



Chemical Changeability of Essential Oils in *Chenpi* and *Qingpi* from the Same Origin by Gas Chromatography-Mass Spectrometry Compiled with Automated Mass Spectral Deconvolution and Identification System

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(Received: 15 January 2013;

Accepted: 11 May 2013)

AJC-13487

Chemical variability of essential oils in *Chenpi* or called pericarpium citri reticulatae and *Qingpi* or called pericarpium citri reticulatae viride from the same source was investigated by gas chromatography-mass spectrometry (GC-MS) compiled with automated mass spectral deconvolution and identification system. *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* were collected once a month during July-December in 2011 from the identical area and the cultivar as *Citrus reticulata* Dahongpao of *C. reticulata* blanco. The oil components were identified by their purified mass spectra and Kováts retention indices (RIs). A total of 67, 77, 66, 51, 46 and 59 compounds representing 89.856, 92.151, 97.881, 97.738, 97.229 and 97.897 % of their total relative contents, respectively in the oils of *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* were discerned. The oils from *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* varied considerably both qualitatively and quantitatively. Chemical changeability of six samples differed with each other, to a degree. The chemotype as D-limonene/ γ -terpinene/linalool (55.111-77.659 %/6.579-7.841 %/1.197-7.773 %) was differentiated for essential oils of *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* from *C. reticulata* Dahongpao. The study manifests automated mass spectral deconvolution and identification system can efficiently enhance the accuracy of quantitative and qualitative analyses by extracting information from chromatograms and mass spectra. The results have some meanings to clarify the similarities and differences in bioactive compounds as volatile oils of *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* and to expound the scientific essence of one material used as two kinds of Chinese materia medica such as *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* in the end.

Key Words: *Chenpi*, *Qingpi*, *Citrus reticulata* blanco, *Pericarpium citri reticulatae viride*, *Pericarpium citri reticulatae*, GC-MS.

INTRODUCTION

Pericarpium citri reticulatae or called *Chenpi* in China is the dried pericarp of the ripe fruits of *Citrus reticulata* Blanco or its cultivars (family rutaceae, genus *citrus* L.). The main cultivars are *C. reticulata* Chachi, *C. reticulata* Dahongpao, *C. erythrosa* Tanaka, *C. reticulata* Tangerina, etc. The drug is subdivided into two classes, known respectively as *Chenpi* and *Guang chenpi*. The latter is from the cultivar of *C. reticulata* Chachi produced in Guangdong Province of China. *Pericarpium citri reticulatae viride* or called *Qingpi* in China is the dry pericarp of the young or immature fruits of *C. reticulata* and its cultivars. The fallen youthful fruit is collected in May and June and known as *Geqingpi*. Whereas the unripe fruit peel is gathered in July and August and called as *Sihuaqingpi*¹⁻⁴. *Pericarpium citri reticulatae* and *Pericarpium*

citri reticulatae viride come from the same material as the desiccated pericarp of *C. reticulata* or its cultivars. They become two kinds of Chinese materia medica because of varied harvest time, which makes them have different effects and indications. They both belong to the *qi*-regulating drug, while pericarpium citri reticulatae is good at regulating the flow of *qi* and energizing the spleen function and pericarpium citri reticulatae viride is used in relaxing the liver and disintegrating stagnated *qi*. They are the representative of one material used as two sorts of Chinese materia medica^{3,4}.

The dissimilar effects and indications between *Pericarpium citri reticulatae* and *Pericarpium citri reticulatae viride* should be associated with their bioactive components. The main active compositions of *Pericarpium citri reticulatae* and *Pericarpium citri reticulatae viride* consist of essential oil and flavonoid. Now, many reports on *Pericarpium citri reticulatae*

and *Pericarpium citri reticulatae viride* focus on flavonoid, but few on essential oils which have strong pharmacologic bioactivities⁵. For instance, D-limonene, as a major component in *Pericarpium citri reticulatae* and *Pericarpium citri reticulatae viride* volatile oils, possesses resolving phlegm and anticancer activities^{4,6}. To our best of knowledge, there is no report is available in literature on the chemical changeability of oils in *Pericarpium citri reticulatae* and *Pericarpium citri reticulatae viride* from the same origin.

