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Application of comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry in the analysis of volatile oil of traditional Chinese medicines

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Abstract

This paper reports comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC × GC–TOF MS) analysis of *Pogostemon cablin Benth* (*Cablin Patchouli*) volatile oil. The suitable column system and operation conditions were chosen on the basis of the properties of composition of the volatile oil. One-dimensional gas chromatography (1D-GC) and GC × GC, GC–MS and GC × GC–TOF MS were compared under appropriate conditions, and the enhanced sensitivity and superior resolution of GC × GC were demonstrated. 394 components were tentatively identified by GC × GC–TOF MS.

Keywords: Gas chromatography, comprehensive two-dimensional; *Pogostemon cablin Benth*; Chinese medicines; Essential oils

1. Introduction

In China traditional Chinese medicines have played an important role in clinical therapy during thousands of years on account of the advantages of definite pharmacological activities and low toxicity [1]. One-dimensional gas chromatography (1D-GC) and GC–mass spectrometry (MS) are very useful methods to analyze volatile oils of traditional Chinese medicines [2–7]. The complexity of isomeric forms and various chemical classes of components with the broad range of terpenes mean that the complete separation may be largely unachievable from 1D-GC. For example, a non-polar column may fail to provide separation between octanol and α-phellandrene. A polar column can give better resolution between monoterpene hydrocarbons and their oxygenated compounds, but fails to separate monoterpene alcohols and esters from sesquiterpene hydrocarbons. The commercial chiral column has sufficient separation power for many of the monoterpenoids of interest and can even separate some sesquiterpenes, but it may fail to separate some other components. For complex samples, choosing different phase column in 1D-GC is just a reshuffling of peaks to give different version of an incompletely resolved analysis. So these problems possibly result in poor matches in the MS library searching, not to saying of obtaining accurate qualitative and quantitative results. With respect to these problems, there is a need to multidimensional gas chromatography.

Comprehensive two-dimensional gas chromatography (GC × GC) is a powerful and versatile analytical tool. It couples two columns with a different separation mechanism via a modulator. The modulator is the key of the system, which traps, focuses, and reinjects the slices into the second column. Its often-stated benefits [8] include enhanced sensitivity, superior resolution, and group separation that facilitate the identification of unknowns. Marriott [9] assured that comprehensive two-dimensional gas chromatography would hold many surprises and much value in respect of new information derived from GC analysis. GC × GC has been successfully applied to the analysis of petroleum samples [10], environmental samples [11] and essential oils [9,12–18]. In our group, this technique has been used to evaluate the quality of volatile oils of traditional Chinese

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medicines [19]. Shellie [12,13] used GC × GC to compare tea tree with Lavender oils, and analyzed lavender essential oils using GC–MS with the correlation of linear retention indices and compared GC–MS with GC × GC. Dimandja [14] used GC × GC to compare peppermint with spearmint. Marriott and Shellie [9,12] expected that GC × GC–TOF MS would play an important future role in the analysis of complex samples. Several applications of GC × GC–TOF MS to petroleum [10], essential oils [16], pesticides [11,20], and cigarette smoking [21,22] were reported.

Pogostemon cablin Benth (or named as *Cablin Patchouli*) is one of widely used traditional Chinese medicines, it has a selective prohibition effect on dermatophytes and a strong prohibitory effect on *Crytococus neoformans, Chaetomium globosum, AS3.963, and Scopulariopsis brevicaulis*, indicating the promise to cure the pneumonia and the chronic meningitis caused by intercurrent infections of these funguses within 8–30% AIDS patients [2–4]. *Pogostemon cablin Benth* volatile oil containing the main effective constituents includes several hundred chemical components and is a very complex natural mixture. It is believed [2,3] that the antifungal activity is closely related with the contents of pogostone, patchoulol, α-patchoulene, and β-patchoulene. Pogostone has been thought as the most important pharmaceutical component in it since 1970's. In general, the determination of *Pogostemon cablin Benth* volatile oil is conducted with GC–MS. Only several tens of compounds were detected [2–7].

