

Origin identification of Phoebe zhennan and Phoebe bournei by SPME-GC-MS

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Abstract

Phoebe zhennan S. Lee (Lauraceae) and *Phoebe bournei* (Hemsl.) Yang are favored for their fine-grain, insect resistance, special fragrance, not easily cracked and deformed, which had been widely used in furniture and construction. Due to the difference in wood quality characteristics and economic value of genus *Phoebe* species from different origins, it is important to establish a fast, efficient, and reliable method to identify the origin of *Phoebe zhennan* and *Phoebe bournei*. Solid phase micro-extraction (SPME) was used as sample preparation approach and gas chromatography-mass spectrometry (GC-MS) technique was employed to analyze the volatile compounds qualitatively. As a result, a total of 40 volatiles were identified by SPME-GC-MS among *Phoebe zhennan* samples from four origins, while 35 substances identified among *Phoebe bournei* samples. Significant difference was detected in the volatiles of *Phoebe zhennan* from different origins except for Guizhou and Yunnan. However, the wood samples of *Phoebe bournei* from all three origins could be discriminated by GC-MS spectroscopy. This finding indicated that genus *Phoebe* species from different origins had different chemical composition, which verified that the SPME-GC-MS technique can be used to identify different species of wood with characteristic compounds.

1. Introduction

Phoebe zhennan S. Lee (Lauraceae) and *Phoebe bournei* (Hemsl.) Yang are trees belonging to the genus *Phoebe* Nees of the Lauraceae family (Zhang et al., 2019). *Phoebe zhennan* is found mainly in western Hubei, northwest Guizhou, and Sichuan, while *Phoebe bournei* is usually cultivated in Guangdong, Fujian, Guangxi, and Guizhou. These two kinds of wood are favored for their fine-grain, insect resistance, special fragrance, not easily cracked and deformed, which had been widely used in furniture and construction (Pan et al., 2018, Wang et al., 2019, Yang et al., 2018). The wood properties of *Phoebe zhennan* and *Phoebe bournei* depending on the origin, growing environment and cultivation method. It is commonly believed that the *Phoebe zhennan* population from Sichuan Province showed the best wood quality traits and have the highest price in comparison to all origins (Liu, 2016).

The macrostructural and microstructural differences of genus *Phoebe* species from different origins are not obvious (Lei et al., 2018). It is very difficult for non-professional identifiers to distinguish among them. However, both *Phoebe zhennan* and *Phoebe bournei* have a special odor, making them suitable for the analysis by gas chromatography-mass spectrometry (GC-MS) approach (Zhou et al., 2015, Xie et al., 2015). The technique of GC-MS has been widely employed to analyze complex and unknown samples because of its rapid, sensitivity, strong qualitative and other superior traits. As a natural polymer composite, wood contains a number of separable extracts. In order to obtain volatile profiles, solid phase microextraction (SPME) technique has been used. SPME was considered as a fast, simple, economical sensitive and solvent free volatile extraction method before the GC-MS analysis. In recent years, the SPME-GC-MS technique has been used analyzing the volatile profiles of wood samples, such as oak (Duval et al., 2013b, Duval et al., 2013a, Vichi et al., 2007), eaglewood (He et al., 2012), and *Machilus pingii* (Xu et al., 2013).

In this study, the technique of SPME- GC-MS was employed for the geographical discrimination of *Phoebe zhennan* and *Phoebe bournei*. Meanwhile, the chemical fingerprint of two species was established and the feasibility of the GC-MS technique for wood origin discrimination will also be explored.

2. Materials And Methods

2.1 Samples

The Latin names, Chinese names, and places of origin of wood samples are presented in Table 1. Fifty-seven specimens were obtained from China National Forestry and Grassland Administration Wildlife Criminal Evidence Identification Center (Nanjing forest police college) including four origins of *Phoebe zhennan* and three origins of *Phoebe bournei*. The trees had an average diameter at breast of 21.7 cm and were 18–28 years old. From each tree, one cores was extracted at 1.3 m stem height by a 12-mm-diameter borer. Wood samples were chopped into pieces and finely powdered to pass a 40-mesh sieve. Then, 1.5 g of wood powder was added into a headspace bottle and extracted by SPME technique.

We declare that this study has the official permission to collect the plant sample and complies with the Chinese legislation. Wood species have been identified by Xiaoming Xue (based on macroscopic characteristics).

Table 1
Listing of samples for testing.