Essential oil of Chinese materia medica is a very complicated system and contains hundreds of chemical components. The overlapped or embedded peaks extensively exist in the total ion chromatogram from volatile oil, which would make the accurately quantitative and qualitative analyses become more difficult. For that reason, the comparative analysis among different samples may be a hard task and even is sometimes impossible. In order to solve this challenge, some researchers⁵ have used chemometric resolution method such as alternative moving window factor analysis, heuristic evolving latent projections, *etc.* The gas chromatography-mass spectrometry (GC-MS) ChemStation is always compiled with automated mass spectral deconvolution and identification system (AMDIS), which is excellent in solving the above problem. AMDIS is a merged set of procedures for first taking out pure component spectra and related information from complex chromatograms and then using this information to establish whether the composition can be recognized as one of the compounds exhibited in a reference library⁷. So, the GC-MS ChemStation and AMDIS are used to do the comparative study among essential oils in *Pericarpium citri reticulatae* and *Pericarpium citri reticulatae viride* and to deconvolve the overlapped or embedded peaks.

In this research, chemical variability of volatile oils in *Pericarpium citri reticulatae* and *Pericarpium citri reticulatae viride* from the same source was originally reported by GC-MS ChemStation compiled with AMDIS. And, AMDIS was first employed to deconvolve the overlapped or embedded peaks in total ion chromatograms of oils from Chinese materia medicas such as *Pericarpium citri reticulatae* and *Pericarpium citri reticulatae viride*.

EXPERIMENTAL

Materials and *n*-alkane standard solution: *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* were collected every month during July to December in 2011 from three adjacent plants growing in Jianlong Town of Bishan County in Chongqing municipality of China. The plants were authenticated as *C. Reticulata* Dahongpao and have the same tree-age. In traditional view, *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* should be collected separately during May to August and September to December. Whereas the color of pericarp collected in 10-17 is yet green due to the particular environment of Jianlong Town, so the sample 10-17 still belongs to *Pericarpium citri reticulatae viride* according to its definition. In this study, samples 7-16, 8-15 and 9-16 belong to *Geqingpi*; 10-17 belongs to *Sihuaqingpi* and they all belong to *Pericarpium citri reticulatae viride*; samples 11-17 and 12-15 belong to *Pericarpium citri*

reticulatae. *n*-Alkane standard solution of C₈-C₂₀ (mixture no. 04070) purchased from Fluka Chemika (Buchs, Switzerland) and the *n*-alkanes of C₂₂-C₂₅ identified in essential oils of samples 7-16 and 8-15 were used to determine Kováts retention indices (RIs) through the equation for temperature-programmed retention indices (PTRIs) proposed by Van den Dool and Kratz⁸.

Essential oil extraction: The six samples were weighted 14.3-22.4 g, respectively and then smashed. The smashed samples were swollen with about 10 times volume (v/w) 150-250 mL of distilled water in a Clevenger-type apparatus for soaking 1 h at 40 °C before extracting volatile oil for 3-4 h⁹. Subsequently, the essential oils were prepared according to the procedure described in the Chinese pharmacopoeia¹. The oils were dried over anhydrous sodium sulphate for 24 h at 4 °C and then stored in separate screw-capped vials in a refrigerator until needed¹⁰. The yields of essential oils (v/w) are < 0.7, 0.7, 2.2, 4.2, 3.4, 2.3% for corresponding samples 7-16, 8-15, 9-16, 10-17, 11-17, 12-15. In order to ensure there is sufficient oil (≥ 0.1 mL) for analysis, the sample's weight should not less than 20 g.

GC-MS analysis: An Agilent 7890A gas chromatograph matched with an Agilent 5975C mass spectrometer with a GC-MS ChemStation data system was used for GC-MS analysis. The gas chromatograph was coupled with a fused silica capillary column HP-5MS 5 % phenyl methyl siloxane (30 m × 0.25 mm i.d., 0.25 μm film thickness). The oven temperature was programmed from 60 °C (3-min hold) to 250 °C at 4 °C min⁻¹. The carrier gas was helium at a constant flow of 1 mL min⁻¹. Injector and ion-source were remained at 280 and 230 °C, individually. Splitting ratio was 20:1. Solvent delay was 3 min. Electron impact mass spectra were taken at 70 eV. Scan at 2.29 scans s⁻¹ from *m/z* 30 to 350 amu¹¹. The injection volume was 1 μL of a 10 % *n*-hexane solution to obtain the appropriate peak intensity¹². The molecular weight 352 of detected compound pentacosane (*n*-C₂₅H₅₂) is beyond 350, so the reasonable *m/z* range should be from 30 to 380 amu.

