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# Comparative analysis of essential oil components in *Pericarpium Citri Reticulatae Viride* and *Pericarpium Citri Reticulatae* by GC–MS combined with chemometric resolution method

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**Abstract**

The similarities and differences of essential oil components in *Pericarpium Citri Reticulatae Viride* (PCRV) and *Pericarpium Citri Reticulatae* (PCR) were investigated by GC–MS combined with a chemometric method, named alternative moving window factor analysis (AMWFA). Furthermore, temperature-programmed retention indices (PTRIs) were used together with mass spectra for identification of the essential oil components. A total of 61 and 59 compounds in the essential oils of PCRV and PCR from three *Citrus* species were identified, which represented 98.15–99.66% and 97.6–99.84% of their total relative contents, respectively. The essential oils from PCRV and PCR significantly differed both qualitatively and quantitatively. The main compound in the essential oils from PCRV and PCR was D-limonene accounting for 65.61–83.14%. The comparative analysis indicates that AMWFA greatly enhanced the accuracy of quantitative and qualitative results by utilizing information from chromatography and mass spectra. The results obtained may be helpful to find out the possibly bioactive compounds of PCRV and PCR.
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*Keywords:* *Pericarpium Citri Reticulatae Viride*; *Pericarpium Citri Reticulatae*; Alternative moving window factor analysis (AMWFA); Essential oil; GC–MS

## 1. Introduction

*Pericarpium Citri Reticulatae Viride* (PCRV) and *Pericarpium Citri Reticulatae* (PCR) have been widely used as traditional Chinese medicines (TCMs) for a long time because of pharmacologic activity, rich resources, low toxicity and costs. PCRV is the dried immature fruits or the dried immature fruits peel of *Citrus Reticulata Blanco* and its cultivars, collected from May to August, while PCR is the dried ripe fruits peel of *Citrus Reticulata Blanco* and its cultivars, gathered from September to December [1]. Their main cultivars are *Citrus Reticulata* '*Chachi*', *Citrus Reticulate* '*Dahongpao*' and *Citrus Erythrosa Tanaka*. PCRV and PCR have been always used as two kinds of TCMs in China because of their different harvest time and different pharmacologic effects. In traditional use in China, PCRV is commonly used to promote the flow of liver Qi, disperse stagnation, while PCR is mostly utilized to eliminate phlegm, strengthen spleen [2]. Moreover, PCR is extensively added to food as a condiment. These differences may be associated with their qualitative and quantitative constituents. The main bioactive constituents of PCRV and PCR consist of essential oil and flavonoid. In the present study, many reports on PCRV and PCR focus on flavonoid [3–8], but few on essential oils which have strong pharmacologic bioactivities [9,10]. For example, D-limonene, as one of the bioactive components in PCRV and PCR volatile oils, has been reported to make the expectoration easy and possess anticancer activity [1,11]. α-Terpineol has been projected to have significant antimicrobial activity [12,13]. Terpinen-4-ol has been demonstrated to have the bacteriostatic activity against several micro-organisms [14]. Up to now, to the best of our knowledge, there is no systematic comparative study on volatile oil of PCRV and PCR from three species: *Citrus Reticulata* '*Chachi*', *Citrus Reticulate* '*Dahongpao*' and *Citrus Erythrosa Tanaka* growing in China. The systematically

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comparative analysis between PCRV and PCR essential oils cannot only help to find out the possibly common and different chemical components but also provide the scientific evidence with correct use of PCRV and PCR.

Essential oil of TCMs is a very complex system and contains hundreds of chemical components. The GC–MS data from essential oil also involves a great number of overlapped and even embedded peaks. These overlapped and embedded peaks may bring about many difficulties when carrying out quantitative and qualitative analysis correctly. On account of these overlapped and embedded peaks, the comparative analysis among different samples may be a hard task and even is sometimes impossible. Chemometric methods, as a very useful assistant tool, use comprehensive chromatographic and spectral information to make it possible to resolve one complex 'black' analytical system clearly and accurately [15]. So far, the methods, such as orthogonal projection resolution (OPR) [16], evolving window orthogonal projection (EWOP) [17], sub-window factor analysis (SFA) [18,19], heuristic evolving latent projections (HELP) [20,21], evolving factor analysis (EFA) [22,23] and multi-component spectra correlative chromatography (MSCC) [24], have been successfully applied to resolve many different real-world samples [25–29]. However, the methods mentioned above can neither resolve the embedded peaks nor simultaneously carry out resolution and comparative analysis between two complex systems. In light of this, we use a novel chemometric method named alternative moving window factor analysis (AMWFA) [30] to resolve overlapped and embedded peaks in essential oil systems of PCRV and PCR and perform comparative analysis simultaneously in order to investigate much more information about the similarities and differences of essential components between PCRV and PCR.

In this study, the essential oils of PCRV and PCR from three species (*Citrus Reticulata* '*Chachi*', *Citrus Reticulate* '*Dahongpao*' and *Citrus Erythrosa Tanaka*) were firstly separated and detected with GC–MS. Then, AMWFA was employed to do the comparative analysis of essential oils between PCRV and PCR and to resolve the overlapped or embedded peaks. The qualitative identification of these chemical components was carried out by mass spectra combined with PTRIs. The quantitative analysis was performed with the overall volume integration method (OVI) [25–27].

## 2. Experimental

### 2.1. Materials and alkane standard solution

PCRV and PCR were collected in August and November, respectively, from Xinhui (*Citrus Reticulata* '*Chachi*'), Zigong (C*itrus Reticulate* '*Dahongpao*') and Changsha (*Citrus Erythrosa Tanaka*). These samples were authenticated by Professor Peishan Xie from Chromap Institute of Herbal Medicine Research SEZ, China. Alkane standard solutions of $C_8$–$C_{20}$ (mixture no. 04070) and $C_{21}$–$C_{40}$ (mixture no. 04071) were purchased from Fluka Chemika (Buchs, Switzerland).

