.05 has no correlation

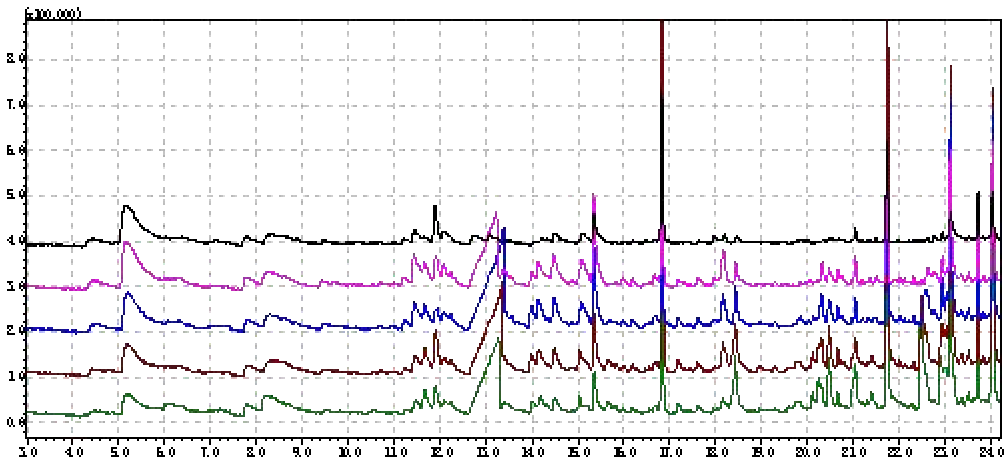
Table S6 Aroma characteristics of 7 Related Compounds

Retention Time	Peak Number	Compound	Aroma Characteristics
11.44	5	1-Octen-3-ol	Earthy odor, Dust odor
18.45	16	Decyl aldehyde	Burnt odor, Grass odor, Citrus odor
20.995	22	Ethyl decanoate	Fruity odor, Grape odor
22.98	25	Eugenyl acetate	Clove odor, Balsam odor, Fruity odor
23.395	28	Hexyl hexanoate	Fruity odor, Grass odor
36.99	44	Ethyl palmitate	Slightly sweet odor, Wax odor
40.09	47	Linoleic acid	Citrus odor



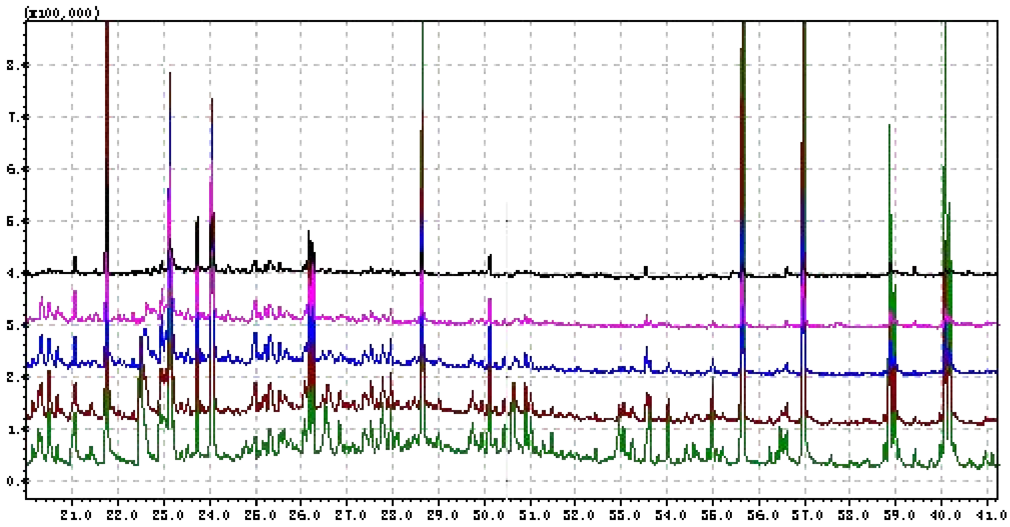
■—85μm PA，■—100μm PDMS，■—65μm PDMS/DVB

Figure S1 GC Chromatograms of Pseudostellariae Radix Sample Extracted with 3 Solid Phase Microextraction