Each peak in 40 peaks which area is bigger than that of other peaks in each sample is analyzed by GC-MS ChemStation and AMDIS. The library searches and spectral matching of the deconvolved pure mass spectra were performed on the National Institute of Standard and Technology (NIST) 08 MS database. The referred RIs are gotten from NIST 08 MS library or the reference¹³. According to the deconvolved chromatogram and mass spectra, the quantitative analysis of each component can be directly calculated by the overall volume integration method (OVI)⁵. They are proportionate to the content of the peak as integration based on total ion chromatogram. The total amount or whole area of each sample is obtained by setting appropriate MS signal integration index, which and some other basic informations can be seen in Table-1.

RESULTS AND DISCUSSION

A really contrast study of the constitutions in essential oils of *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* could be implemented because they are from the same origin such as the cultivar and environment. Likewise, extraction conditions were identical for all samples. So, the

TABLE-1
MS SIGNAL INTEGRATION INDEX AND SOME OTHER BASIC INFORMATIONS

	7-16	8-15	9-16	10-17	11-17	12-15
Initial peak width	0.1	0.1	0.1	0.1	0.1	0.1
Initial threshold	3.6	6.7	5.4	5.3	5.8	5.1
Total detected peaks	179	179	176	179	180	183
Total area	15656130	28938793	31382578	25772756	21596727	32048202
Max intensity of TIC	125000	152000	166000	161000	149000	162000
Detected compounds by AMDIS	241	290	133	78	53	107