### 2.2. Extraction of essential oil

After the samples were dried for 2 h at 35 °C and smashed, 30 g of sample was swollen with 500 ml of distilled water in a standard extractor for extracting volatile oil for 3 h. Then, the essential oils were prepared according to the procedure described in the Chinese pharmacopoeia [2]. The essential oils were dried over anhydrous sodium sulphate until the last traces of water were removed and then stored in the dark glass bottle at 4 °C prior to GC–MS analysis.

### 2.3. GC–MS analysis of volatile oil

A Shimadzu GC-2010 gas chromatograph (Kyoto, Japan) coupled with a Shimadzu QP2010 mass spectrometer was used for GC–MS analysis. The gas chromatograph was fitted with a fused silica capillary column OV-1 (30 mm × 0.25 mm i.d., 0.25 μm film thickness) which was purchased from Chromatographic Center of College of Chemistry and Chemical Engineering, Nanjing University of Technology (Nanjing, China). The following oven temperature program was initiated at 65 °C, increased at the rate of 6 °C/min to 260 °C. The carrier gas was helium at a constant flow of 1 ml/min. Injector, interface and ion-source were kept at 300, 250 and 230 °C, respectively. Splitting ratio was 20:1. Electron impact mass spectra were taken at 70 eV. Scan at 0.2 scans $s^{-1}$ from *m/z* 30 to 500 amu.

### 2.4. Retention indices

*Van den Dool* and *Kratz* [31] proposed a quasi-linear equation for temperature-programmed retention indices as follows:

$$\boldsymbol{I}_x = 100_n + 100\left[\frac{\boldsymbol{t}_x - \boldsymbol{t}_n}{\boldsymbol{t}_{n+1} - \boldsymbol{t}_n}\right] \quad (1)$$

where $I_x$ is the temperature-programmed retention index of the interest, and $t_n$, $t_{n+1}$, $t_x$ are the retention times in minute of the two standard *n*-alkanes containing *n* and $n+1$ carbons and the interest, respectively. This equation was used to calculate retention indices in the present work, linear temperature-programmed GC operating conditions.

### 2.5. Resolution by AMWFA

AMWFA is an extensive and conjoint version of multi-component spectral correlative chromatography (MSCC) [24] and sub-window factor analysis (SFA) [18,19], which is used to do the fast comparison between two complex systems. It could determine the number of common components and then identify their corresponding spectra semi-automatically according to the selective information hidden in two systems. First, common rank map obtained when scanning in AMWFA with the moving window technique was employed to determine whether the two comparative peak clusters have the same compounds and the number of common components. Then, the pure spectra and chromatograms of common compounds could be resolved by AMWFA. Finally, the common components could be analyzed

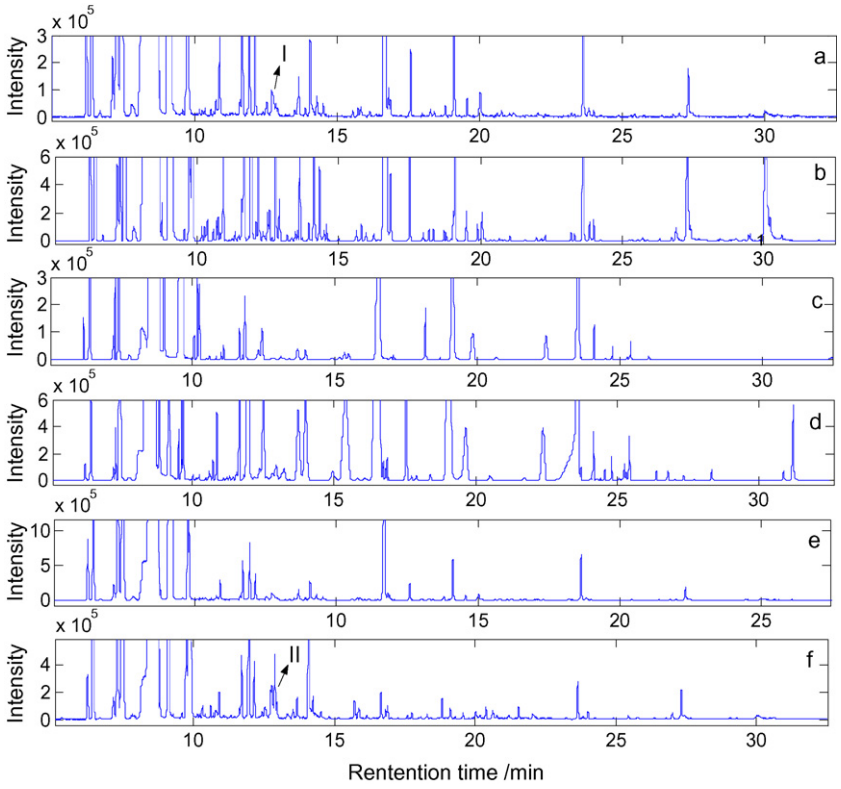


Fig. 1. GC–MS TIC of PCRV and PCR. (a) PCRV of *Citrus Reticulata* '*Chazhi*'; (b) PCR of *Citrus Reticulata* '*Chachi*'; (c) PCRV of *Citrus Erythrosa Tanaka*; (d) PCR of *Citrus Erythrosa Tanaka*; (e) PCRV of *Citrus Reticulate* '*Dahongpao*'; (f) PCR of *Citrus Reticulate* '*Dahongpao*'.

quantitatively and qualitatively. For more detail about AMWFA, the reader could refer to the reference [30].

All data analysis was carried out on a Pentium IV (Intel) personal computer. All programs of the chemometric resolution methods were coded in MATLAB 6.5 for windows. The library searches and spectral matching of the resolved pure components were conducted on the National Institute of Standard and Technology (NIST) 107 MS database containing 107 886 compounds. The exactly qualitative results were obtained with the help of PTRIs.