Latin name	Chinese name	Places of origin	Number of test samples
<i>Phoebe zhennan</i>	Zhennan	Hubei (Hefeng)	6
		Sichuan (Leshan, Gulan, and Dazhu)	9
		Yunnan (Zhaotong)	5
		Guizhou (Suiyang, Fanjing Mountain, Guandu, and Zunyi)	17
<i>Phoebe bournei</i>	Mingnan	Guizhou (Fanjing Mountain)	5
		Hubei (Laifeng)	6
		Guangxi (Baise, Jinxiu)	9

2.2 GC-MS instrument and conditions

GC-MS analysis was performed on an Agilent 7000B System (Agilent Technologies, USA). Separation was achieve using a DB-5MS column of 30 m × 0.25 mm × 0.25 μm (L × R × T) with a temperature program shown in Table 2. Using helium as the carrier gas with a column flow rate of 1 min/L. Mass spectrometry conditions included the scanning range of 33 ~ 450 amu, EI ionization mode, 70ev electron energy, and the National Institute of Standards and Technique 17 (NIST17; Gaithersburg, MD, USA) MS library. A mixture of 1 μL n-alkane (C7–C25) was run under the same capillary column and GC-MS conditions to obtain the retention index (RI). The peak area was chosen as the analytical signal for the relative amount. The relative contents of each volatiles were calculated by area normalization, and the average value of all tested wood samples was taken. Principal component analysis (PCA) was used in this study to obtain a preliminary overview of the general similarities and variations between the different origins of *Phoebe zhennan* and *Phoebe bournei*.

Table 2
Temperature program setting.

Temperature (°C)	Rate (°C/min)	Hold (min)	Total (min)
60	-	1	1
120	10	0	7
160	5	6	21
210	10	3	29

3. Results And Discussion

3.1 Results of GC-MS analysis

The volatile compounds extracted from *Phoebe zhennan* and *Phoebe bournei* were separated by GC-MS, in total 52 kinds of chemical composition (peak area above 0.5%) were scrutinized (Table 3, Table 4), including alkanes, olefins, alcohols, naphthalene, and aldehydes compounds.

Figure 1 shown the total ion chromatograms of *Phoebe zhennan*, the results clearly exhibited the different characteristic peaks of the samples from four origins. The characteristic peaks of the wood samples from Hubei and Sichuan mainly appeared above 6 min while the samples from Yunnan and Guizhou mainly appeared between 9 min and 25 min. We observed that the spectra of wood samples from Yunnan and Guizhou could not be used for origin identification because their characteristics peak are similar in number and intensity. Given the relative geographical proximity and the similar climatic conditions of the two origins, the samples from Hubei and Sichuan have similar volatile characteristics.

Figure 2 shown the total ion chromatogram of *Phoebe bournei* obtained from GC-MS, it can be seen that the characteristic peaks of all specimens mainly appeared between 9 min and 24 min, but the intensity of the peaks differed among the three origins. Results demonstrated that three origins of *Phoebe bournei* can be readily distinguished by visual inspection. In total, 35 volatiles were identified and presented in Table 4.

3.2 The difference analysis among four origins of *Phoebe zhennan*

The retention time, chemical name, relative content, RI, and CAS number are presented in Table 3. Wood samples of *Phoebe zhennan* from Hubei, Sichuan, Yunnan, and Guizhou Province were extracted by SPME technique. In this study, 40 kinds of chemical composition have been identified, indicating that this technique was suitable for the wood extraction with specific odors.

Among all the identified compounds, Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- was the constitutes found with the highest proportion in the *Phoebe zhennan* samples of the total chromatogram area. The trans-Verbenol and Copaene, were the second and the third most abundant volatiles, respectively. The relative content of α -Cubebene, Ylangene, Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1 α ,2 β ,4 β)]-, (-)-Aristolene, γ -Muurolene, Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)-, and (3R,5aS,9aR)-2,2,5a,9-

Tetramethyl-3,4,5,5a,6,7-hexahydro-2H-3,9a-methanobenzo[b]oxepine were significantly different in *Phoebe zhennan* samples from the four origins.

Six compounds were identified only in wood samples from Yunnan and Guizhou: trans-Verbenol, Cedrol, 2-Naphthalenemethanol, decahydro- $\alpha,\alpha,4a$ -trimethyl-8-methylene-, [2R-(2 α ,4 $\alpha\alpha$,8 $\alpha\beta$)]-, 2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydro- $\alpha,\alpha,4a,8$ -tetramethyl-, (2R-cis)-, Agarospirol and 2-((2R,8R,8aS)-8,8a-Dimethyl-1,2,3,4,6,7,8,8a-octahydronaphthalen-2-yl)propan-2-ol. It suggests that these substances may be thought of as characteristic components for the origin identification of *Phoebe zhennan* in Yunnan-Guizhou Plateau populations.

Figure 3 shows the 2-dimensional scatter plots of PCA based on the GC-MS data. Since the sum of PC1 and PC2 in the 3-dimensional scatter plot was 61.6% (Fig. 3a), a 3-dimensional scatter plot was presented (Fig. 3b). Most compounds identified were associated with positive scores on PC1 whereas 14 chemical compositions have negative scores. The highest positive PC1 loadings were observed assigned to Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)- and Bicyclo[3.1.1]heptan-2-one, 6,6-dimethyl-, (1R)-. These data are directly related to differences in the content of volatile components in woods.