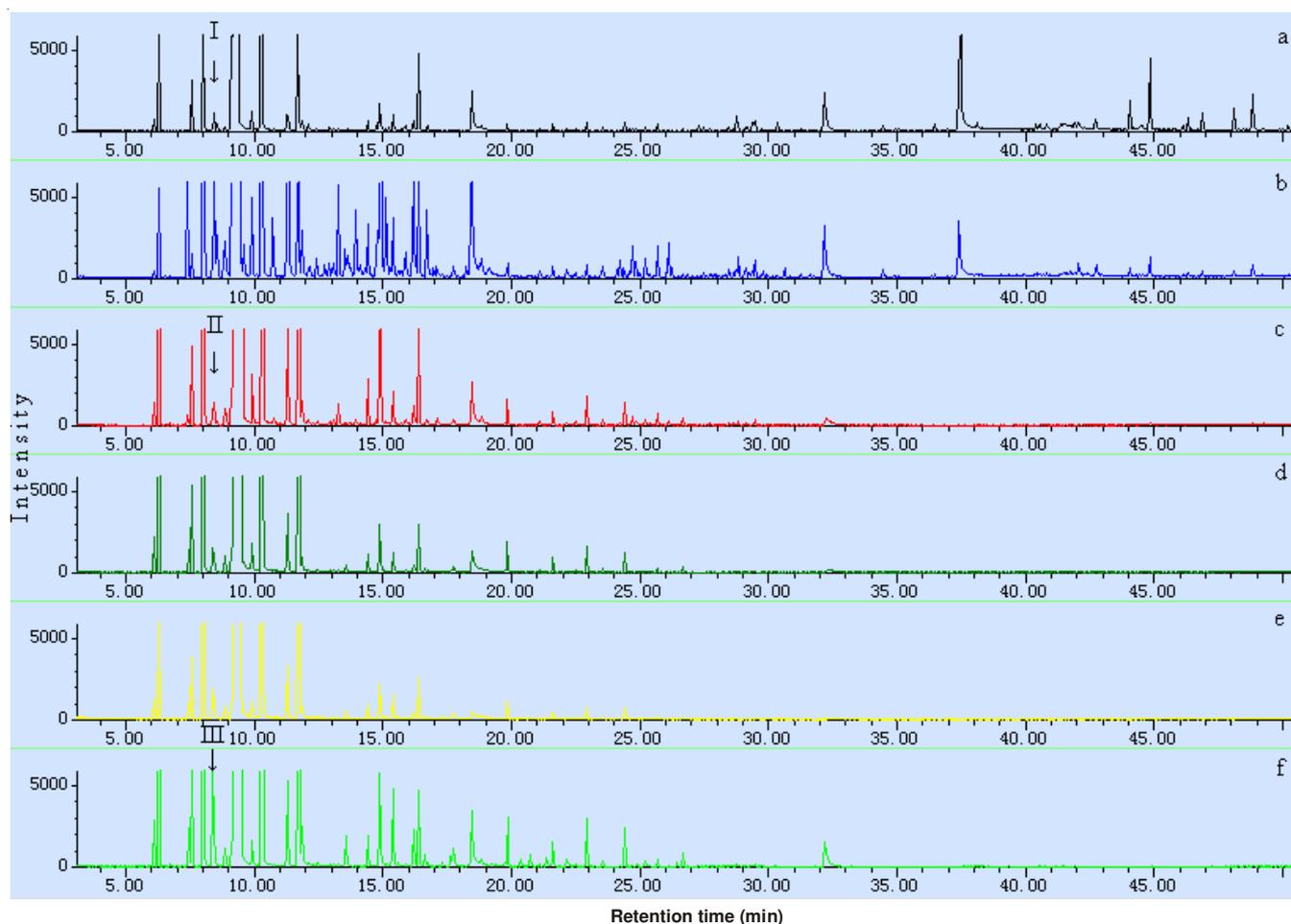


Fig. 1. Magnified total ion chromatograms of PCRV and PCR gathered at the different time. a 7-16; b 8-15; c 9-16; d 10-17; e 11-17; f 12-15

impacts of hereditary, environmental and technological factors on the chemical components of oils were thought inappreciable¹⁴.

Deconvolution of overlapped peaks by AMDIS: Fig. 1 shows the magnified total ion chromatograms of essential oils of *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae*. All the total ion chromatograms are very complex analytical systems and include many overlapped peaks. Because of these, the simple search through the MS database will sometimes fail since the original mass spectrum from super-imposed peaks cannot get a good matching index with that of a pure component in the NIST 08 MS database.

Peak clusters I, II and III in Fig. 1 are such instances. The total ion chromatograms of peak clusters I, II and III are shown in Fig. 2. It seems that cluster I is overlapped by two peaks, cluster II is a broad single peak and cluster III is a singular peak. Moreover, at some dissimilar scanning points, the MS

spectra correspond to different compounds with low matching indices. Namely, the three peak clusters are overlapped seriously and even possibly comprise the embedded peaks. So, it is necessary to deconvolve the overlapped peaks for doing the comparative investigation among these analytical systems rightly.

In order to demonstrate how to identify the compounds correctly, these three peak clusters are used as a case to display the deconvolution process by AMDIS. It can be seen in Fig. 2 that cluster I contains two components, cluster II and III both contain three components through the analysis of AMDIS.

The extracted or purified spectrum of detected component R1 at 8.386 min of sample 7-16 was selected, afterwards the function of going to NIST MS Program was chosen and the optimum counterpart is octanal whose match probability is as high as 92.7%. Whereas if the initial spectrum at that time point was selected and later done the same procedure,

the best match is still octanal but its match probability falls to 58.6 %, which shows the extracted spectrum is purer than the original spectrum at that time point. So the component R1 can be ascertained as octanal. The similar conclusion can be made for the component discerned at 8.386 min of sample 9-16 or at 8.379 min of specimen 12-15.

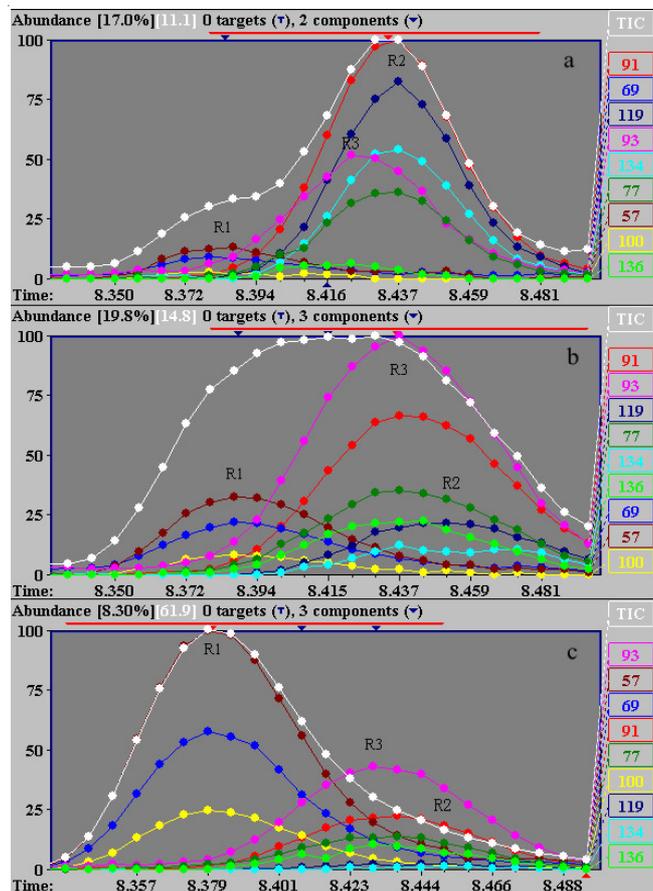


Fig. 2. Analytic results of total ion chromatograms from 8.33 to 8.50 min by AMDIS. a. 7-16; b. 9-16; c. 12-15