## 3. Results and discussion

### 3.1. Resolution of overlapped peaks by AMWFA

Fig. 1 shows the total ion chromatograms (TICs) of the essential oils of PCRV and PCR. All the TICs from PCRV and PCR samples are very complex analytical systems. Although many chromatographic peaks are baseline separated, there still exist some overlapped peaks. Because of these, the simple search with the MS database will definitely fail because the mass spectrum

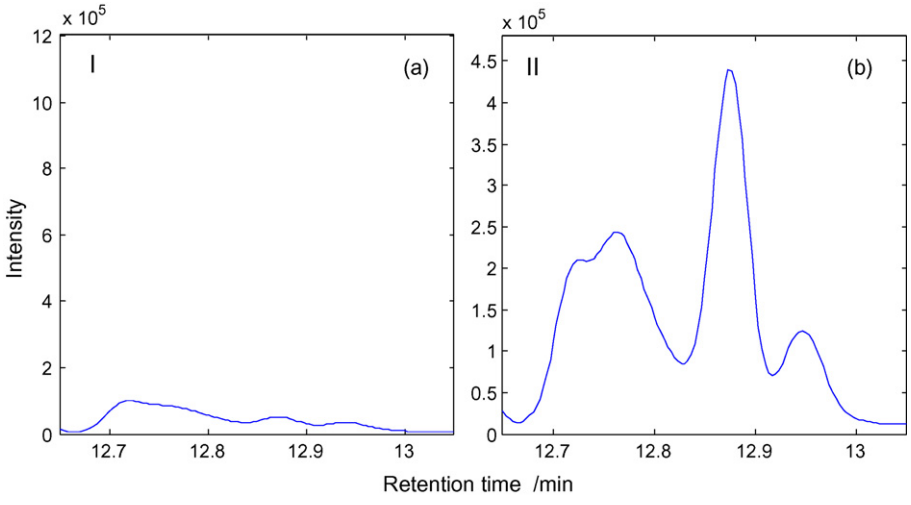


Fig. 2. Total ion chromatograms of PCRV and PCR of 12.65 to 13.05 min. (a) PCRV of *Citrus Reticulata* '*Chachi*'; (b) PCR of *Citrus Reticulate* '*Dahongpao*'.

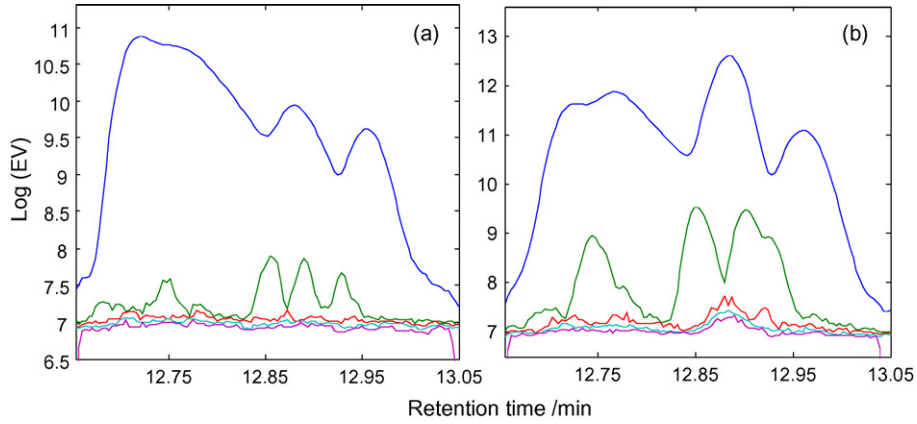


Fig. 3. Rank map curve of PCRV and PCR of 12.65–13.05 min. (a) PCRV of *Citrus Reticulata* '*Chachi*'; (b) PCR of *Citrus Reticulate* '*Dahongpao*'.

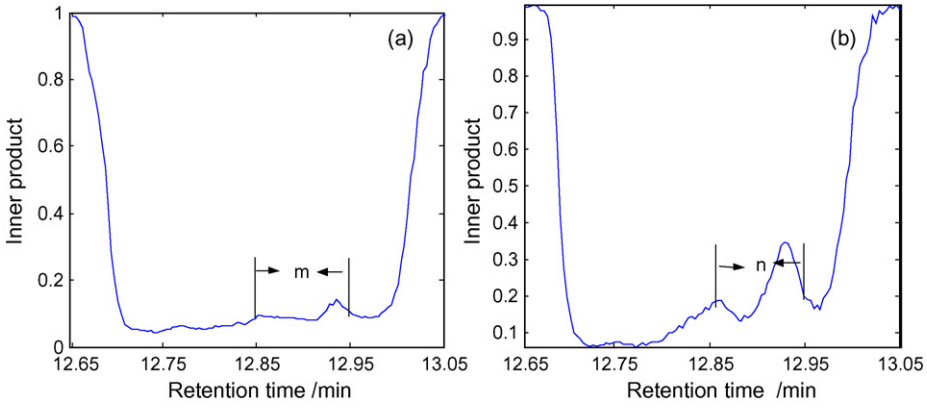


Fig. 4. The results of MSCC (a) and IP-MSCC (b) analysis.

of mixtures measured can never get good matching index with that of a pure component in the NIST MS database. Peak clusters I and II in Fig. 1 are such examples. The TICs of Peak clusters I and II are shown in Fig. 2. It seems that both of the two peak clusters consist of four components which are not baseline separated. Furthermore, at different scanning points, the 'four components' get different compounds with low matching indices, that is to say, the two peak clusters are overlapped seriously and even possibly contain the embedded peaks. Besides, qualitative analysis becomes difficult by PTRIs or mass similarity search. So, it is necessary to resolve the overlapped peaks before we do the comparative analysis between these analytical systems.

In order to project how to identify the compounds correctly, these two peak clusters are taken as an example to illustrate the resolution procedure by AMWFA. The results obtained from fixed size moving window evolving factor analysis (FSWMEFA) [32] are shown in Fig. 3. It can be seen that both peak cluster I and peak cluster II contain in fact five chemical components. In order to see whether the two peak clusters contain the same components, MSCC and inverse projection MSCC (IP-MSCC) are first employed. Here, we regard peak cluster I as the base matrix, and the peak cluster II as the target matrix. The results obtained by MSCC and IP-MSCC are shown in Fig. 4.

