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GC–MS fingerprint of *Pogostemon cablin* in China

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Abstract

Pogostemon cablin, originating in Malaysia and India, is cultivated in southern China including Guangdong and Hainan Province, which was called GuangHuoXiang to differentiate it from the HuoXiang of the north, the species *Agastache rugosa*, that it resembles. Essential oil of *P. cablin* mainly contributes to the pharmacological activities and the therapeutic properties of the essential oils are directly correlated with their qualitative and quantitative composition. For controlling the quality, standard fingerprint of *P. cablin* collected from different regions was developed by using GC–MS. Nine compounds including β-patchoulene, caryophyllene, α-guaiene, seychellene, β-guaiene, δ-guaiene, spathulenol, patchouli alcohol and pogostone were identified among 10 main peaks in *P. cablin*. Hierarchical clustering analysis based on characteristics of 10 investigated peaks in GC profiles showed that 18 samples were divided into three main clusters, patchouliol-type, pogostone-type and an interim-type, which was the one between the two chemotypes. The simulative mean chromatogram for the three types *P. cablin* was generated using the *Computer Aided Similarity Evaluation System*. The fingerprint can help to distinguish the substitute or adulterant, and further assess the differences of *P. cablin* grown in various areas of China.
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Keywords: GC–MS fingerprint; *Pogostemon cablin*; Quality control; Patchouli alcohol; Pogostone

1. Introduction

The quality control has always been the key issue in the development of herbal medicines. Fingerprint technique is a powerful tool and has been widely accepted as a useful approach for the evaluation and quality control of multi-component herbal materials and their finished products [1,2]. There have been a number of reports regarding the use of HPLC, GC, GC × GC, CE, HPTLC and IR fingerprints on the quality assessment of some herbal medicines and their raw materials [3–11].

Patchouli [*Pogostemon cablin* (Blanco) Benth.], originating in Malaysia and India, was imported into China for perfume and medicine during untold centuries, and the south China province Guangdong finally started growing its own around the eleventh century. It was called GuangHuoXiang to differentiate it from the HuoXiang of the north, the species *Agastache rugosa* that it resembles. Up to date, Patchouli is widespread in southern China, including Guangdong (Guangzhou, Gaoyao, Zhanjiang) and Hainan Province. The plant has been used as Chinese herbal medicine to remove dampness, relieve summer-heat, exterior syndrome, stop vomiting and stimulate the appetite [12]. Essential oil of *P. cablin* collected by steam distillation mainly contributes to the pharmacological activities [13], and the therapeutic properties of the essential oils are directly correlated with their qualitative and quantitative composition which is obviously different derived from various cultivation regions of Patchouli [14,15].

The aim of this study is to develop a characteristic fingerprint of *P. cablin* by using GC–MS to control the quality of the raw herb. This fingerprint can help distinguish the substitute or adulterant, and further assess the differences of *P. cablin* grown in various areas of China.

2. Materials and methods

2.1. Materials and chemicals

Fourteen batches of *P. cablin* were obtained from Huodao (two batches), Pudao and Liantang of Gaoyao, Yingli and Longmen (two batches) of Leizhou, Chengyue (three batches) of

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Suixi, Longdong of Guang zhou, Xuwen and Wuchuan, Guangdong Province, and Wancheng of Wanning, Hainan Province, respectively. Three batches of commercial sample for *P. cablin* were obtained from Gaoyao, Zhanjiang of Guangdong and Wanning of Hainan, respectively. A standard *P. cablin* material was obtained from National Institute for the Control of Pharmaceutical and Biological Products, China and *A. rugosa* was collected from Sichuan Province. All samples were collected ourselves. The botanical origins of all the materials were identified morphologically and microscopically by Dr. Cao Hui. The voucher specimens of samples were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macau, China.

Patchouli alcohol and pogostone were separated and purified ourselves. The structures were confirmed by comparing UV, MS and NMR data with literature [16,17]. Methanol for GC was purchased from Merck (Darmstadt, Germany).

2.2. Pressurized liquid extraction

Pressurized liquid extractions (PLE) were performed on a Dionex ASE 200 (Dionex Corp., Sunnyvale, CA, USA) system. In brief, raw materials of *P. cablin* were dried at 35 °C for 20 h and were ground into powder of 0.154 mm. Powder of 0.5 g Patchouli with stem and leaf (8:2) was mixed with diatomaceous earth (1.5 g) and placed into 11-ml stainless steel extraction cell, respectively. The use of a dispersion agent, such as diatomaceous earth, is recommended in order to reduce the solvent volume used for the extraction. The sample was extracted under the optimized conditions: solvent, methanol; temperature, 80 °C; particle size, 0.154 mm; static extraction time, 15 min; pressure, 1500 p.s.i.; static cycle, 1 and 60% of the flush volume. Then, extract was transferred to a 25 ml volumetric flask which was brought up to its volume with extraction solvent and filtered through a 0.45 μm Econofilter (Agilent Technologies) prior to injection into the GC–MS system.