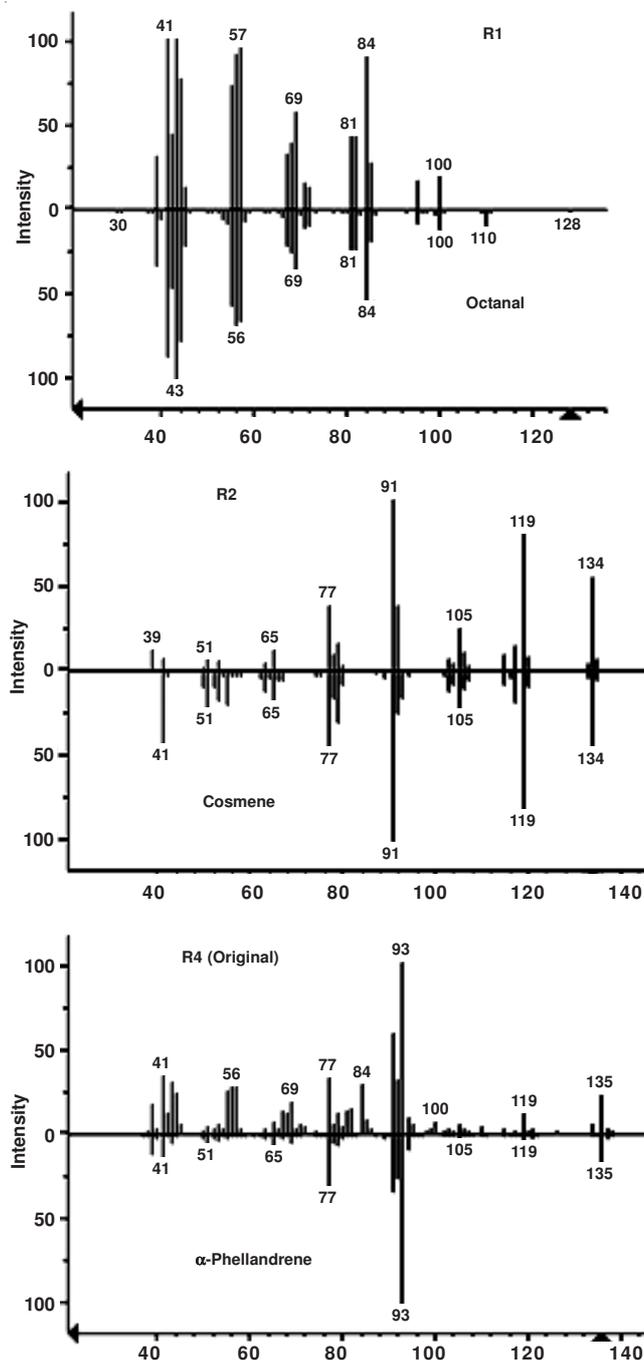
Alike procedure was done to the recognized constituent R2 at 8.437 min of sample 7-16. The optimal match is 2,6-dimethyl-1,3,5,7-octatetraene, *E,E*- or called cosmene. While the finest counterpart of the detected component R3 at 8.437 min of sample 9-16 is α -phellandrene when a similar process was done. That is to say, compounds cosmene and α -phellandrene have nearly the same retention time, so their MS spectra should be overlapped or even embedded.

The original spectrum of detected component R4 at 8.415 min of sample 9-16 has both the characteristic fragment ion peaks of α -phellandrene and octanal and its match probability with compound α -phellandrene is only 4.21 %. The best counterpart of its extracted spectrum is 7-azanorbornene, which is unreasonable and so the obtained spectrum is illogical too. Hence, the composition R4 doesn't exist in nature.