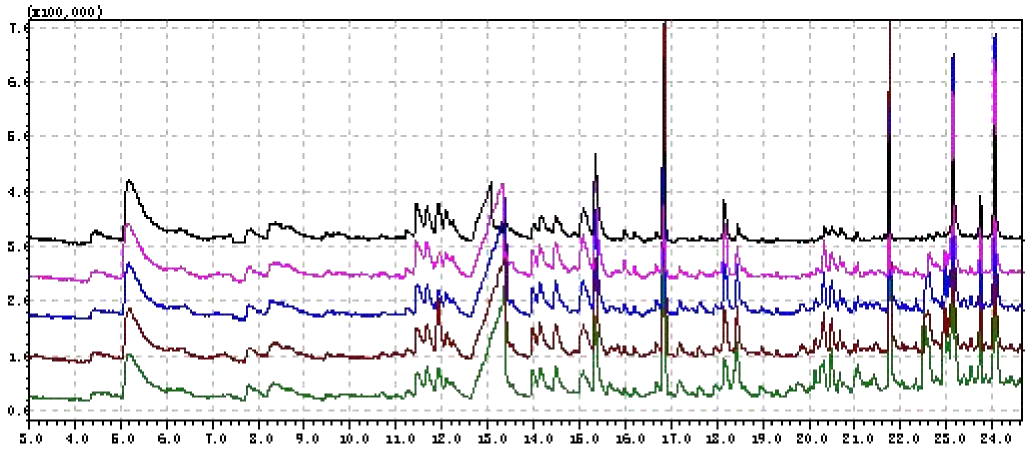
■—50 °C，■—60 °C，■—70 °C，■—80 °C，■—90 °C

Figure S2 GC Chromatograms of Pseudostellariae Radix Sample with Retention Time of 3-24 min at 5 Temperatures



■—50 °C，■—60 °C，■—70 °C，■—80 °C，■—90 °C

Figure S3 GC Chromatograms of Pseudostellariae Radix Sample with Retention Time of 20-41 min at 5 Temperatures



■—10 min，■—20 min，■—30 min，■—40 min，■—50 min

Figure S4 GC Chromatograms of Pseudostellariae Radix Sample with Retention Time of 3-24 min at 5 Time of Extraction

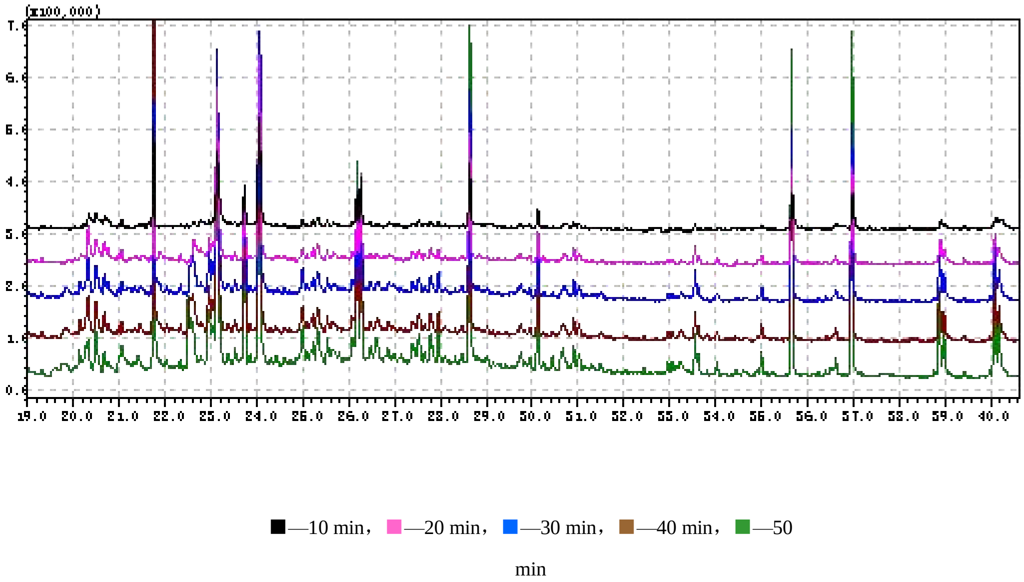


Figure S5 GC Chromatograms of Pseudostellariae Radix Sample with Retention Time of 19-40 min at 5 Time of Extraction

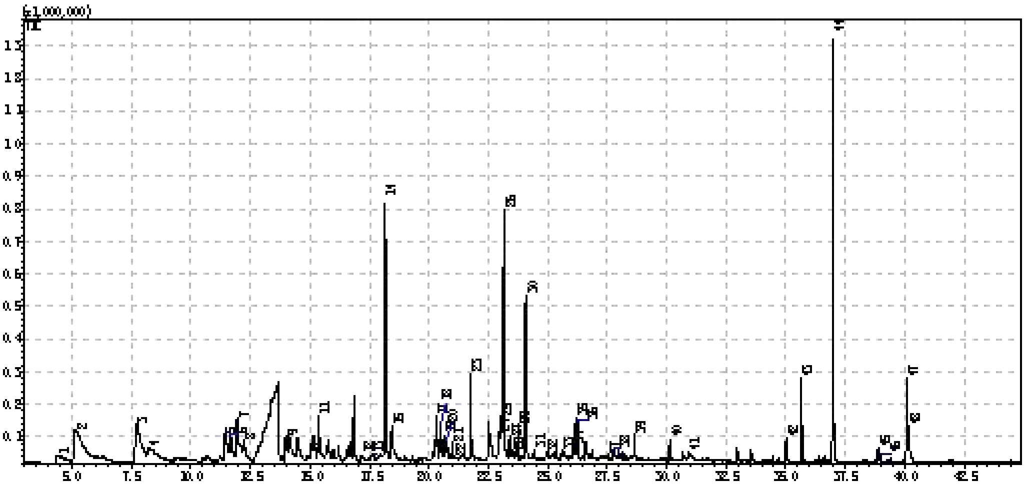


Figure S6 Total Ion Chromatogram (TIC) of Pseudostellariae Radix Sample