2.3. GC–MS analysis

GC–MS was performed with an Agilent 6890 gas chromatography instrument coupled to an Agilent 5973 mass spectrometer and an Agilent ChemStation software (Agilent Technologies, Palo Alto, CA, USA). Compounds were separated on a 30 m × 0.25 mm i.d. capillary column coated with 0.25 μm film 5% phenyl methyl siloxane. The column temperature was at 80 °C for injection, temperature program began at 10 °C min^{-1} to 110 °C, then at 3 °C min^{-1} to 120 °C and held for 10 min. Furthermore, the temperature was increased at 2 °C min^{-1} to 134 °C, at 1 °C min^{-1} to 143 °C and held for 5 min, then at 5 °C min^{-1} to 240 °C, finally at 20 °C min^{-1} to 280 °C and held for 5 min. Split injection (2 μl) was conducted with a split ratio of 1:10 and helium was used as carrier gas of 1.0 ml min^{-1} flow-rate. The spectrometers were operated in electron-impact (EI) mode, the scan range was 40–550 amu, the ionization energy was 70 eV and the scan rate was 0.34 s per scan. The inlet, ionization source temperature were 250 °C and 280 °C, respectively.

2.4. Statistical software package

The statistical software package is SPSS 11.5 for windows (SPSS Inc, Chicago, IL, USA), which comprise a number of "procedures"—graphical, statistical, reporting, processing and tabulating procedures—that enable simple and rapid data evaluation. A method named as the nearest neighbor was applied, and Cosine, which is a pattern similarity measure, was selected as measurement for hierarchical cluster analysis. The correlation coefficients and the similarities of entire chromatographic patterns among tested samples, and the simulative mean chromatogram were calculated and generated using the Computer Aided Similarity Evaluation System (CASES, MATLAB 5.3 based, version 1.240, developed by Research Center for Modernization of Chinese Medicine, Central South University, China).

3. Results and discussions

3.1. Identification of components in patchouli

The gas chromatographic method is used almost exclusively for the qualitative analysis of the volatiles. Retention times were utilized as primary criterion for the peaks identification. However, the identification is practically difficult because the analyte can elute at the same time with other compounds in the sample. The mass spectrometer used as chromatographic detector offers additional data for the identification of the separated compounds. The most frequent identification method is the comparison of the recorded spectra with an MS library and/or reference standard compound.

Total ion chromatograms of PLE extracts from typical material of Patchouli were shown in Fig. 1. All the main components were separated completely, and 9 of 10 peaks were identified according to the mass spectrum of each component. By comparing with literatures [16–22] and standards, peaks 1–9 were identified as β-patchoulene, caryophyllene, α-guaiene, seychellene, β-guaiene, δ-guaiene, spathulenol, patchouli alcohol and pogostone, respectively (Fig. 2). The results are summarized in Table 1.

3.2. Validation of the method

To develop characteristic fingerprint, the experimental procedure was validated and contents of patchouli alcohol and pogostone in *P. cablin* were determined. The selected ion monitoring (SIM) method was used for the quantification of patchouli alcohol (*m/z* 222) and pogostone (*m/z* 168).

The calibration curves, which obtained from the selected ions peak area, for patchouli alcohol and pogostone were linear over the range 3.45–276.36 ng and 5.15–257.50 ng absolute on column, with the coefficients of correlation (*r*) were 0.9992 and 0.9994, respectively. The injection precision for patchouli alcohol and pogostone was determined by injecting successively standard for five times. The relative standard deviation of patchouli alcohol and pogostone was 2.57% and 1.84%, respectively.