In this paper, GC × GC–TOF MS has been used to analyze *Pogostemon cablin Benth* volatile oil. The suitable column system and the operation conditions were optimized on the basis of the characteristic of composition of *Pogostemon cablin Benth* volatile oil. 1D-GC and GC × GC, GC–MS, and GC × GC–TOF MS were compared under appropriate conditions. The enhanced sensitivity and superior resolution of GC × GC were demonstrated, the number of components identified by GC × GC–TOF MS is much more than that reported before.

2. Experimental

2.1. 1D-GC and GC–MS system

A model Agilent 6890 GC equipped with an FID was operated at a data acquisition rate of 100 Hz. A Shimadzu QP 5000 GC–MS with Class-5000 Chromatography Workstation software was used. The MS scan parameters included a mass range of *m/z* 40–400, a scan interval of 0.5 s, a scan speed of 1000 amu s^{-1}, and a detector voltage of 1.2 kV. The column used in two instruments was a 60 m × 0.25 mm i.d. × 0.25 μm SOLGELWAX (SGE, Australia). The GC and GC–MS were operated under a temperature programmed condition from 70 (held 3 min) to 200 °C(held 35 min) at 3 °C min^{-1}. The carrier gas used was helium. The column head pressure was at a constant pressure of 125 kPa.

2.2. GC × GC–TOF MS apparatus and column systems

GC × GC–TOF MS system consisted of an Agilent 6890 GC, a Pegasus III time-of-flight mass spectrometer (Leco Corp., St Josephs, MI) and a cold-jet modulator KT-2001 Retrofit prototype (Zoex Corp, Lincoln, NE). The modulator consisted of two hot jets and two cold jets. Nitrogen gas is cooled by heat exchange through copper tubing immersed in liquid nitrogen outside the GC system and is delivered to the cold jets which trap and focus the analytes eluting from the first column. Two hot jets heated the analytes, which were assembled in the modulator tube, to the second column for injection. The detailed description of the setup and its operation can be found in the literatures [19,21,22].

The column sets and operation conditions used in this research were listed in Table 1. The columns were connected by means of a press-fit connector. Two columns were installed in a same oven.

A time-of-flight mass spectrometer Pegasus III (Leco Corp., St Josephs, MI) was used to acquire mass spectrum data from GC × GC using 70 eV electron impact ionization, and was operated at an acquisition rate of 50 spectra s^{-1}. The ion-source temperature was 220 °C and the transfer interface temperature was 250 °C. The scan mass range was from mass 33 to 450.

2.3. Data conversion

Either Chemstation data (100 Hz) or TOF MS data (50 Hz) can be exported in ASCII file format (*csv files). Then the *csv files were converted to *bin files by a homemade conversion program based on the modulation frequency and sampling rate. The *bin files were translated into the *hdf files by Transform software to generate a contour plot. The components can be quantified by Zoex software (Zoex Corp, Lincoln, NE, USA).

2.4. Generating the peak table

The GC × GC–TOF MS software of the LECO Pegasus was used to find all the peaks in the raw GC × GC chromatogram with the signal-to-noise ratio higher than 100 and in the mass range of *m/z* 33–450. The mass threshold value was 10. The maximum number of peaks that can be listed in the peak table was 8000. The workstation can automatically give the parameters such as similarity, reverse, and probability of peaks through comparing them with the compounds in the library. The results were combined in a peak table [21].

2.5. Sample

Pogostemon cablin Benth was from Shenyang Pharmaceutical University of China. The volatile oil was extracted with the steam distillation method described in the Chinese Pharmacopoeia (1995) [23].

Table 1
GC × GC experimental conditions

	First column	Second column
Set 1		
Length (m)	50	2.5
Diameter (mm)	0.2	0.1
Stationary phase	DB-Petro[a]	DB-17ht[b]
Film thickness (μm)	0.5	0.1
Temperature program	70 °C (3 min) → 3 °C min^{-1} → 240 °C (20 min)	70 °C(3 min) → 3 °C min^{-1} → 240 °C(20 min)
Set 2		
Length (m)	60	3.0
Diameter (mm)	0.25	0.1
Stationary phase	DB-WAX[c]	DB-1701[d]
Film thickness (μm)	0.25	0.4
Temperature program	70 °C (3 min) → 3 °C min^{-1} → 200 °C (35 min)	70 °C (3 min) → 3 °C min^{-1} → 200 °C (35 min)
Set 3		
Length (m)	60	3.0
Diameter (mm)	0.25	0.1
Stationary phase	SOLGELWAX[e]	Cyclodex-B[f]
Film thickness (μm)	0.25	0.1
Temperature program	70 °C (3 min) → 3 °C min^{-1} → 200 °C (35 min)	70 °C (3 min) → 3 °C min^{-1} → 200 °C (35 min)