In view of Fig. 3, samples from Yunnan seem to be closest to the samples from Guizhou, indicating that they can be easily confused. In contrast, the samples from Sichuan and Hubei exhibited independent clusters away from the Guizhou and Yunnan samples. This implies that the samples from Yunnan are chemically similar to Guizhou. The Guizhou Province and Yunnan Province are located in southwestern China, and shown typical karst landforms (Cheng et al., 2020). The region is located in a typical monsoon climate zone, with the highest moisture content in summer and the lowest moisture in winter. The monsoon's strength or weakness, and advancement or retreat, often result in the occurrence of flooding or droughts (Zheng, 2015). It is unreasonable to divide *Phoebe zhennan* provenance through administrative regions. Because of the close geographical proximity and the similar climatic conditions, it seems reasonable to combine wood samples from Yunnan and Guizhou into a single category.

Table 3
Chemical composition of extractives analyzed by GC-MS (*Phoebe zhennan*).

RT (min)	Chemical name	Relative content (%)				RI	CAS No.
		Hubei	Sichuan	Yunnan	Guizhou		
5.98	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	-	1.19 (0.1)	-	-	993	7785-70-8
6.3	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)-	3.93 (0.1)	4.17 (0.5)	0.79	1.01	960	36262-09-6
7.32	o-Cymene	2.09 (0.2)	3.35 (0.4)	-	-	1035	527-84-4
8.4	Benzene, 1-methyl-4-(1-methylethenyl)-	1.44	1.57 (0.1)	-	-	1102	1195-32-0
9.04	3-Cyclopentene-1-acetaldehyde, 2,2,3-trimethyl-, (R)-	2.37	-	-	-	1137	4501-58-0
9.31	Bicyclo[3.1.1]heptan-2-one, 6,6-dimethyl-, (1R)-	1.05	1.61 (0.1)	-	-	1153	38651-65-9
9.43	trans-Verbenol	-	-	8.97 (0.3)	10.23 (0.1)	1159	1820-09-3
9.75	Pinocarvone	2.07 (0.1)	2.06 (0.1)	0.73	0.81	1178	30460-92-5
10.39	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-	2.52 (0.1)	2.46 (0.1)	1.83 (0.1)	1.85	1213	515-00-4
10.67	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-	17.24 (0.4)	22.74 (0.6)	14.76 (0.4)	14.67 (0.2)	1226	80-57-9
13.46	α -Cubebene	6.78 (0.1)	3.02 (0.1)	4.50	3.34 (0.1)	1362	17699-14-8
13.97	Ylangene	2.18	1.68	3.82 (0.1)	1.14	1387	14912-44-8
14.1	Copaene	7.27 (0.1)	6.97	5.73 (0.2)	7.23 (0.1)	1392	3856-25-5
14.39	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1 α ,2 β ,4 β)]-	6.61 (0.1)	3.48 (0.1)	3.89 (0.1)	3.00	1406	515-13-9
14.85	cis- α -Bergamotene	1.50	1.19 (0.1)	0.82	1.23	1425	18252-46-5
15.01	1H-3a,7-Methanoazulene,2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3 α ,3 $\alpha\beta$,7 β ,8 $\alpha\alpha$)]-	2.69 (0.1)	2.77 (0.1)	2.06 (0.1)	2.89 (0.1)	1432	469-61-4
15.11	Caryophyllene	1.52	1.94 (0.1)	1.06	1.20	1437	87-44-5

Relative content deviations lower than 0.1% were not listed in the table.

RT (min)	Chemical name	Relative content (%)				RI	CAS No.
		Hubei	Sichuan	Yunnan	Guizhou		
15.41	cis-Thujopsene	4.08 (0.1)	5.71 (0.1)	4.65 (0.2)	5.89	1449	470-40-6
15.55	(-)-Aristolene	3.12	3.96 (0.1)	2.41 (0.1)	3.32 (0.1)	1455	6831-16-9
15.91	Humulene	1.04	1.04	-	0.95	1471	6753-98-6
16.4	γ-Muurolene	2.76 (0.08)	3.11 (0.6)	2.52	2.85	1492	30021-74-0
16.75	β-Guaiene	-	0.88	0.74	0.72	1505	88-84-6
16.89	Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1α,7β,8αα)]-	2.00	1.49	-	-	1509	4630-07-3
16.96	(3R,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol	0.96	-	2.07	2.37 (0.1)	1512	38230-60-3
17.19	2H-3,9a-Methano-1-benzoxepin,octahydro-2,2,5a,9-tetramethyl-, [3R-(3α,5αα,9α,9αα)]-	-	1.30	0.81	-	1520	5956-9-2
17.27	Benzene, 1-methyl-4-(1,2,2-trimethylcyclopentyl)-, (R)-	0.90	1.30	1.28	1.82 (0.3)	1521	16982-00-6
17.65	Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)-	6.98 (0.2)	6.41 (0.2)	3.72	5.27 (0.1)	1533	483-76-1
17.98	Cubenene	1.59 (0.1)	-	-	-	1544	29837-12-5
18.35	α-Calacorene	0.92	1.21	-	0.69	1556	21391-99-1
18.54	(3R,5aS,9aR)-2,2,5a,9-Tetramethyl-3,4,5,5a,6,7-hexahydro-2H-3,9a-methanobenzo[b]oxepine	1.65	2.35 (0.1)	3.18	2.61	1563	5956-12-7
19.71	1-((1R,2R,3R)-2-(3-Isopropylfuran-2-yl)-3-methylcyclopentyl)ethanone	2.6 (0.1)	2.98 (0.6)	-	-	1599	1143-45-9
19.82	Caryophyllene oxide	1.35 (0.1)	-	3.27	3.01 (0.1)	1602	1139-30-6
20.68	Cedrol	-	-	2.13	2.27	1627	77-53-2

Relative content deviations lower than 0.1% were not listed in the table.