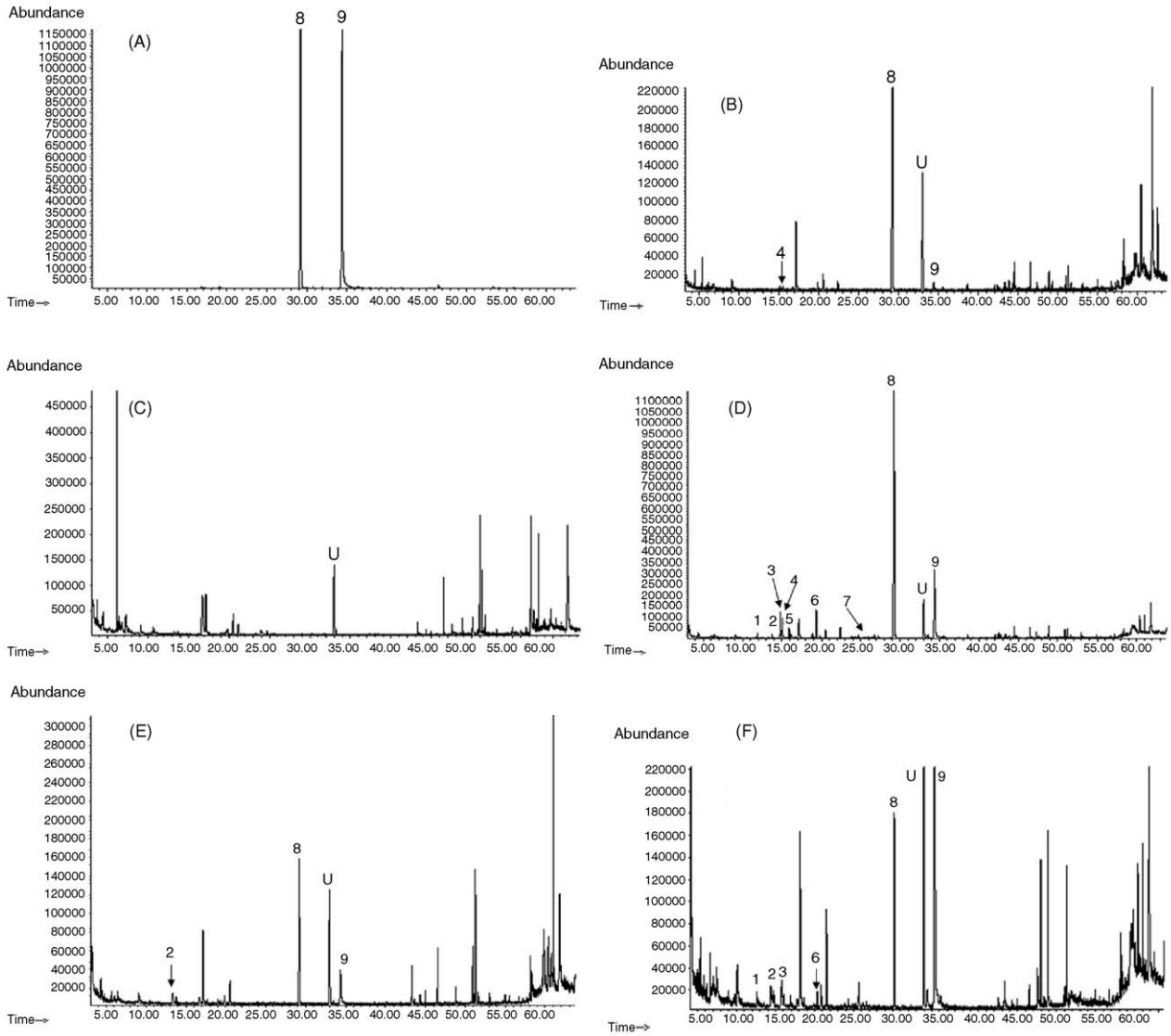

Fig. 1. GC–MS total ion chromatograms for different samples. (A) Mixture of standards, (8) patchouli alcohol and (9) pogostone; (B) standard material of *Pogostemon cablin* obtained from National Institute for the Control of Pharmaceutical and Biological Products, China; (C) *Agastache rugosa* collected from Sichuan Province; *Pogostemon cablin* collected from (D) Pudao of Gaoyao; (E) Longdong of Guangzhou; and (F) Liangtang of Gaoyao, Guangdong Province, respectively. (1) β-Patchoulene, (2) caryophyllene, (3) α-guaiene, (4) seychellene, (5) β-guaiene, (6) δ-guaiene, (7) spathulenol, (U) unknown.