From Fig. 4a, it seems that the mass spectra features of the compounds in peak cluster I are highly correlated with that in peak cluster II. The only suspicious part is a small peak at retention time 12.95, which seems to suggest different compounds in the two peak clusters in this region. However, Fig. 4b gives a much clear result for this. From Fig. 4b, one can see a rather big peak at almost the same region. The reason for this may be that the concentration of compounds in peak cluster I was far smaller than that in peak cluster II (see also the scales of Fig. 2). Such

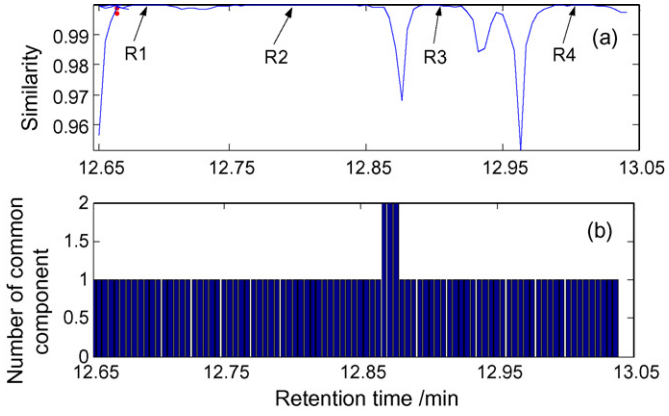


Fig. 5. The results of resolution of common component R1, R2, R3 and R4. (a) Mass auto-correlative curve from AMWFA; (b) common rank map from AMWFA.

a result indicates that there must be some small part different for the two peak clusters even though they are highly correlated. Thus, whether section m (marked in Fig. 4a) and region n (marked in Fig. 4b) have the common compounds remains a problem.

In order to get more detail information for the two peak clusters, the moving window searching with a fixed window size three is conducted on peak cluster II. As a result of this, spectral auto-correlative curve and common rank map are acquired by AMWFA, which are shown in Fig. 5a and b, respectively. Common rank map provides us with some usefully selective section of the common compounds according to their eigenvalues being equal to 1 [30]. In the spectral auto-correlative curve, four parts in plot marked R1, R2, R3 and R4 with correlation coefficient close to 1 indicate that there are four common compounds in these two peak clusters. Then the pure spectra of the four common components can be easily obtained from the corresponding region R1, R2, R3 and R4 in spectral auto-correlative curve. After obtaining the pure MS spectra, the qualitative analysis can be conducted by similarity research in NIST 107 mass library. It is found that the common compound R1 is 2,5,5-trimethyl-1,6-heptadiene with similarity index (SI) equal to 0.994, and R2 is Citronellol (SI = 0.959), and R3 is *cis*-Citral (SI = 0.90), and R4 is (+)-Carvone (SI = 0.993). The pure mass and standard spectra of the four common compounds are displayed in Fig. 6.

In order to further investigate the different parts in detail in these two peak clusters, the component stripping technique is employed to eliminate the components with selective information by HELP method [20,21]. The correlative results of MSCC and IP-MSCC of section m and region n are shown in Fig. 7. It tells us that the retention area of the same compound in section m and region n is the latter region (12.91–12.94 min) of MSCC in Fig. 7a and the former section (12.89–12.93 min) of IP-MSCC in Fig. 7b. Concerning the different compounds, their