RT (min)	Chemical name	Relative content (%)				RI	CAS No.
		Hubei	Sichuan	Yunnan	Guizhou		
20.82	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	2.58 (0.1)	-	0.75	0.82	1631	19888-34-7
21.12	2-Naphthalenemethanol,decahydro- $\alpha,\alpha,4a$ -trimethyl-8-methylene,[2R-(2 $\alpha,4\alpha,8\alpha\beta$)]-	-	-	2.60 (0.1)	1.80	1639	473-15-4
21.29	2-Naphthalenemethanol,1,2,3,4,4a,5,6,7-octahydro- $\alpha,\alpha,4a,8$ -tetramethyl-(2R-cis)-	-	-	1.47	1.70	1644	1209-71-8
21.64	Longiverbenone	1.60 (0.1)	1.26 (0.1)	0.90	-	1654	64180-68-3
21.84	Agarospirol	-	-	6.48 (0.1)	6.83 (0.2)	1659	1460-73-7
22.39	2-((2R,8R,8aS)-8,8a-Dimethyl-1,2,3,4,6,7,8,8a-octahydronaphthalen-2-yl)propan-2-ol	-	-	5.55	5.79 (0.1)	1661	20489-45-6
23.1	8-Isopropyl-1,5-dimethyltricyclo[4.4.0.0 ^{2,7}]dec-4-en-3-one	4.62 (0.2)	6.81 (0.5)	6.51 (0.2)	2.69	1696	1209-91-2
Relative content deviations lower than 0.1% were not listed in the table.							

3.3 The difference analysis among three origins of *Phoebe bournei*

The relative content of the same ingredient in *Phoebe bournei* samples from the three origins were different. There were 25 common volatile compounds among the three kinds of wood specimens (Table 4). The main components of *Phoebe bournei* samples from Guangxi were Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- (13.09%), trans-Verbenol (8.40%), Copaene (7.00%), 2-((2R,8R,8aS)-8,8a-Dimethyl-1,2,3,4,6,7,8,8a-octahydronaphthalen-2-yl)propan-2-ol (6.86%), 8-Isopropyl-1,5-dimethyltricyclo[4.4.0.0^{2,7}]dec-4-en-3-one (6.81), and Agarospirol (6.06%), while Guizhou origins were Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- (13.09%), trans-Verbenol (10.24%) and Agarospirol (6.40%). Analysis also revealed the presence of compounds in Hubei origin with Bicyclo[3.1.1]hept-3-en-2-one,4,6,6-trimethyl- (19.63%), trans-Verbenol (13.52%) and cis-Thujopsene (6.49%) being the most predominant.

In addition to these volatiles, it is worth noting the presence of Borneol, Cubenol, Ylangenal, since these unique compounds can be used as evidence for the identification of the tree origins. Cubenol and Ylangenal were found exclusively in *Phoebe bournei* of Guangxi origin and Borneol was only found in Guizhou origin, a geographical classification could be achieved using a detect of these compounds. GC-MS analyzing and comparing characteristic components of fingerprint can provide theory basis for the identification of *Phoebe bournei* origins.

Figure 4 shown the scatter plot with the top two and three PCs based on the relative content based on the volatiles listed in Table 4. The first three components represented 70% of total variance, with PC1 accounting for 44.5%, and PC2 accounting for 21.9% of the variance, easily distinguishing three clusters of wood with similar chemical

types. Since same origins of *Phoebe bournei* samples exhibited close clustering, the origin of *Phoebe bournei* wood samples could be identified through PCA of the chemical compounds data from GC-MS analysis.

Table 4
Chemical composition of extractives analyzed by GC-MS (*Phoebe bournei*).