Table 1
Mass data of 10 compounds identified from *Pogostemon cablin*

Compound	Rt (min)	Mass data[a]
β-Patchoulene	11.89	204(M^+, 76), 189(81), 161(100), 133(37), 105(51), 91(36), 41(20)
Caryophyllene	13.78	161(M^+, 30), 147(50), 133(95), 119(37), 105(31), 93(100), 91(71), 79(58), 69(53), 55(27), 41(49)
α-Guaiene	14.82	204(M^+, 60), 189(49), 161(31), 148(43), 147(96), 133(59), 121(39), 107(82), 105(100), 93(64), 81(44), 79(44), 41(30)
Seychellene	15.06	204(M^+, 28), 189(17), 175(6), 161(6), 147(10), 133(101), 122(100), 107(14),
β-Guaiene	15.90	204(M^+, 50), 189(35), 161(32), 147(15), 135(100), 133(22), 119(28), 107(91), 105(47), 93(65), 91(46), 81(12), 79(18), 55(18), 41(25)
δ-Guaiene	19.35	204(M^+, 33), 189(62), 161(20), 148(42), 147(46), 135(55), 121(34), 108(95), 107(100), 93(97), 79(50), 67(25), 55(27), 41(34)
Spathulenol	26.76	220(M^+, 7), 187(32), 162(39), 159(35), 145(32), 121(39), 107(45), 105(34), 93(57), 91(36), 81(37), 69(32), 43(100), 41(40)
Patchoulialcohol	29.21	222(M^+, 100), 207(35), 189(15), 179(20), 161(40), 138(77), 125(45), 109(34), 98(54), 95(37), 83(60), 81(52), 67(30), 55(29), 43(36), 41(38)
Unknown	32.98	168(M^+, 57), 125(8), 111(21), 97(35), 83(45), 69(43), 55(100), 41(30)
Pogostone	34.44	224(M^+, 36), 209(6), 181(35), 168(100), 153(93), 85(16), 69(12), 43(29), 41(9)

[a] *m/z*, relative intensity shown in parenthesis, and the ion of relative intensity 100 was used for the quantification.

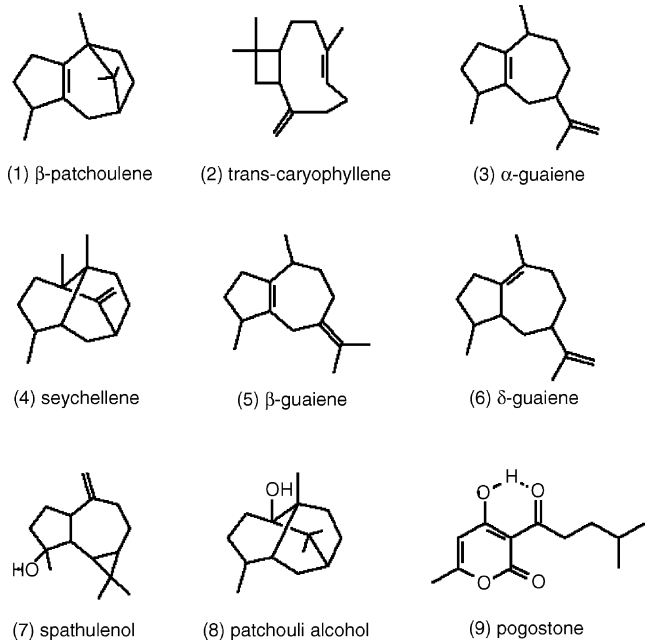

Fig. 2. The structures of nine identified compounds in *Pogostemon cablin*.

The stability of patchouli alcohol and pogostone was also determined by inject freshly prepared standard solution for three times at 0 h, 1 h, 2 h, 4 h, 8 h and 12 h, respectively. The R.S.D. of patchouli alcohol and pogostone was 2.52% and 3.97%, respectively. Thus, the quantitation of components such as patchouli alcohol and pogostone in Patchouli could be preformed within 12 h after the sample extraction.

In order to determine the recovery for the analytes, a known amount of patchouli alcohol and pogostone was added into Patchouli sample and extracted at specified conditions mentioned above. The extracted material was subjected to GC–MS, and the content of the analytes was calibrated. The recovery of patchouli alcohol and pogostone was 97.6% and 96.9% with relative standard deviation (R.S.D.) of 4.01% and 4.22%, respectively, where $n = 5$.

The contents of patchouli alcohol and pogostone in different Patchouli were determined by using the calibrated GC–MS. Table 2 shows the summary results on the contents of investigated compounds. The results showed that there is great variation for the contents of investigated components in Patchouli from different areas of China. And patchouli alcohol and pogostone were not detected in *A. rugosa*.