Carrier gas in all column systems: helium, constant pressure: 607 kPa, modulation time: 5 s.
[a] DB-Petro (J&W Scientific, Folsom, CA, USA), a 100% dimethylpolysiloxane.
[b] DB-17ht (J&W), a 50% phenyl-methylpolysiloxane.
[c] DB-WAX(J&W) a polyethylene glycol.
[d] DB-1701(J&W), a (14%-cyanopropyl-phenyl)-methylpolysiloxane.
[e] SOLGEWAX(SGE), a polyethlene glycol in a Sol–Gel matrix.
[f] Cyclodex-B (SGE), a permethylated β-cyclodextrin in OV 1701.

3. Results and discussion

3.1. Selection of GC × GC and GC × GC–TOF MS operational conditions

GC × GC is based on familiar GC principle, the factors that affect 1D-GC separation also affect GC × GC separation. Many authors have investigated the effect of temperature [11,15], modulation time [11], and column combination [11] on the GC × GC separation. In this paper, great attention was paid to the optimization of the modulation time and the combination of two columns. The modulation of the peaks eluting from the first column should be of a frequency that leads to several fractions delivered to the second column, and all compounds entering into the second column in one specific fraction should elute during the same modulation time in order to prevent co-elution with the components of the next fraction. If temperature program rate is too fast a good separation result cannot be achieved. The experiments found that the suitable temperature programmed rate for the analysis of volatile oils of traditional Chinese medicines is 2–3 °C min^{-1} and the proper modulation time is 5 s.

In GC × GC, generally, the first dimension is a thick film or long non-polar column and the second is a short, thin, moderately polar or polar column. Here, we used a 50 m or 60 m column as first dimensional column to resolve the components in *Pogostemon cablin Benth* volatile oil as much as possible. The columns in set 1 with the non-polar one on first dimension and the polar one on second dimension were tested at first. The total GC × GC–TOF MS contour plot was given in Fig. 1A. It is noted that the peaks eluting in the time from 2200 to 3600 s overlapped heavily. Based on TOF MS qualitative analysis information, it was found that these components in zone 3 are mainly moderately polar components.

To improve the resolution, a polar stationary phase column was used as the first dimensional column in column set 2 and 3, a chiral column was employed as the second dimensional column in set 3. The total GC × GC–TOF MS contour plots were given in Fig. 1B and Fig. 1C, respectively. It is obvious that the column sets 2 and 3 can provide a better separation than the column set 1. When comparing Fig. 1B with Fig. 1C, it is observed that the widths of the peaks in Fig. 1B are larger than those in Fig. 1C. The reason is that the stationary phase of the second dimensional column of set 2 is thicker than that of set 3. So the retention time of components in the second dimensional column of set 2 is longer than that in the second of set 3. This may result in the resolution loss on column set 2. In addition, the chiral column is a good choice for separating the isomeric components in volatile oils of traditional Chinese medicines. Based on the peak table it is discovered that the number of constituents identified by TOF MS in Fig. 1C is more than that in Fig. 1B. So we chose column set 3 to investigate the detailed constituents of *Pogostemon cablin Benth* volatile oil.