RT (min)	Chemical name	Relative content (%)			RI	CAS No.
		Guangxi	Guizhou	Hubei		
6.3	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)-		1.18	2.02 (0.1)	960	36262-09-6
7.38	Benzene, 1-methyl-3-(1-methylethyl)-		1.30	1.58 (0.1)	1038	535-77-3
9.43	trans-Verbenol	8.40 (0.4)	10.24 (0.3)	13.52 (0.4)	1159	1820-09-3
9.75	Pinocarvone		1.29	2.05 (0.3)	1178	30460-92-5
9.87	Borneol		0.77 (0.1)		1184	507-70-0
10.21	Benzenemethanol, $\alpha,\alpha,4$ -trimethyl-		0.86	1.06 (0.1)	1203	1197-01-9
10.39	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-	1.68	2.54 (0.1)	3.15	1213	515-00-4
10.67	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-	13.09 (0.5)	18.90 (0.3)	19.63 (0.4)	1226	80-57-9
13.46	α -Cubebene	1.77	1.70	1.84	1362	17699-14-8
13.96	1,2,4-Metheno-1H-indene, octahydro-1,7a-dimethyl-5-(1-methylethyl)-, [1S-(1 α ,2 α ,3 $\alpha\beta$,4 α ,5 α ,7 $\alpha\beta$,8S*)]-	1.33 (0.1)	0.72	1.42 (0.1)	1386	22469-52-9
14.1	Copaene	7.00 (0.1)	5.61 (0.1)	5.19 (0.1)	1392	3856-25-5
14.39	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1 α ,2 β ,4 β)]-	4.73 (0.1)	2.76 (0.1)	2.60 (0.1)	1406	515-13-9
14.85	cis- α -Bergamotene	1.02	0.94	0.84	1425	18252-46-5
15.01	1H-3a,7-Methanoazulene,2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3 α ,3 $\alpha\alpha$,7 α ,8 $\alpha\alpha$)]-	1.86 (0.1)	2.11 (0.1)	2.38	1432	469-61-4
15.11	Caryophyllene	2.20 (0.2)	2.65 (0.1)	1.91	1437	87-44-5
15.41	cis-Thujopsene	4.69	5.37 (0.3)	6.49 (0.1)	1449	470-40-6
15.55	(-)-Aristolene	2.13	2.30	2.26 (0.1)	1455	6831-16-9

Relative content deviations lower than 0.1% were not listed in the table.

RT (min)	Chemical name	Relative content (%)			RI	CAS No.
		Guangxi	Guizhou	Hubei		
15.91	Humulene	0.98	1.00	0.73	1471	6753-98-6
16.39	(1S,4aR,8aS)-1-Isopropyl-7-methyl-4-methylene-1,2,3,4,4a,5,6,8a-octahydronaphthalene	2.26 (0.1)	1.55	1.5 (0.1)	1492	6980-46-7
16.99	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	0.98	0.81 (0.1)	0.59	1513	483-75-0
17.19	2H-3,9a-Methano-1-benzoxepin,octahydro-2,2,5a,9-tetramethyl-, [3R-(3 α ,5 $\alpha\alpha$,9 α ,9 $\alpha\alpha$)]-	1.17	0.79 (0.1)		1520	5956-09-2
17.27	Benzene, 1-methyl-4-(1,2,2-trimethylcyclopentyl)-, (R)-	1.09 (0.1)	1.27 (0.1)	1.97 (0.1)	1522	16982-00-6
17.7	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-	5.93 (0.1)	4.67 (0.1)	3.20 (0.1)	1535	483-77-2
18.54	Cyclohexanemethanol, 4-ethenyl- α,α ,4-trimethyl-3-(1-methylethenyl)-, [1R-(1 α ,3 α ,4 β)]-	5.22 (0.2)	3.95 (0.1)	2.77 (0.1)	1562	639-99-6
19.82	Caryophyllene oxide	2.09 (0.1)	1.71 (0.1)	1.36	1602	1139-30-6
20.03	Isospathulenol	0.91 (0.1)		0.59	1608	88395-46-4
20.68	Cedrol	1.23 (0.1)	1.00 (0.1)	0.85	1627	77-53-2
21.12	2-Naphthalenemethanol,decahydro- α,α ,4a-trimethyl-8-methylene-, [2R-(2 α ,4 $\alpha\alpha$,8 $\alpha\beta$)]-	2.99 (0.2)	2.56	2.92 (0.1)	1639	473-15-4
21.29	2-Naphthalenemethanol,1,2,3,4,4a,5,6,7-octahydro- α,α ,4a,8-tetramethyl-, (2R-cis)-	1.70	2.00 (0.1)	1.28 (0.1)	1644	1209-71-8
21.5	Cubenol	1.10 (0.1)			1650	21284-22-0
21.66	Ylangenal	0.81			1654	41610-68-8
21.84	Agarospinol	6.06 (0.2)	6.40 (0.2)	4.37 (0.1)	1659	1460-73-7
22.39	2-((2R,8R,8aS)-8,8a-Dimethyl-1,2,3,4,6,7,8,8a-octahydronaphthalen-2-yl)propan-2-ol	6.86 (0.5)	5.41 (0.2)	4.38 (0.2)	1675	20489-45-6
22.85	Cyclohexanemethanol, 4-ethenyl- α,α ,4-trimethyl-3-(1-methylethenyl)-, acetate, [1R-(1 α ,3 α ,4 β)]-	1.18 (0.1)	0.64 (0.1)		1688	60031-93-8
23.1	8-Isopropyl-1,5-dimethyltricyclo[4.4.0.0 ^{2,7}]dec-4-en-3-one	6.81 (0.3)	4.35 (0.4)	4.19 (0.2)	1696	1209-91-2
Relative content deviations lower than 0.1% were not listed in the table.						