3.3. Fingerprint of P. cablin

In China, *P. cablin* cultivated in Guangzhou and Gaoyao regions of Guangdong Province was locally named as "Shipai Huoxiang"; While, that cultivated in Wuchuan, Suixi and Leizhou regions of Guangdong Province and Wanning region of Hainan Province was locally named as "Hainan Huoxiang". Traditionally, the former is considered as authentic herb. And the latter is not usually used as medicinal plants though the yield of volatile oil is much higher [18]. Our study showed that the chemical variation is obvious among *P. cablin* material from different regions. Fig. 3A shows the results of hierarchical cluster analysis based on 10 peaks characteristics from GC profiles of the tested 18 samples of *P. cablin*, which are divided into three main clusters, are different from *A. rugosa*. It was reported that there were two chemotypes, i. e. pogostone-type and patchouliol-type, of *P. cablin* [23,24]. Our results showed there was a cluster, named interim-type, between two chemotypes. Among the peaks of chromatograms for cluster analysis of samples, two typical peaks of patchouli alcohol and pogostone were optimized based on cluster analysis of 10 peaks and then principle component analysis. Using the peaks characteristics of the two compounds, hierarchical cluster analysis of the tested 18 samples was performed as before. The result was very similar to the one derived from 10 peaks characteristics, which was in good accordance with the two chemotypes (Fig. 3B). Therefore, the characteristics of peaks, especially patchouli alcohol and pogostone, from GC profiles could be used as markers for quality control of *P. cablin*.

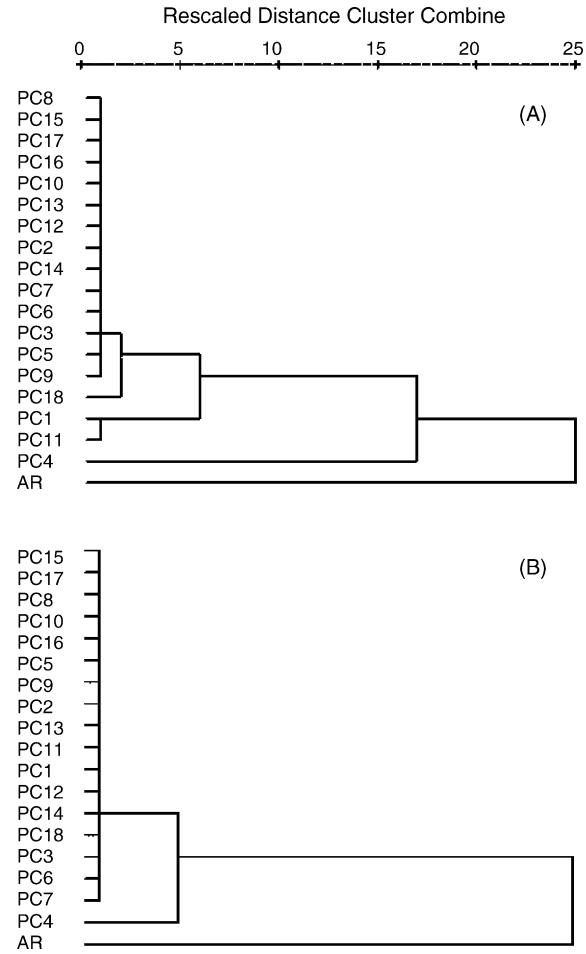

Fig. 3. Dendrograms resulting from single linkage between groups hierarchical cluster analysis. The hierarchical clustering was done by SPSS software. A method named as the nearest neighbor was applied, and Cosine was selected as measurement. (A) Dendrogram resulting from 10 peaks, their retention times and peak area, derived from GC fingerprints of the tested 18 Patchouli samples. (B) Dendrogram resulting from the characteristics of two peaks, patchouli alcohol and pogostone, derived from GC profiles of the tested 18 Patchouli samples. PC1-PC18 was the same as that described in Table 2. AR is *Agastache rugosa* collected from Sichuan Province.

Table 2
Absolute (mg/g) and relative contents (%) of investigated compounds in *Pogostemon cablin* collected from different regions of China