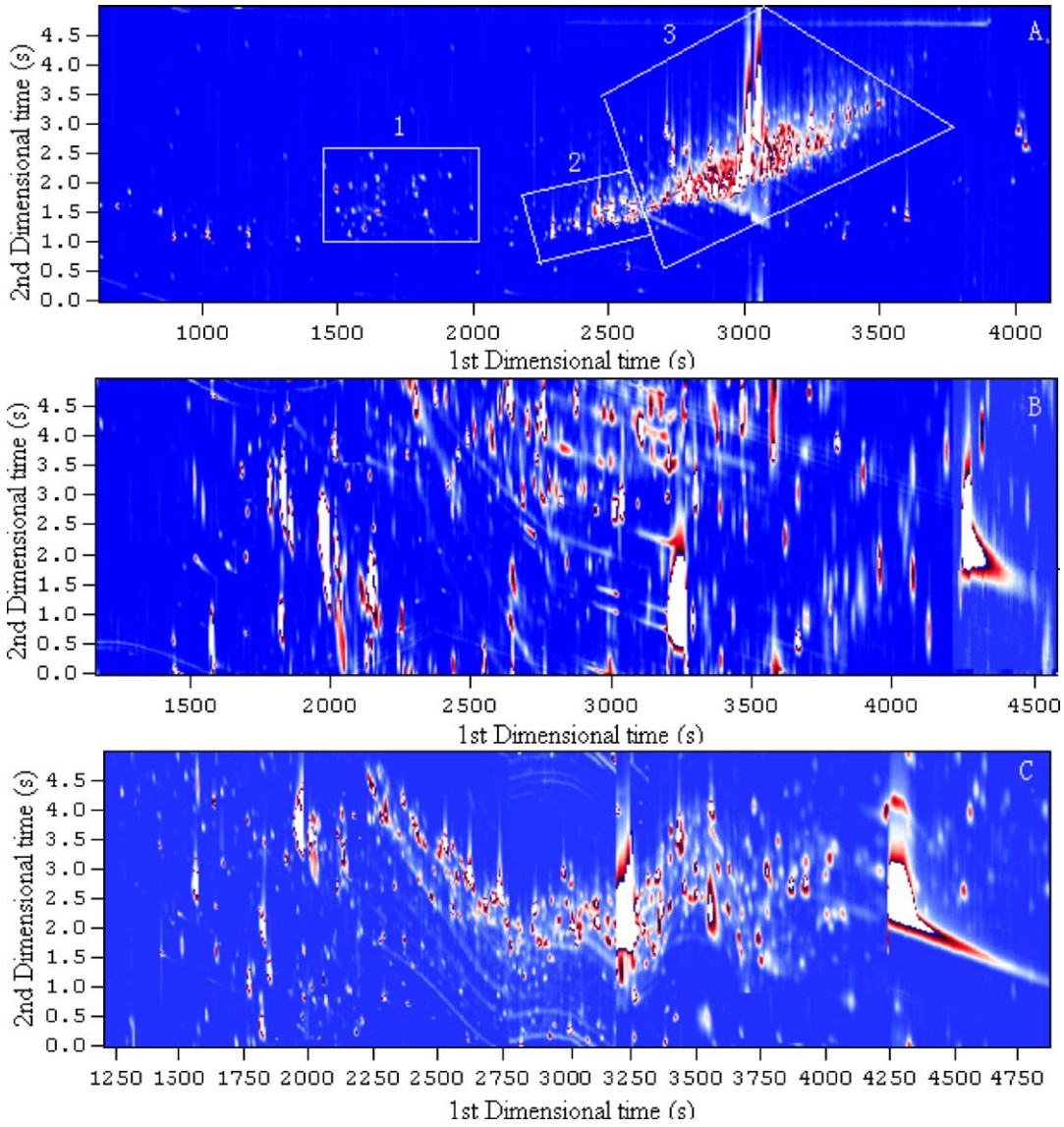

Fig. 1. The GC × GC–TOF MS contour plots of *Pogostemon cablin Benth* volatile oil on different column systems. (A) Column set 1; (B) column set 2; (C) column set 3 in Table 1. Zones 1, 2 and 3 in (A) are mainly oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes and pogostone, respectively.