3.4 The difference analysis between *Phoebe zhennan* and *Phoebe bournei*

Phoebe zhennan was considered to be the most authentic Gold Phoebe, which has a much greater economic value than *Phoebe bournei*. As shown in Table 3 and Table 4, the relative contents in *Phoebe zhennan* samples were characterized by higher levels of α -Cubebene and Cedrol compared to *Phoebe bournei*, which allowed the differentiation between these two species. Interestingly, a high content of compounds such as Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- and Copaene could not be used as discriminating variables, since not all origins of genus *Phoebe* contained these substances.

Figure 5 shown the PCA results for the identified compounds listed in Table 3 and Table 4, and samples of *Phoebe zhennan* clearly developed independent clusters away from the *Phoebe bournei* on the PCA score plot. First two components explained approximately 60% of the total variability (40.5% for PC1 and 16.7% for PC2).

To distinguish *Phoebe zhennan* and *Phoebe bournei*, the volatiles only detected in one species were defined as characteristic compounds. As shown in Table 5 and Table 6, 17 kinds of specific phytoconstituents were identified in *Phoebe zhennan* wood samples, while 12 kinds of volatiles were only identified and treated as characteristic compounds in *Phoebe bournei*. In summary, *Phoebe zhennan* and *Phoebe bournei* can be identified successfully according to chemical composition.

4. Conclusions

In this study, SPME-GC-MS technique has been used to analyze *Phoebe zhennan* and *Phoebe bournei* wood samples. In total, 40 volatiles were identified in *Phoebe zhennan* samples and 35 substances identified in *Phoebe bournei*, these phytoconstituents were classified into alkanes, olefins, alcohols, naphthalene, and aldehydes compounds, with Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- showed the highest relative content in both species.

The origins of *Phoebe zhennan* and *Phoebe bournei* were well-separated based on GC-MS analysis, which indicated that volatile compounds play an important role in the geographical discrimination of wood samples. Meanwhile, a PCA scatterplot was able to separate genus *Phoebe* species from different origins. Furthermore, our experimental data suggested that the chemical composition of *Phoebe zhennan* from Yunnan and Guizhou are highly similar. It seems reasonable to combine Yunnan origin and Guizhou origin into a single category.

Because *Phoebe zhennan* and *Phoebe bournei* wood are expensive, it's valuable to establish a rapid and micro-destructive identification method. Although the prediction of *Phoebe zhennan* and *Phoebe bournei* by GC-MS technique and statistical analysis obtained high accuracy, given our limited number of samples, this approach may not work for all origins.

We also believe that the wood origin identification would be much more accuracy if more detailed analyses are performed. Therefore, it is necessary to combine chemical constituents of woods with other wood properties (such as vessels distribution, ring width or density, etc.) to make sure the classification results are accurate and satisfactorily reliable.

Declarations

Statement

On behalf of all authors, I declare that our research has no conflict of interest.

Availability of data and materials

The datasets generated and analyzed during the current study are available in the Figshare repository, [<https://doi.org/10.6084/m9.figshare.20410650.v1>]