Samples	β-Patchoulene	Caryophyllene	α-Guaiene	Seychellene	β-Guaiene	δ-Guaiene	Spathulenol	Patchouli alcohol (Pat)	Unknown	Pogostone (Pog)	Pat/Pog[a]
PC1[b]	–[c]	+[d]	+	+	–	+	–	1.84[e]	+	0.31	5.95
	–	3.65[f]	1.66	1.15	–	0.85	–	49.69	34.64	8.36	
PC2	+	+	+	+	+	+	+	5.85	+	1.04	5.62
	0.42	0.78	5.51	3.60	1.79	8.48	1.01	61.22	6.30	10.90	
PC3	+	+	+	+	+	+	+	4.56	+	1.48	3.09
	5.83	0.54	4.52	3.40	1.71	5.41	0.40	56.55	8.58	18.31	
PC4	+	+	+	–	–	+	–	1.85	+	2.63	0.70
	1.68	1.39	1.55	–	–	1.23	–	22.93	38.55	32.67	
PC5	+	+	+	+	+	+	+	6.78	+	1.38	4.91
	0.52	1.41	7.80	3.60	2.45	12.51	0.95	50.01	10.56	10.19	
PC6	+	+	+	+	+	+	+	7.40	+	2.24	3.30
	0.53	1.58	7.41	3.32	1.98	8.02	1.98	53.24	5.80	16.16	
PC7	+	+	+	+	+	+	+	4.06	+	1.52	2.67
	0.24	0.74	5.27	3.16	1.38	9.34	0.50	56.15	2.22	21.01	
PC8	+	+	+	+	+	+	+	6.54	+	2.24	2.91
	0.46	1.28	6.93	2.83	1.86	10.64	0.92	51.66	5.70	17.73	
PC9	+	+	+	+	+	+	+	7.45	–	1.52	4.90
	0.75	2.09	10.23	4.59	3.28	10.30	2.88	54.68	–	11.17	
PC10	+	+	+	+	+	+	+	5.11	+	1.08	4.74
	0.34	0.63	4.11	2.60	1.42	5.89	0.59	59.69	12.17	12.58	
PC11	–	+	–	–	–	–	–	1.77	+	0.26	6.79
	–	1.64	–	–	–	–	–	51.27	39.54	7.55	
PC12	+	+	+	+	+	+	+	4.75	+	0.62	7.62
	0.48	1.24	7.01	3.68	2.04	6.84	2.58	58.67	9.78	7.70	
PC13	+	+	+	+	+	+	+	5.00	+	0.53	9.50
	0.52	1.19	7.37	3.27	1.99	7.24	2.61	57.35	12.43	6.04	
PC14	+	+	+	+	+	+	+	6.51	+	1.02	6.39
	0.69	1.32	7.73	4.28	2.68	11.766	1.024	54.78	7.15	8.58	
PC15	+	+	+	+	+	+	+	6.46	+	1.12	5.78
	0.44	1.07	6.45	4.17	1.79	9.485	1.235	59.10	6.05	10.22	
PC16	+	+	+	+	+	+	+	4.66	+	1.01	4.64
	0.41	0.68	4.59	3.56	1.50	6.310	0.938	60.50	8.47	13.05	
PC17	+	+	+	+	+	+	+	3.67	+	0.66	5.57
	0.33	0.75	5.04	2.76	1.59	7.949	0.845	60.31	9.58	10.84	
PC18	–	–	–	+	–	–	–	2.55	+	±[g]	–
	–	–	–	0.49	–	–	–	78.15	21.36	±	–

[a] Pat/Pog, the content ration of patchouli alcohol and pogostone.
[b] PC1-PC18 are *P. cablin* derived from Huodao (two batches), Pudao and Liantang of Gaoyao, Yingli and Longmen (two batches) of Leizhou, Chengyue (three batches) of Suixi, Longdong of Guang zhou, Xuwen and Wuchuan, Guangdong Province, Wancheng of Wanning, Hainan Province, Gaoyao and Zhanjiang of Guangdong, Wanning of Hainan and a standard *Pogostemon cablin* material, obtained from National Institute for the Control of Pharmaceutical and Biological Products, China, respectively.
[c] Undetected.
[d] Absolute content was not determined without standard compound.
[e] The data was presented as average of three replicates (R.S.D. < 3%).
[f] The percentage of peak area in 10 investigated compounds.
[g] Under the limit of quantification.

Fig. 4A shows the standardized TIC profiles for 18 samples of *P. cablin*. The correlation coefficient of each chromatogram to their simulative mean chromatogram was 0.982 ± 0.010 (mean ± S.D., patchouliol-type of *P. cablin*, $n = 14$), except that of four samples was 0.871 (standard raw material), 0.708, 0.620 and 0.502 (pogostone-type of *P. cablin*), repectively. The RRT and RPA of each characteristic peak to reference peak (patchouli alcohol), which was the highest peak in the chromatogram and the major compound of the volatile components in samples of *P. cablin*, were also calculated (Table 3). The data indicated that the content ratio of patchouli alcohol and pogostone was similar in patchouliol-type of *P. cablin* and different in a few samples, as well as pogostone-type of *P. cablin*. Therefore, there are two typical types and an interim-type of GC–MS fingerprintit for *P. cablin* (Fig. 4B). The former also correspond to the genotypes of plastid *matK* gene and nuclear 18S rRNA gene of different samples [25]. Unfortunately, pogostone-type sample, which is considered as authentic herbal material, is rare because the cultivation has been greatly decreased with urban area of Guangzhou city enlarge. Actually, the variation