3.2. Comparison of 1D-GC and GC × GC

To compare 1D-GC with GC × GC, in 1D-GC we also used the SOLGEWAX (60 m × 0.25 mm i.d. × 0.25 μm) column that was the same as the first column of set 3, in the meantime, all operational conditions were kept the same. Because of the lower peak capacity in 1D-GC, many components overlapped and many components with a minor content were also embedded by other large peaks and noise. Fortunately, in GC × GC these phenomena were greatly reduced. Fig. 2 shows a detailed example on resolution improvement in GC × GC. Fig. 2A is a part of 1D-GC chromatogram from first dimension, Fig. 2C is a part of GC × GC chromatogram. It is discovered that one overlapped peak in Fig. 2A from 1D-GC can be separated into four individual peaks from four components by GC × GC (Fig. 2C). Under the same operational conditions, 79 peaks were got with GC, while about 800 peaks were resolved with GC × GC.

Regardless of what kind of columns, 1D chromatographic retention is more or less the integration of many factors, such as dispersion force, induction force, orientation forces and polarity effect etc., true group separation cannot be achieved. However, in GC × GC, the combination of a non-polar column on the first dimension and a polar column on the second dimension provides two independent separation mechanisms. On the first dimension the separation was carried out according to the vapor pressure of the analytes, and on the second dimension according to their polarities. The orthogonal two-dimensional separation makes compounds with certain similar characteristic group together.