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Tables

Tables 5 to 6 are available in the Supplementary Files section

Figures

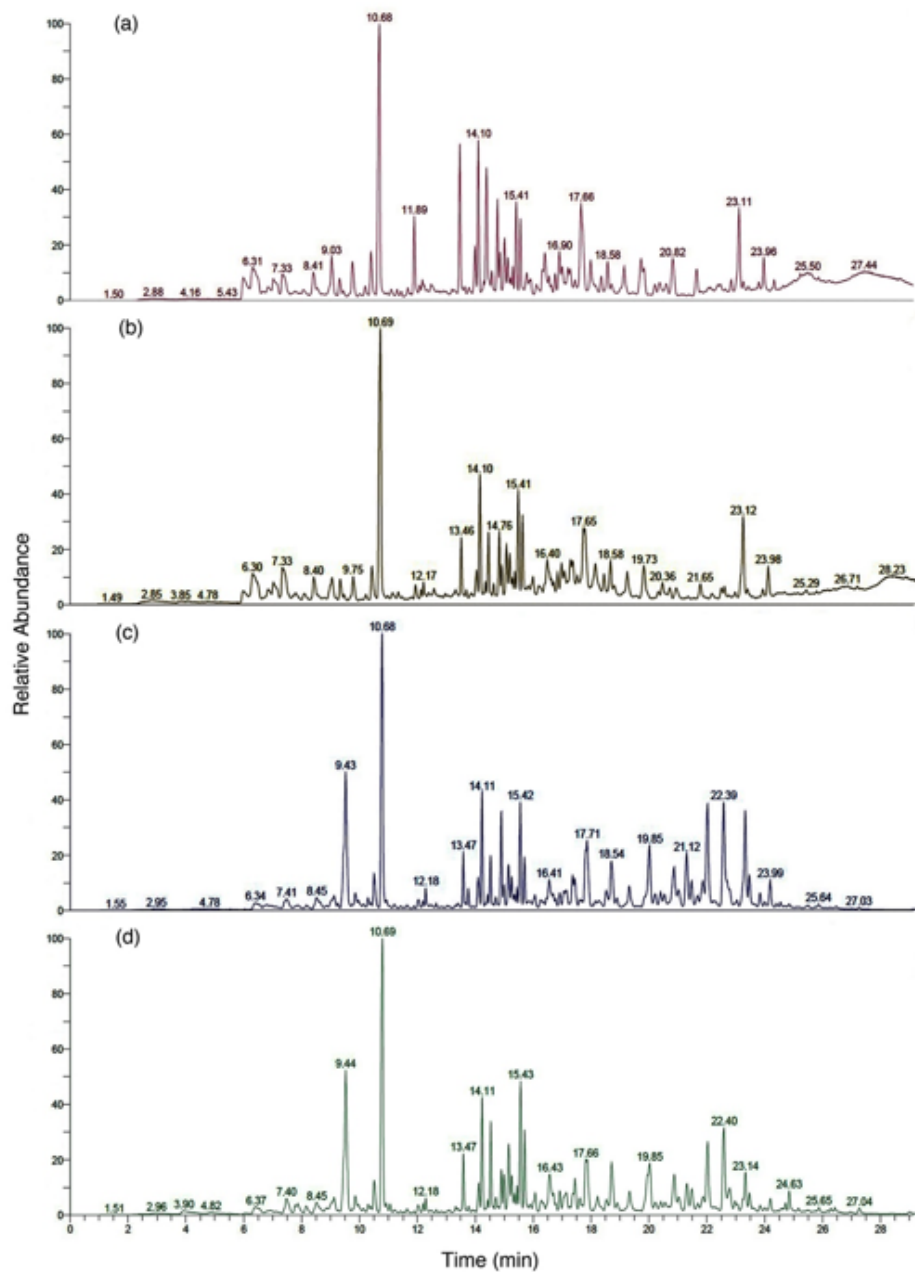


Figure 1

GC-MS total ion chromatogram of *Phoebe zhennan*. (a) GC-MS total ion chromatogram of specimens from Hubei. (b) GC-MS total ion chromatogram of specimens from Sichuan. (c) GC-MS total ion chromatogram of specimens from Yunnan. (d) GC-MS total ion chromatogram of specimens from Guizhou.

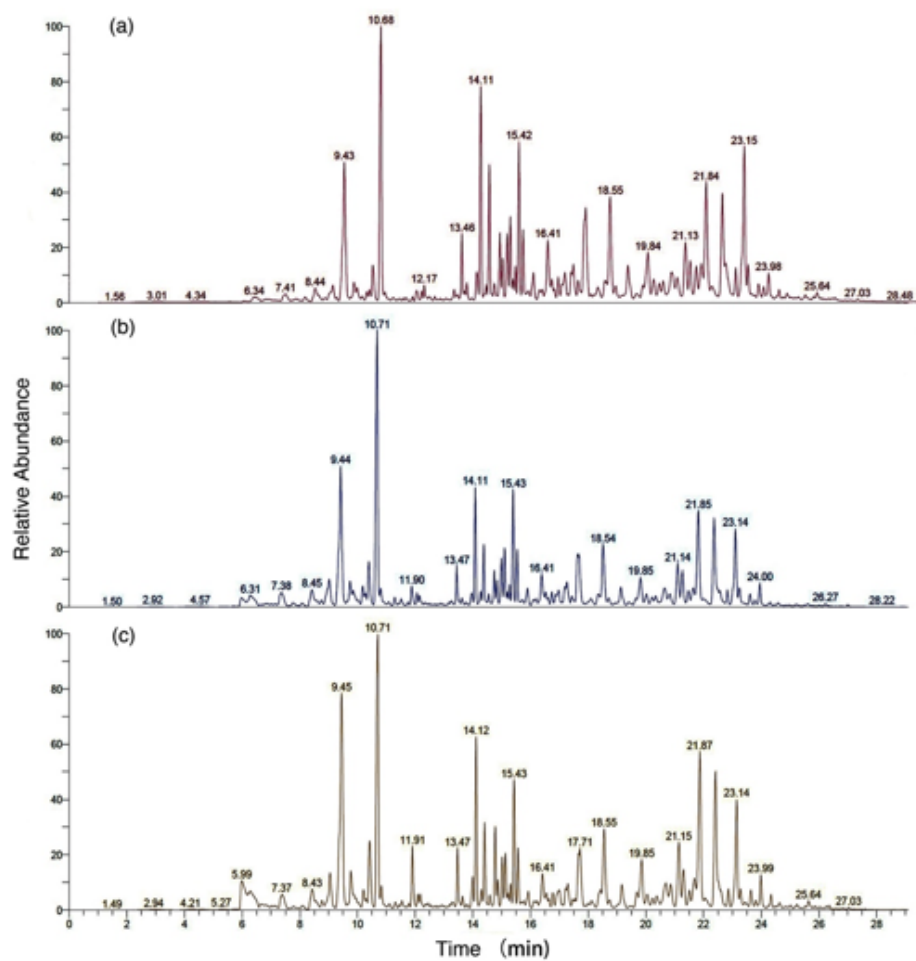


Figure 2

GC-MS total ion chromatogram of *Phoebe bournei*. (a) GC-MS total ion chromatogram of specimens from Guizhou. (b) GC-MS total ion chromatogram of specimens from Hubei. (c) GC-MS total ion chromatogram of specimens from Guangxi.