Likewise, the best match of discerned component at 8.430 min of sample 12-15 is α -phellandrene too. The original spectrum of the recognized constituent at 8.408 min of specimen 12-15 still has both the typical fragment ion peaks of α -phellandrene and octanal. Its extracted spectrum is unreasonable and the counterpart to the extracted spectrum is

irrational, as well. So, the detected component at that time point doesn't exist also. The corresponding spectra can be seen in Fig. 3.

The representative fragment ion peaks (m/z): 57, 69, 100; 93, 77, 136; 91, 119, 134 of respective compounds octanal, α -phellandrene, cosmene were selected in AMDIS. In sample 7-16, it is clearly observed that the characteristic peaks of octanal or cosmene and their intensity variability is identical, respectively. However, the peaks: 93, 77 and 136 of α -phellandrene should be noticed. The abundance changeability of 93 and 136 is different from that of the representative ion peaks of cosmene, whereas the intensity variability of peak 77 is nearly the same as that of the typical ion peaks of cosmene, which is because 77 is the common peak of cosmene and α -phellandrene. In this case, the content of α -phellandrene is



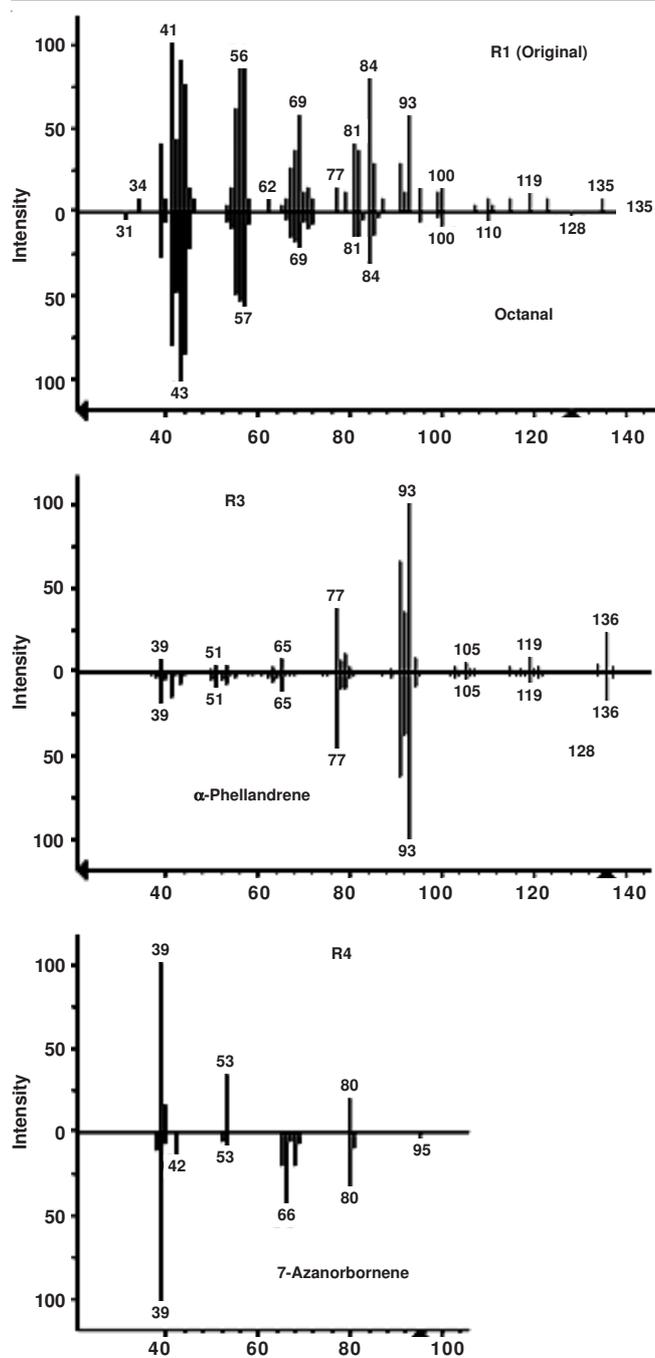


Fig. 3. Extracted spectra of R1, R2, R3 and R4, the original spectra of R1 and R4 and their corresponding standard mass spectra

much lower than cosmene's, which can be seen from the abundances of peaks 136 and 134 and the intensities of 77 in the standard mass spectra of compounds cosmene and α -phellandrene are almost equivalent (about 50), so 77 is mainly effected by cosmene. Peak 93 is the mutual peak of cosmene and α -phellandrene too, but 93 is the base peak in the standard mass spectrum of α -phellandrene, while its intensity is only about 16 in the normal mass spectrum of cosemen. Therefore, peak 93 is actually mainly affected by α -phellandrene. Peak 136 is only owned by α -phellandrene, so it is not affected by cosmene. In conclusion, the total ion chromatogram of sample 7-16 from 8.33 to 8.50 min contains 3 compounds such as octanal, α -phellandrene and cosmene, in reality. The compound

α -phellandrene in sample 7-16 was not detected by AMDIS because its RT is so close to cosmene's and its content is very low.