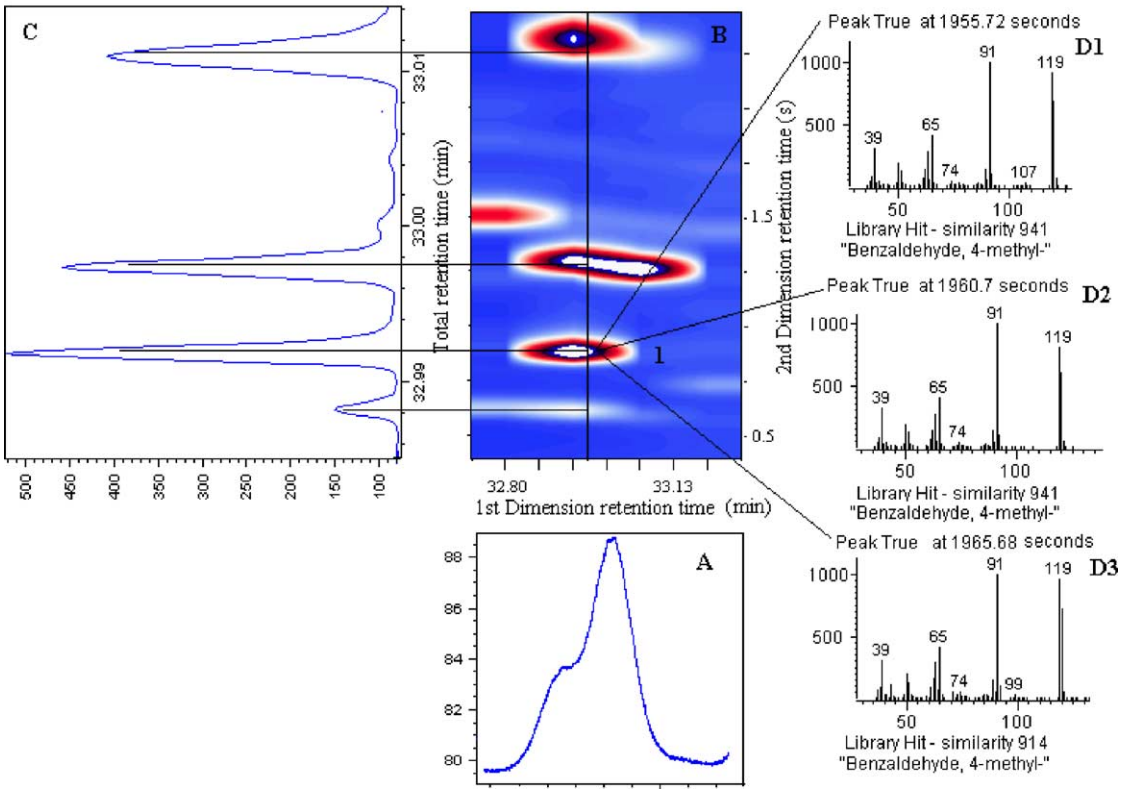

Fig. 2. Detailed comparison of 1D-GC with GC × GC under the same injection amount, split ratio and the same velocity of carrier gas (70 °C). (A) Detail of 1D-GC chromatogram; (B) detail of GC × GC contour plot; (C) detail of GC × GC chromatogram. The vertical line at 32.96 min indicates the second dimension chromatogram that is shown in (C). In (B) peak 1 was modulated three times by GC × GC, therefore, identified three times by TOF MS, the spectra D1, D2, D3 were corresponding deconvoluted mass spectrum. Column: 1D-GC, SOLGELWAX (60 m × 0.25 mm i.d. × 0.25 μm); GC × GC, first: SOLGELWAX (60 m × 0.25 mm i.d. × 0.25 μm), second: cyclodex-B (3 m × 0.1 mm i.d. × 0.1 μm). The modulation time of GC × GC was 5 s. The Chemstation acquired data at 100 Hz for both 1D GC and GC × GC.

Because a similar molecular structure corresponds to a similar pharmaceutical effect, and certain pharmaceutically active effect of a traditional Chinese medicine is related to certain kinds of components with specific molecular skeleton, the special groups will be directly related to the pharmaceutically active effect [24], they can be used to fast assess the quality of traditional Chinese medicines [19,24].

The *Pogostemon cablin Benth* volatile oil mainly includes three types of components which can be seen in Fig. 1A. Based on GC × GC–TOF MS, it can be known that the peaks eluting from 1400 to 2000 s are oxygenated monoterpenes (zone 1), the peaks eluting from 2250 to 2700 s are mainly sesquiterpenes (zone 2), and the peaks from 2700 to 3600 s are mainly oxygenated sesquiterpenes as well as pogostone (zone 3). These oxygenated sesquiterpenes are mainly ketones, alcohols, and ethers. Terpenes that derive from the head-to-tail linkage of the "isoprene" moiety have carbon ranges from C_{10} to C_{40}. The monoterpenes have two isoprene units and 10 carbon atoms. The sesquiterpenes have three isoprene and 15 carbon atoms. It was found that a lot of saturated and unsaturated cyclic hydrocarbons and oxygenated compounds constitute the *Pogostemon cablin Benth* oil.

3.3. Comparison of GC–MS and GC × GC–TOF MS

Based on GC–MS, several tens of components were detected with the similarity over 800. Using GC × GC–TOF MS the quantity of the components whose similarity is over 800 was up to 394. Many overlapped and embedded peaks in 1D-GC chromatogram are difficult to get a good match index from the NIST MS database. By contrast, in GC × GC–TOF MS system, there are largely completely resolved components (Fig. 1C). So the spectra are cleaner and more precisely represent the pure components than those in GC–MS. In the meantime, in GC × GC–TOF MS a peak is modulated several times, in turns a component can be detected several times by TOF MS. For example, in Fig. 2B peak 1 was modulated three times and identified three times by TOF MS software, too. The deconvoluted mass spectra can be seen in D1, D2, and D3 of Fig. 2, the similarity is 941, 941, and 914, respectively. GC × GC–TOF MS can provide three kinds of orthogonal information including two-dimensional retention times and the MS spectra data, the identification results become more reliable.

3.4. Qualitative analysis of Pogostemon cablin Benth volatile oil

By using similar method [21], based on the peak table, 394 components with the similarity over 800 were identified in Fig. 1C. The identification information including retention times, similarity, reverse, and probability, makes the reliability of analysis greatly increased. The similarity and reverse factors indicate how well a mass spectrum matches the library spectrum, but the isomers have similar mass spectra, so the probability is used to determine whether the peaks with the same name belong to one compound or several compounds. According to our experience and literature data [21], the similarity and reverse number above 800 and 900, respectively, indicate that an acquired mass spectrum usually shows a good match with the library spectrum. The probability value above 9000 means that the mass spectrum is highly unique. In this paper, many new compounds have been tentatively identified. Perhaps, they are very useful for further pharmaceutical research.

394 compounds tentatively identified include 82 alcohols, 21 esters, 27 ethers, 27 acids, 38 aldehydes, 100 ketones, 82 hydrocarbons, and 17 other components.