Table 3
Relative retention time (RT_R) and relative peak area (RPA) of investigated components in *Pogostemon cablin* collected from different regions of China using patchouli alcohol as reference compound

Samples[a]		β-Patchoulene	Caryophyllene	α-Guaiene	Seychellene	β-Guaiene	δ-Guaiene	Spathulenol	Patchouli alcohol	Unknown	Pogostone
PC1	RT_R	–	0.47	0.51	0.52	–	0.66	–	1.00	1.13	1.18
	RPA	0	7.35	3.33	2.32	0	1.72	0	100	69.70	34.09
PC2	RT_R	0.41	0.47	0.51	0.52	0.54	0.66	0.92	1.00	1.13	1.18
	RPA	0.68	1.27	9.00	5.88	2.93	13.85	1.65	100	10.29	15.82
PC3	RT_R	0.41	0.47	0.51	0.52	0.54	0.66	0.92	1.00	1.13	1.18
	RPA	1.03	0.95	7.99	6.02	3.03	9.56	0.71	100	15.18	33.41
PC4	RT_R	0.40	0.47	0.51	–	–	0.66	–	1.00	1.13	1.18
	RPA	7.33	6.08	6.75	0	0	5.37	0	100	168.16	392.16
PC5	RT_R	0.41	0.47	0.51	0.52	0.54	0.66	0.92	1.00	1.13	1.18
	RPA	1.04	2.82	15.60	7.19	4.89	25.01	1.90	100	21.12	18.49
PC6	RT_R	0.41	0.47	0.51	0.52	0.54	0.66	0.92	1.00	1.13	1.18
	RPA	1.00	2.96	13.91	6.24	3.72	15.06	3.71	100	10.89	28.12
PC7	RT_R	0.41	0.47	0.51	0.52	0.55	0.66	0.92	1.00	1.13	1.18
	RPA	0.43	1.31	9.39	5.63	2.45	16.63	0.89	100	3.96	40.23
PC8	RT_R	0.41	0.47	0.51	0.52	0.54	0.66	0.92	1.00	1.13	1.18
	RPA	0.89	2.47	13.42	5.47	3.59	20.60	1.78	100	11.03	31.22
PC9	RT_R	0.41	0.47	0.51	0.52	0.54	0.66	0.92	1.00	–	1.18
	RPA	1.38	3.82	18.78	8.39	5.99	18.84	5.26	100	0	18.20
PC10	RT_R	0.41	0.47	0.51	0.52	0.54	0.66	0.92	1.00	1.13	1.18
	RPA	0.56	1.05	6.88	4.35	2.38	9.86	0.99	100	20.38	20.68
PC11	RT_R	–	0.47	–	–	–	–	–	1.00	1.13	1.18
	RPA	0	3.20	0	0	0	0	0	100	77.13	30.90
PC12	RT_R	0.41	0.47	0.51	0.52	0.55	0.66	0.92	1.00	1.13	1.18
	RPA	0.81	2.12	11.94	6.28	3.47	11.65	4.39	100	16.67	11.48
PC13	RT_R	0.41	0.47	0.51	0.52	0.54	0.66	0.92	1.00	1.13	1.18
	RPA	0.90	2.08	12.85	5.71	3.47	12.62	4.55	100	21.67	9.31
PC14	RT_R	0.41	0.47	0.51	0.52	0.54	0.66	0.92	1.00	1.13	1.18
	RPA	1.26	2.41	14.11	7.81	4.90	21.48	1.87	100	13.06	14.15
PC15	RT_R	0.41	0.47	0.51	0.52	0.54	0.66	0.92	1.00	1.13	1.18
	RPA	0.75	1.81	10.92	7.05	3.02	16.05	2.09	100	10.23	15.16
PC16	RT_R	0.41	0.47	0.51	0.52	0.54	0.66	0.92	1.00	1.13	1.18
	RPA	0.68	1.12	7.59	5.88	2.48	10.43	1.55	100	14.00	21.16
PC17	RT_R	0.41	0.47	0.51	0.52	0.54	0.66	0.92	1.00	1.13	1.18
	RPA	0.54	1.25	8.35	4.58	2.64	13.18	1.40	100	15.89	18.06
PC18	RT_R	–	–	0.52	–	–	–	–	1.00	1.13	1.18
	RPA	0	0	0.63	0	0	0	0	100	27.33	0[b]
Average	RT_R	0.41	0.47	0.51	0.52	0.54	0.66	0.92	1.00	1.13	1.18
	RPA	1.07	2.45	9.52	4.93	2.72	12.33	1.82	100	29.26	41.81
R.S.D. (%)	RT_R	0.63 ($n = 15$)	0.00 ($n = 17$)	0.48 ($n = 17$)	0.00 ($n = 15$)	0.67 ($n = 14$)	0.00 ($n = 16$)	0.00 ($n = 14$)	–	0.00 ($n = 17$)	0.00 ($n = 18$)
	RPA	150.62 ($n = 18$)	74.65 ($n = 18$)	52.65 ($n = 18$)	53.40 ($n = 18$)	64.98 ($n = 18$)	58.40 ($n = 18$)	90.30 ($n = 18$)	–	137.07 ($n = 18$)	210.51 ($n = 18$)