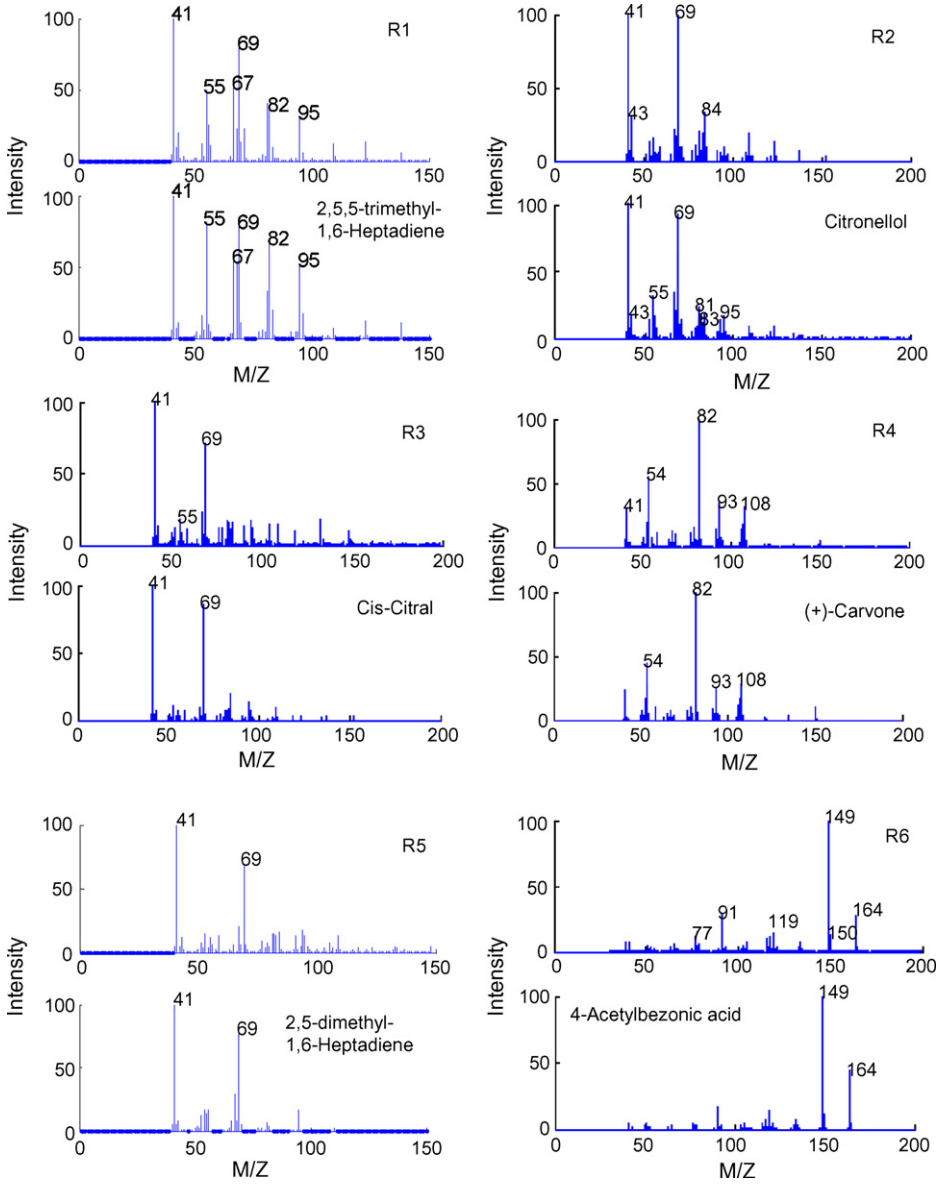


Fig. 6. Resolved mass spectra of components R1, R2, R3, R4, R5 and R6, and their corresponding standard mass spectra.

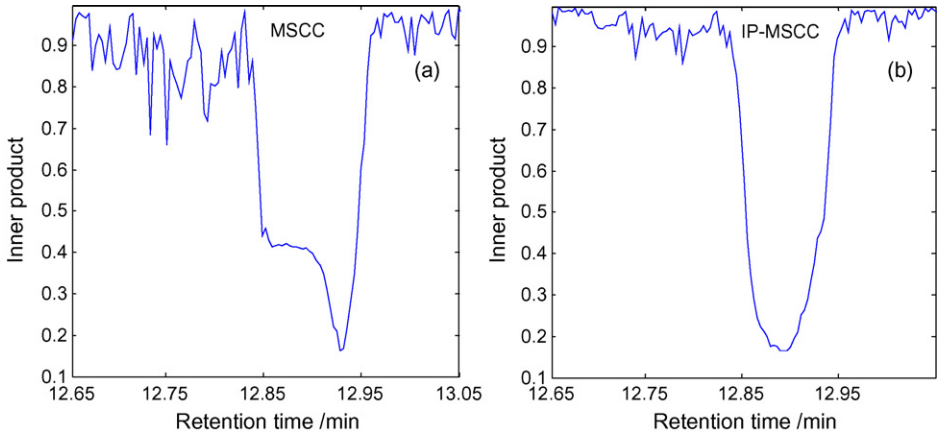


Fig. 7. The results of MSCC (a) and IP-MSC C (b) analysis.

pure mass spectra can be acquired by using component stripping technology by HELP [20,21], which are shown in Fig. 6. Through similarity matching in NIST 107 mass database, two different chemical components named R5 in peak cluster I and R6 in peak cluster II, respectively, could be tentatively identified as 2,5-dimethyl-1,6-heptadiene and 4-acetylbenzoic acid with similarity indices 0.95 and 0.994, respectively.

With all the spectra available, the pure chromatograms of the two peak clusters can be easily calculated by least squares technique. The results are shown in Fig. 8. Their retention indices of the components according to their eluted times provided by pure chromatogram can also be calculated through Eq. (1).

Other peaks in the studied samples are determined qualitatively in the same way as described above. The tentatively qualitative results of constituents of essential oils from PCRV and PCR are shown in Table 1. In addition, the retention indices, listed in order of elution on an OV-1 column, are given in Table 1, too.

### 3.2. Quantitative analysis of chemical components of essential oils from PCRV and PCR

According to the resolved chromatogram and mass spectra, the quantitative analysis of each component can be directly calculated by the overall volume integration (OVI) [25–27]. They are proportional to the content of the peak as integration based on TIC.

The quantitative results of essential components from PCRV and PCR essential oils are shown in Table 1. In essential oils of PCRV from *Citrus Reticulata* '*Chachi*', *Citrus Reticulate* '*Dahongpao*' and *Citrus Erythrosa Tanaka*, 48, 51 and 49 components were determined representing 99.66%, 98.56% and 98.15% of the total relative content, respectively. The essential oils obtained from PCR from *Citrus Reticulata* '*Chachi*', *Citrus Reticulate* '*Dahongpao*' and *Citrus Erythrosa Tanaka* were composed of 51, 49 and 48 compounds representing 99.17%, 99.84% and 97.6%, respectively.

### 3.3. Comparison of volatile components between PCRV and PCR

#### 3.3.1. Components analysis of essential oils of PCRV and PCR

As can be seen in Table 1, Monoterpenes represented the main compound family in all extracts. Their abundance varied in the ranges from 76.88% to 95.72% of the total relative percents. All the samples were characterized by a very high amount of D-limonene varied from 65.61% to

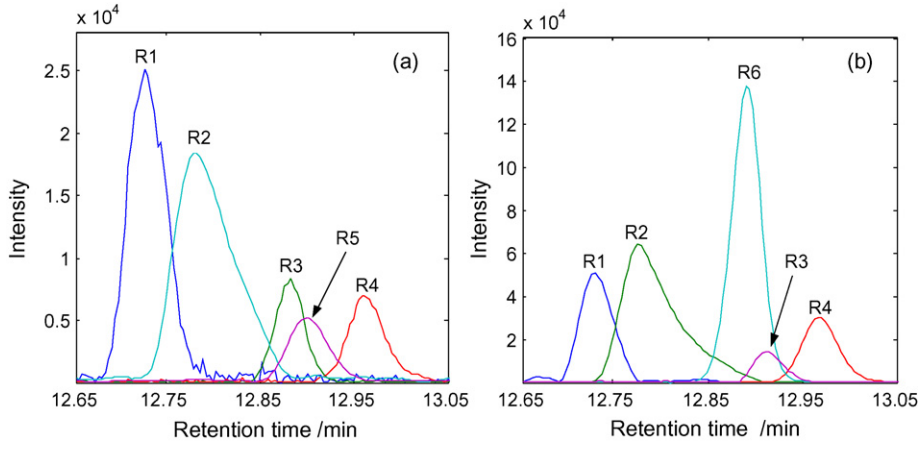


Fig. 8. Resolved chromatographic curves of PCRV and PCR of 12.65–13.05 min. (a) PCRV of *Citrus Reticulata* '*Chachi*'; (b) PCR of *Citrus Reticulate* '*Dahongpao*'.