Abiding by the identical principle to analyze the total ion chromatogram of sample 12-15, we can verify the existence of octanal and α -phellandrene again and we can see the presence of cosmene by its typical peaks, but as the same reason mentioned above it cannot be discerned by AMDIS. Correspondingly, to the total ion chromatogram of sample 9-16, it is confirmed the presence of octanal, α -phellandrene and cosmen once more. To our knowledge, compound cosmene was never reported as a component in essential oils of pericarpium citri reticulatae viride and pericarpium citri reticulatae or the peels of *C. reticulata* or its cultivars formerly, its structure can be seen in Fig. 4. Their RIs can also be determined by their RTs provided by their characteristic fragment ion peaks.

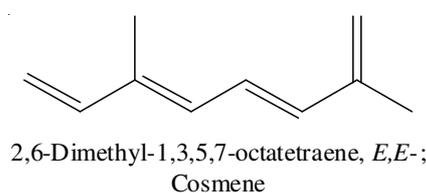


Fig. 4. Structure of cosmene

Furthermore, from the intensity variability of peaks 57, 136 and 134, the amount changeability of compounds octanal, α -phellandrene and cosmene can be confirmed (Table-2). The content ratios of octanal/ α -phellandrene/cosmene are 0.26:< 0.05:1, 0.26:0.23:1, 1.54:2.96:1, 12.6:18.2: < 1, 30.1:9: < 1, 63.6:16.7: < 1 for corresponding specimen.

Other peaks in the total ion chromatograms are determined qualitatively in the same way as described above. The tentatively qualitative results of constituents in essential oils from pericarpium citri reticulatae viride and pericarpium citri reticulatae are exhibited in Table-2.

Quantitative analysis of chemical compositions in essential oils of *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae*: The quantitative results are shown in Table-2. In essential oils of pericarpium citri reticulatae viride, 67, 77, 66 and 51 components representing 89.856, 92.151, 97.881 and 97.738 % of the total relative content, respectively were determined. Oils of *Pericarpium citri reticulatae* were composed of 46 and 59 compounds constituting 97.229 % and 97.897 %, separately. The total components content of sample 7-16 or 8-15 as 89.856 % or 92.151 %, respectively, is a little lower than the other four specimens. As it can be seen in Table-1, the number of detected compounds by AMDIS is 241 or 290 as to corresponding sample 7-16 or 8-15, which is much more than the other four samples. In other words, there still exist more unidentified low content compounds in sample 7-16 or 8-15 than the other four specimens' and their total relative amount is a little higher than the other four samples'.

Chemical changeability of essential oils in *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae*: As is shown in Table-2, Figs. 5 and 6, the essential oils from *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* significantly differed from each other both