Among 82 alcohols from C_3 to C_{15} there are 22 saturated linear compounds, seven unsaturated linear compounds and 53 saturated or partly unsaturated cyclic components including 20 oxygenated monoterpenes, 21 oxygenated sesquiterpenes, and eight phenyl cyclic components.

Among 21 esters from C_4 to C_{16} there are nine linear saturated esters, three unsaturated linear ester, nine cyclic esters.

There are 27 saturated or partly unsaturated cyclic ethers from C_7 to C_{15} including 10 phenyl cyclic components, one oxygenated monoterpenes, and 10 oxygenated sesquiterpenes.

There are 27 acids from C_2 to C_{11} consisting of 8 one double-bond unsaturated and 19 saturated linear acids.

Among 38 aldehydes there are six saturated and 16 unsaturated linear aldehydes, 16 saturated or partly unsaturated cyclic aldehydes including 12 phenyl cyclic components and three oxygenated monoterpene.

Table 2
Thirty three components that were identified in this paper and reported earlier by other researchers

	Retention time (s)	$n^{\dagger}$	Name	Formula	Similarity	Reverse	Probability	CAS
1	685.78–710.14	6	(−)-β-Pinene	$C_{10}H_{16}$	940	940	4229	18,172–67–3
2	719.86	1	β-Phellandrene	$C_{10}H_{16}$	875	876	2754	555–10–2
3	774.22	1	β-Pinene	$C_{10}H_{16}$	845	845	3011	127–91–3
4	823.58–828.56	2	Heptanal	$C_7H_{14}O$	896	896	8019	111–71–7
5	855.18–865.10	3	Limonene	$C_{10}H_{16}$	895	895	4093	138–86–3
6	875.84–884.86	3	Eucalyptol	$C_{10}H_{18}O$	941	941	8996	470–82–6
7	1277.84–1282.82	2	3-Octanol	$C_8H_{18}O$	949	951	5347	20,296–29–1
8	1304.02–1313.94	3	Nonanal	$C_9H_{18}O$	911	911	6811	124–19–6
9	1505.34–1515.24	3	δ-Elemene	$C_{15}H_{24}$	927	933	4524	20,307–84–0
10	1556.30–1576.02	6	β-Patchoulene	$C_{15}H_{24}$	915	924	3427	514–51–2
11	1632.76–1642.66	3	(−)-Camphor	$C_{10}H_{16}O$	915	925	5909	464–48–2
12	1645.30	1	β-Bourbonene	$C_{15}H_{24}$	841	903	7830	5,208–59–3
13	1788.62–1833.68	9	β-Elemen	$C_{15}H_{24}$	946	946	5336	515–13–9
14	1815.64–1840.30	6	α-Guaiene	$C_{15}H_{24}$	918	918	3294	3,691–12–1
15	1827.04–1836.98	3	4-Terpinenol	$C_{10}H_{18}O$	910	910	7479	562–74–3
16	1839.92–1849.9	3	Caryophyllene	$C_{15}H_{24}$	886	889	2527	87–44–5
17	1939.02–1969.2	6	α-Patchoulene	$C_{15}H_{24}$	890	891	4210	560–32–7
18	1991.34	1	(−)-α-Terpineol	$C_{10}H_{18}O$	806	806	2996	
19	2018.82–2028.74	3	α-Caryophyllene	$C_{15}H_{24}$	912	912	6281	6,753–98–6
20	2124.16–2134.08	3	δ-Guaiene	$C_{15}H_{24}$	900	901	2452	3,691–11–0
21	2168.00	1	β-Selinene	$C_{15}H_{24}$	870	905	1185	17,066–67–0
22	2218.34–2223.28	2	δ-Cadinene	$C_{15}H_{24}$	838	866	2082	483–76–1
23	2275.28–2285.24	3	Myrtenol	$C_{10}H_{16}O$	925	927	7590	515–00–4
24	2711.42–2766.30	16	Caryophyllene oxide	$C_{15}H_{24}O$	905	905	6233	1,139–30–6
25	2905.36–2920.32	4	Elemol	$C_{15}H_{26}O$	918	924	5578	639–99–6
26	2955.70–3170.34	26	α-Elemenone	$C_{15}H_{22}O$	899	937	4545	
27	3060.92–3120.92	9	Globulol	$C_{15}H_{26}O$	837	848	2236	
28	3081.32–3111.32	6	Epiglobulol	$C_{15}H_{26}O$	827	827	2286	
29	3171.5–3271.38	40	Patchouli alcohol	$C_{15}H_{26}O$	927	930	8224	5,986–55–0
30	3361.16–3366.14	2	(−)-Spathulenol	$C_{15}H_{24}O$	809	809	2869	77,171–55–2
31	3407.46–3417.40	3	Ledene oxide-(I)	$C_{15}H_{24}O$	833	837	1410	
32	3681.78–3691.72	3	cis-Farnesol	$C_{15}H_{26}O$	850	858	2308	3,790–71–4
33	4250.28–4700.15	60	Pogostone	$C_{12}H_{16}O_4$	900	830	8932	