Table 1
The quantitative and qualitative results of the essential oils in PCRV and PCR

| Peak number | Compounds | Molecular formula | Relative content (%) | | | | | | *I* |
|---|---|---|---|---|---|---|---|---|---|
| | | | *Citrus* '*Chachi*' PCRV | *Reticulate* PCR | *Citrus* '*Dahongpao*' PCRV | *Reticulata* PCR | *Citrus Tanaka* PCRV | *Erythrosa* PCR | |
| 1 | α-Thujene | $C_{10}H_{16}$ | 0.43 | 0.33 | 0.09 | 0.13 | 0.15 | 0.07 | 931 |
| 2 | α-Pinene | $C_{10}H_{16}$ | 1.67 | 1.05 | 0.44 | 0.82 | 0.77 | 0.35 | 940 |
| 3 | Camphene | $C_{10}H_{16}$ | tr | 0.01 | 0.01 | nd | nd | nd | 981 |
| 4 | Sabinene | $C_{10}H_{16}$ | 0.14 | 0.14 | 0.07 | 0.09 | 0.1 | 0.06 | 986 |
| 5 | β-Pinene | $C_{10}H_{16}$ | 1.5 | 1.02 | 0.34 | 0.09 | 0.42 | 0.28 | 987 |
| 6 | Octanal | $C_8H_{16}O$ | 0.12 | tr | 0.1 | tr | 0.02 | 0.1 | 994 |
| 7 | β-Myrcene | $C_{10}H_{16}$ | 1.2 | 1.6 | 1.72 | 0.59 | 1.8 | 1.75 | 1003 |
| 8 | α-Phellandrene | $C_{10}H_{16}$ | 0.05 | 0.05 | 0.01 | 0.02 | 0.02 | 0.01 | 1012 |
| 9 | *p*-Cymene | $C_{10}H_{14}$ | 1.43 | 0.1 | 0.4 | nd | 0.57 | 0.41 | 1019 |
| 10 | D-Limonene | $C_{10}H_{16}$ | 65.61 | 73.39 | 67.83 | 82.2 | 83.14 | 77.19 | 1035 |
| 11 | β-*cis*-Ocimene | $C_{10}H_{16}$ | 0.04 | 0.03 | 0.19 | 0.08 | 0.42 | 0.38 | 1051 |
| 12 | γ-Terpinen | $C_{10}H_{16}$ | 22.38 | 10.78 | 5.46 | 7.05 | 5.53 | 4.65 | 1065 |
| 13 | 1-Octanol | $C_8H_{18}O$ | nd | nd | nd | tr | nd | nd | 1077 |
| 14 | Isopropenyltoluene | $C_{10}H_{12}$ | 0.01 | 0.02 | 0.03 | nd | 0.09 | 0.14 | 1094 |
| 15 | (+)-4-Carene | $C_{10}H_{16}$ | 1.19 | 0.87 | 0.32 | 0.25 | 0.33 | 0.32 | 1085 |
| 16 | Nonanal | $C_9H_{18}O$ | nd | tr | 0.01 | 0.01 | 0.1 | 0.36 | 1104 |
| 17 | Linalool | $C_{10}H_{18}O$ | 0.35 | 0.7 | 15.99 | 5.77 | 0.27 | 0.78 | 1106 |
| 18 | Linderol | $C_{10}H_{18}O$ | tr | nd | tr | nd | nd | nd | 1124 |
| 19 | (*R*)-3,7-Dimethyl-6-octenal | $C_{10}H_{18}O$ | nd | 0.02 | 0.05 | 0.01 | nd | nd | 1138 |
| 20 | Camphor | $C_{10}H_{16}$ | nd | 0.01 | tr | 0.01 | 0.03 | 0.02 | 1149 |
| 21 | Citronellal | $C_{10}H_{18}O$ | 0.1 | 0.1 | 0.09 | 0.14 | 0.05 | 0.18 | 1153 |
| 22 | Nonanol | $C_9H_{20}O$ | 0.01 | 0.02 | nd | 0.01 | 0.01 | tr | 1168 |
| 23 | 1-Terpinen-4-ol | $C_{10}H_{18}O$ | 0.2 | 0.78 | 0.45 | 0.1 | 0.14 | 0.35 | 1182 |
| 24 | α-Terpineol | $C_{10}H_{18}O$ | 0.33 | 1.45 | 0.98 | 0.22 | 0.28 | 0.85 | 1196 |
| 25 | *n*-Decanal | $C_{10}H_{20}O$ | 0.13 | 0.24 | 0.23 | 0.24 | 0.11 | 0.3 | 1207 |
| 26 | 2,5,5-Trimethyl-1,6-heptadiene | $C_{10}H_{18}$ | 0.08 | 0.05 | tr | 0.13 | 0.01 | 0.01 | 1211 |
| 27 | Citronellol | $C_{10}H_{20}O$ | 0.04 | 0.02 | 0.02 | 0.07 | tr | tr | 1225 |
| 28 | *cis*-Citral | $C_{10}H_{16}O$ | 0.02 | 0.01 | tr | tr | tr | tr | 1227 |
| 29 | (+)-Carvone | $C_{10}H_{14}O$ | nd | 0.01 | tr | 0.17 | tr | 0.01 | 1228 |
| 30 | 2,5-Dimethyl-1,6-heptadiene | $C_9H_{16}$ | 0.01 | nd | tr | nd | tr | nd | 1228 |
| 31 | 4-Acetylbenzoic acid | $C9H_8O_3$ | 0.02 | tr | tr | 0.02 | tr | tr | 1236 |
| 32 | β-Citronellol | $C_{10}H_{20}O$ | 0.01 | 0.44 | 0.26 | 0.12 | 0.06 | 0.27 | 1243 |
| 33 | Nerol | $C_{10}H_{18}O$ | 0.07 | nd | nd | nd | nd | nd | 1248 |
| 34 | Thymol methyl ether | $C_{11}H_{16}O$ | 0.01 | nd | 0.48 | 0.09 | nd | nd | 1255 |
| 35 | Neral | $C_{10}H_{16}O$ | nd | nd | nd | tr | 0.04 | 0.3 | 1262 |
| 36 | 2-Decenal | $C_{10}H_{18}O$ | nd | tr | nd | tr | 0.01 | 0.01 | 1272 |
| 37 | Geranial | $C_{10}H_{16}O$ | tr | 0.05 | 0.27 | 0.44 | 0.03 | 0.08 | 1279 |
| 38 | Perillaldehyde | $C_{10}H_{14}O$ | 0.04 | 0.24 | 0.16 | nd | 0.03 | 0.59 | 1294 |
| 39 | Decanol | $C_{10}H_{22}O$ | 0.01 | 0.02 | 0.01 | nd | nd | nd | 1303 |
| 40 | Thymol | $C_{10}H_{14}O$ | 0.1 | 0.31 | 1.93 | 0.28 | 2.12 | 4.86 | 1308 |
| 41 | Bornyl acetate | $C_{12}H_{20}O_2$ | tr | tr | nd | 0.01 | tr | 0.05 | 1318 |
| 42 | Carvacrol | $C_{10}H_{14}O$ | 0.02 | 0.1 | tr | nd | 0.01 | 0.06 | 1341 |
| 43 | *n*-Undecanal | $C_{11}H_{22}O$ | tr | 0.02 | tr | 0.01 | 0.02 | 0.05 | 1354 |
| 44 | Cavrbenol | $C_{10}H_{16}O$ | nd | nd | nd | 0.01 | tr | nd | 1365 |
| 45 | Citronellyl butyrate | $C_{14}H_{26}O_2$ | tr | tr | tr | 0.02 | tr | tr | 1380 |
| 46 | δ-Elemene | $C_{15}H_{24}$ | nd | nd | 0.07 | 0.04 | 0.17 | 0.2 | 1381 |
| 47 | Neryl acetate | $C_{12}H_{20}O_2$ | 0.01 | 0.02 | 0.01 | 0.03 | tr | 0.01 | 1396 |
| 48 | Geraniol acetate | $C_{12}H_{20}O_2$ | nd | 0.01 | nd | 0.01 | 0.01 | 0.02 | 1409 |
| 49 | Decanoic acid | $C_{10}H_{20}O_2$ | nd | 0.01 | 0.1 | 0.01 | nd | 0.01 | 1437 |
| 50 | Copaene | $C_{15}H_{24}$ | tr | 0.01 | nd | 0.03 | 0.01 | 0.02 | 1458 |
| 51 | Benzoicacid, 2-(methylamino)-methyl ester | $C_9H_{11}NO_2$ | 1.69 | 3.84 | nd | 0.1 | 0.92 | 2.06 | 1484 |
| 52 | β-Elemene | $C_{15}H_{24}$ | 0.03 | nd | 0.04 | 0.04 | 0.08 | 0.13 | 1494 |
| 53 | Caryophyllene | $C_{15}H_{24}$ | 0.07 | 0.14 | nd | tr | 0.01 | 0.04 | 1509 |
| 54 | γ-Muurolene | $C_{15}H_{24}$ | tr | 0.01 | tr | 0.03 | tr | tr | 1527 |
| 55 | Undecyl acetate | $C_{13}H_{26}O_2$ | tr | 0.01 | tr | nd | 0.12 | 0.13 | 1565 |
| 56 | α-Farnesene | $C_{15}H_{24}$ | 0.19 | 0.36 | 0.01 | 0.1 | nd | nd | 1583 |