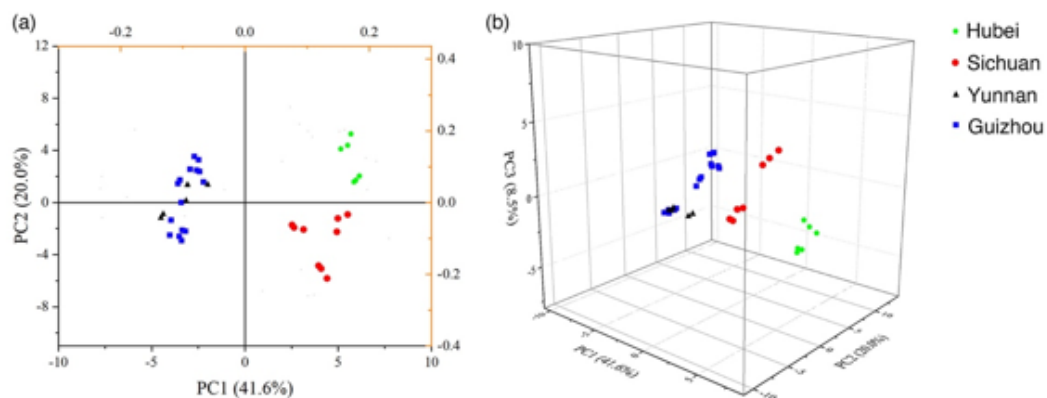


Figure 3

Scatter plot of top the (a) two and (b) three PCs of the *Phoebe zhennan* from four origins based on GC-MS data.

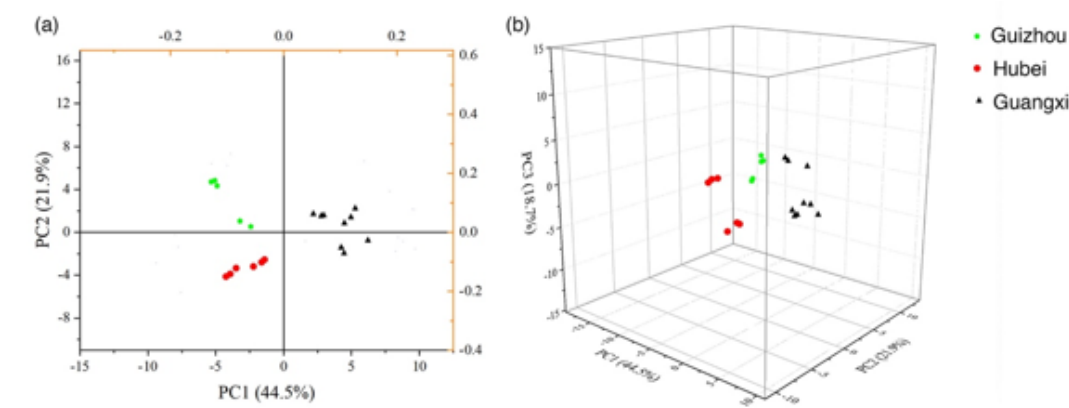


Figure 4

Scatter plot of top the (a) two and (b) three PCs of the *Phoebe bournei* from three origins based on GC-MS data.

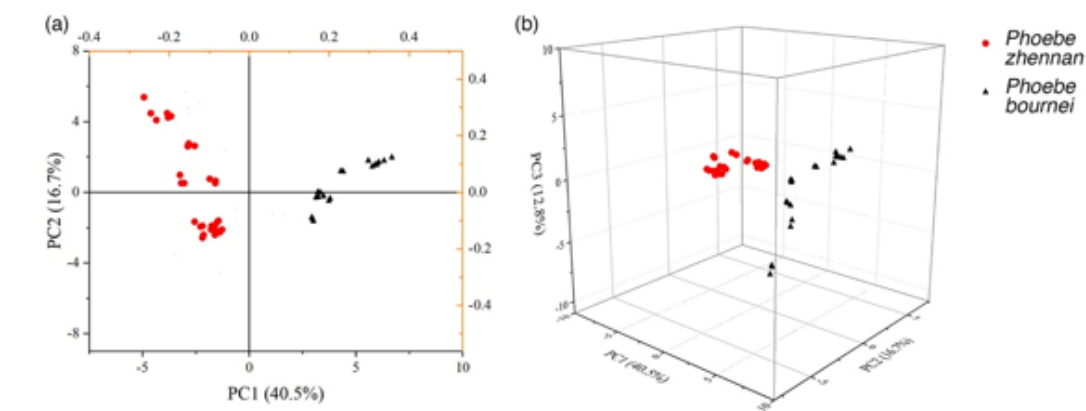


Figure 5

Scatter plot of top the (a) two and (b) three PCs of *Phoebe zhennan* and *Phoebe bournei* wood samples based on GC-MS data.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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