Note: $n^{\dagger}$ is times of a peak injected to the second dimension. The complete information on all identified compounds is available for those investigators that are specially interested by e-mail.

Among the 100 ketones there are 26 saturated linear ketones, 17 unsaturated linear ketones, and 57 with saturated or partly unsaturated cyclic ketones consisting of 11 oxygenated monoterpenes, 15 oxygenated sesquiterpenes, and nine phenyl cyclic components.

There are 82 hydrocarbons from C_3 to C_{15} including five saturated and unsaturated linear hydrocarbons, 77 saturated or partly unsaturated cyclic hydrocarbons consisting of eight monoterpenes, 29 sesquiterpenes, 19 phenyl cyclic components, 14 naphthyl components, and four indenyl components.

Table 2 listed 33 of 394 compounds that were also reported earlier by other researchers [5,8,9,11,12]. We can provide all other data by e-mail if you are on request.

3.5. Quantitative analysis of Pogostemon cablin Benth volatile oil based on the peak volume normalization method

It is different from 1D-GC, in GC × GC, the content of a component is direct proportion to its peak volume. It has been reported that quantitation in comprehensive two-dimensional GC has several potential advantages over 1D-GC [25].

Based on their components, *Pogostemon cablin Benth* volatile oils from different regions can be classified into three types, they have a characteristic of high level of pogostone [3], high level of patchouli alcohol [2,5], and high level of patchouli alcohol and pogostone [6,7], respectively. The oil analyzed in this work belongs to the third type. Because it is not easy to get the standard samples of the components of interest, as an approximation comparison, we suppose that these compounds of interest have same response factors. Based on the zone volume normalization it was found that the contents of pogostone, patchoulol, α-patchoulene, and β-patchoulene are 24%, 28%, 6%, and 2%, respectively. The contents of oxygenated monoterpenes (zone 1), sesquiterpenes (zone 2), and oxygenated sesquiterpenes and pogostone (zone 3) marked in Fig. 1A are 4%, 18%, and 72%, respectively.

4. Conclusions

The column system with a 60 m × 0.25 mm × 0.25 μm polar column as first dimension and a 3 m × 0.1 mm × 0.1 μm chiral column as second column was a good choice for the detailed component analysis of *Pogostemon cablin Benth* volatile oil. Under the same operational conditions, 79 peaks were detected with GC, while about 800 peaks were resolved with GC × GC. GC × GC–TOF MS not only tentatively identified 394 peaks (much more than 27 peaks tentatively identified by GC–MS), but also provided several kinds of identification information that make the result more reliable. Among 394 components there are 100 ketones, 27 ethers, 21 esters, 27 acids, 82 alcohols, 38 aldehydes, 82 hydrocarbons, and 17 other components.

It was found from this study that GC × GC–TOF MS is a powerful separation and identification tool for the analysis of complex volatile oils of traditional Chinese medicines. GC × GC–TOF MS can give the information about the formula and structure, can provide the opportunity for differentiating different volatile oils, can give the subtle differences of the oils from different areas, and can find new compounds that have the possible pharmaceutical effect on some diseases. So in the future GC × GC–TOF MS will play an important role in the analysis of volatile oils of traditional Chinese medicines.

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