Table 1 (*Continued*)

| Peak number | Compounds | Molecular formula | Relative content (%) | | | | | | *I* |
|---|---|---|---|---|---|---|---|---|---|
| | | | *Citrus* '*Chachi*' PCRV | *Reticulate* PCR | *Citrus* '*Dahongpao*' PCRV | *Reticulata* PCR | *Citrus Tanaka* PCRV | *Erythrosa* PCR | |
| 57 | δ-Cadinene | $C_{15}H_{24}$ | 0.01 | 0.04 | 0.01 | tr | 0.01 | tr | 1588 |
| 58 | Elemol | $C_{15}H_{26}O$ | 0.02 | 0.02 | 0.01 | 0.01 | nd | tr | 1649 |
| 59 | Germacrene | $C_{15}H_{24}$ | nd | 0.01 | 0.06 | 0.02 | 0.13 | 0.12 | 1715 |
| 60 | α-Sinensal | $C_{15}H_{22}O$ | 0.26 | 0.7 | 0.23 | 0.14 | nd | nd | 1746 |
| 61 | Hexadecanoic acid | $C_{16}H_{32}O_2$ | 0.06 | 0.01 | tr | 0.09 | 0.01 | 0.02 | 1772 |
| Total | | | 99.66 | 99.17 | 98.48 | 99.84 | 98.15 | 97.6 | |

*Notes*: nd, not detected; tr (trace), relative content <0.01%.

83.14%, which was identical to other citrus oils reported by Blanco Tirado [9]. The oxygenated compounds were relatively poor, the total of which accounted for 3.62–21.38%. In oxygenated compounds, the highest content of Linalool (15.99%) was found in the essential oil of PCRV of *Citrus Reticulate* '*Dahongpao*'. The sesquiterpene amounts were lower than that of oxygenated compounds and only represented 0.21–0.97%. Compared with the volatile oils of *Citrus Reticulata* '*Chachi*' and *Citrus Reticulate* '*Dahongpao*', the volatile oils of *Citrus Erythrosa Tanaka* had much more sesquiterpene compounds.