[a] PC1-PC18 were the same as that described in Table 2.
[b] Under the limit of quantification.

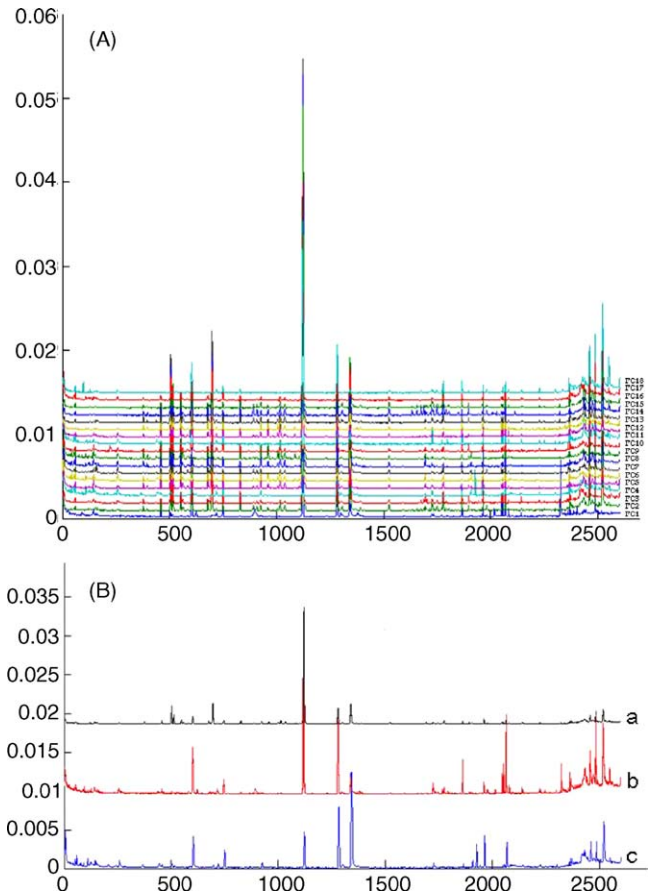

Fig. 4. Profiles of (A) standardized TIC for 18 different samples and (B) the three typical fingerprint of *Pogostemon cablin* in China. PC1–PC18 was the same as that described in Table 2. (a) The simulative mean chromatogram for patchouliol-type of *Pogostemon cablin*; (b) the simulative mean chromatogram for interim-type of *Pogostemon cablin*; and (c) the chromatogram for pogostone-type of *Pogostemon cablin*.

of chemical components in *P. cablin* maybe mainly derived from the difference of their genotypes. Further study is in progress.

Patchouli alcohol has been known for well over a century. It is a major constitute of the pungent oil from the East Indian shrub *P. cablin* and widely used in fragrances [17]. Patchouli alcohol was considered as Ca^{2+} antagonist [26] and antibacterial component [27] in *P. cablin*. In addition, pogostone was also considered as antibacterial agent [28]. It is thought that interaction of multiple chemical compounds contributes to the therapeutic effects of Chinese medicines. However, the overall clinical efficacy of two chemotypes of *P. cablin* has not been determined. Therefore, comparison of chemical components and pharmacological activities of two chemotypes of *P. cablin* is helpful to elucidate the mechanism of therapeutic effects and active components of Patchouli.

4. Conclusion

The GC–MS fingerprints of two typical chemotypes, patchouliol-type and pogostone-type, and an interim-type of *P. cablin* were developed, which suggested that comparison of their pharmacological activities is necessary to elucidate the mechanism of therapeutic effects and active components, as well as the desired cultivation of Patchouli.

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