### 3.3.2. Comparative analysis of essential oils of PCRV and PCR

As is shown in Table 1, although most of compounds presented in the essential oils of PCRV and PCR are the same, their contents are different. These differences of contents of chemical components may lead to different pharmacologic effects of PCRV and PCR to some extent, especially the main bioactive compounds such as γ-terpinen, Linalool, and so on. In fact, the amounts of bioactive compounds play more important role in curing some diseases in TCMs use in China. Moreover, some components could be found in PCRV or PCR but not in another's. For example, Nerol was only found in PCRV, while 1-octanol in PCR though its content was small. All differences of constituents of essential oils between PCRV and PCR further confirm that PCRV and PCR must be used as two kinds of TCMs to some extent.

## 4. Conclusion

In this paper, by using the chemometric method and PTRIs, the essential oils of PCRV and PCR were analyzed and then compared regarding their qualitative and relatively quantitative characteristics. The comparative results of essential oils between PCRV and PCR were significant to help us to use the two TCMs better and correctly. Furthermore, the chemometric method could greatly enhance the accuracy of quantitative and qualitative results together with PTRIs. The comparison also showed that AMWFA could be a convenient and fast tool for doing comparative analysis in complicated systems or 'black' analytical systems.

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## References

[1] Y.J. Shen, Pharmacology of Traditional Chinese Medicine, Shanghai Publishing House of Science and Technology, Shanghai, 2002, pp. 108–111.
[2] Pharmacopoeia Committee of the People's Republic of China, Pharmacopoeia of the People's Republic of China, Publishing House of People's Health, Beijing, 2000.
[3] F.M. Areias, A.C. Rego, C.R. Oliveira, Biochem. Pharmacol. 62 (2001) 111–118.
[4] N.M. Borradaile, K.K. Carroll, E.M. Kurowska, Lipids 34 (1999) 591–598.
[5] J.B. Harborne, C.A. Williams, Phytochemistry 55 (2000) 481–504.
[6] J.A. Manthey, N. Guthrie, K. Girohmann, Curr. Med. Chem. 8 (2001) 135–153.
[7] M. Zhang, H.T. Ji, J.S. Wang, D.H. Qian, Acta Pharm. Sin. 21 (2000) 253–256.
[8] D.D. Wang, J. Wang, X.H. Huang, Y. Tu, K.Y. Ni, J. Pharm. Biomed. Anal. 44 (2007) 63–69.
[9] C. Blanco Tirado, E.E. Stashenko, M.Y. Combariza, J.R. Martinez, J. Chromatogr. A 697 (1995) 501–513.
[10] G. Flamini, M. Tebano, P.L. Cioni, Anal. Chim. Acta 589 (2007) 120–124.
[11] M. Asamoto, T. Ota, H. Toriyama-Baba, et al., Jpn. J. Cancer Res. 93 (2002) 32–35.
[12] C.F. Carson, T.V. Riley, J. Appl. Bacteriol. 74 (1995) 264–269.
[13] S. Cosentino, C.I.G. Tuberoso, B. Pisano, M. Satta, V. Mascia, E. Arzedi, F. Palmas, Lett. Appl. Microbiol. 29 (1999) 130–135.
[14] S. Barel, R. Segal, J. Yashphe, J. Ethnopharmacol. 33 (1991) 187–191.
[15] Y.Z. Liang, O.M. Kvalheim, R. Manne, Chemom. Intell. Lab. Syst. 18 (1993) 235–250.
[16] Y.Z. Liang, O.M. Kvalheim, Anal. Chim. Acta 292 (1994) 5–15.
[17] C.J. Xu, J.H. Jiang, Y.Z. Liang, Analyst 124 (1999) 1471–1476.
[18] R. Manne, H.L. Shen, Y.Z. Liang, Chemom. Intell. Lab. Syst. 45 (1999) 171–176.
[19] H.L. Shen, R. Manne, Q.S. Xu, D.Z. Chen, Y.Z. Liang, Chemom. Intell. Lab. Syst. 45 (1999) 323–328.
[20] O.M. Kvalheim, Y.Z. Liang, Anal. Chem. 64 (1992) 936–945.
[21] Y.Z. Liang, O.M. Kvalheim, H.R. Keller, D.L. Massart, P. Kiechle, F. Erni, Anal. Chem. 64 (1992) 946–953.
[22] M. Maeder, Anal. Chem. 59 (1987) 527–530.
[23] M. Maeder, A. Zilian, Chemom. Intell. Lab. Syst. 3 (1988) 205–213.

[24] Y. Hu, Y.Z. Liang, B.Y. Li, X.N. Li, Y.P. Du, J. Agric. Food Chem. 52 (2004) 7771–7776.
[25] F. Gong, Y.Q. Peng, H. Cui, Y.Z. Liang, A.K.M. Leung, F.T. Chau, Acta Pharm. Sin. 34 (1999) 214–217.
[26] F. Gong, Y.G. Peng, H. Cui, Y.Z. Liang, A.K.M. Leung, F.T. Chau, Chem. J. Chin. Univ. 20 (1999) 199–203.
[27] H.L. Shen, Y.Z. Liang, R.Q. Yu, X.C. Li, X.X. Sun, Sci. Chin. Ser. B 41 (1998) 21–29.
[28] F.Q. Guo, Y.Z. Liang, L.F. Huang, C.J. Xu, Chem. J. Chin. Univ. 25 (2004) 430–435.
[29] F.Q. Guo, Y.Z. Liang, C.J. Xu, X.N. Li, L.F. Huang, J. Pharm. Biomed. Anal. 35 (2004) 469–478.
[30] Z.D. Zeng, Y.Z. Liang, Y.L. Wang, X.N. Li, et al., J. Chromatogr. A 1107 (2006) 273–285.
[31] H. Van den Dool, P.D. Kratz, J. Chromatogr. 11 (1963) 463–471.
[32] H.R. Keller, D.L. Massart, Anal. Chim. Acta 246 (1991) 379–390.