TABLE-2
CHEMICAL CONSTITUENTS IN ESSENTIAL OILS OF PCR_V AND PCR¹⁵

No.	RI	Components	Relative content (%)					
			PCR _V				PCR	
			7-16	<i>Geqingpi</i>	9-16	10-17	11-17	12-15
1	926	α -Thujene	0.12	0.031	0.121	0.221	0.152	0.236
2	933	α -Pinene	1.22	0.479	1.046	1.297	0.899	1.264
3	948	Camphene	0.016	0.02	0.031	0.02	0.01	0.01
4	971	2-Ethenyltetrahydro- 2,6,6-trimethyl-2 <i>H</i> -pyran	0.011	0.583	0.06	Nd	0.013	tr
5	973	Sabinen	0.013	tr	0.04	0.154	0.123	0.2
6	976	β -Pinene	0.53	0.155	0.465	0.579	0.483	0.652
7	991	β -Myrcene	1.336	1.7	2.773	2.862	2.492	2.529
8	1004	Octanal	0.052	0.155	0.083	0.126	0.301	0.636
9	1005	α -Phellandrene	tr	0.136	0.16	0.182	0.09	0.167
10	1006	Cosmene	0.2	0.6	0.054	tr	tr	tr
11	1016	1,4- Cineol	Nd	0.073	Nd	Nd	Nd	Nd
12	1018	α -Terpinene	0.059	0.338	0.219	0.19	0.184	0.206
13	1021	Isomyrcenol	tr	0.089	Nd	Nd	Nd	Nd
14	1026	<i>p</i> -Cymene	4.014	2.176	0.667	0.393	0.265	0.273
15	1033	D-Limonene	58.909	55.111	74.087	77.659	77.628	70.552
16	1039	β - <i>cis</i> -Ocimene	tr	0.28	0.05	0.011	tr	0.012
17	1048	Tetrahydro-2,2-dimethyl-5-(1-methyl-1-propenyl)furan	Nd	0.088	Nd	Nd	Nd	Nd
18	1049	β - <i>trans</i> -Ocimene	0.315	0.467	0.34	0.208	0.21	0.167
19	1059	γ Terpinene	6.717	6.579	7.841	6.994	7.282	7.093
20	1089	Terpinolene	0.179	1.536	0.567	0.493	0.53	0.52
21	1090	<i>p</i> - α -Dimethyl styrene	0.243	1.225	0.14	tr	tr	0.019
22	1101	Linalool	1.291	1.197	3.394	3.123	3.99	7.773
23	1105	Nonanal	tr	0.11	0.114	0.07	0.05	0.025
24	1106	Hotrienol	0.231	0.29	0.11	tr	tr	tr
25	1113	1,3,8- <i>p</i> -Menthatriene	0.144	0.074	0.03	Nd	Nd	Nd
26	1122	Myrcenol	Nd	0.15	Nd	Nd	Nd	Nd
27	1146	β -Terpineol	0.048	0.648	0.165	0.038	Nd	Nd
28	1154	Ocimenol	Nd	0.212	Nd	Nd	Nd	Nd
29	1155	Citronellal	Nd	Nd	Nd	0.096	0.105	0.196
30	1156	3,6-Dihydro-4-methyl-2-(2-methyl-1-propenyl)-2 <i>H</i> -pyran	Nd	0.099	Nd	Nd	Nd	Nd
31	1157	4-Isopropenylcyclohexanone	0.04	0.127	0.015	Nd	Nd	Nd
32	1179	Terpinen-4-ol	0.168	0.402	0.304	0.17	0.144	0.19
33	1188	<i>p</i> -Cymen-8-ol	0.036	0.167	0.023	Nd	Nd	Nd
34	1189	2-Methylene-5-(1- methylethenyl)cyclohexanol,	0.13	0.421	0.052	Nd	Nd	Nd
35	1192	α -Terpineol	0.426	5.979	1.254	0.427	0.4	0.61
36	1199	γ Terpineol	Nd	0.612	0.038	Nd	Nd	Nd
37	1207	Decanal	0.315	0.505	0.263	0.23	0.28	0.51
38	1219	<i>p</i> -Menth-1-en-9-al	0.034	0.036	0.019	Nd	Nd	0.01
39	1221	<i>cis</i> -Carveol	0.064	0.25	0.015	tr	Nd	tr
40	1230	Nerol	Nd	Nd	0.181	Nd	Nd	Nd
41	1230	β -Citronellol	Nd	Nd	tr	0.09	0.087	0.271
42	1236	Methyl thymyl ether	0.966	1.07	0.648	0.37	0.383	0.452
43	1243	<i>cis</i> -Citral	Nd	Nd	0.01	0.04	0.035	0.087
44	1246	(+)- Carvone	0.135	0.52	0.062	0.02	Nd	0.032
45	1257	<i>trans</i> -Geraniol	Nd	0.144	0.11	Nd	Nd	0.022
46	1273	<i>trans</i> -Citral	Nd	Nd	0.03	0.038	0.025	0.091
47	1276	Perillaldehyde	0.03	0.138	0.092	0.1	0.09	0.164
48	1297	Thymol	1.332	2.616	0.82	0.559	0.337	0.783
49	1308	Undecanal	tr	tr	0.026	0.012	tr	0.028
50	1339	δ -Elemene	0.119	0.116	0.183	0.215	0.165	0.318
51	1355	Citronellol acetate	Nd	Nd	Nd	Nd	tr	0.062
52	1366	Nerol acetate	0.027	Nd	Nd	tr	0.017	0.093
53	1378	Copaene	0.047	0.056	0.04	0.022	0.01	0.023
54	1386	Geraniol acetate	Nd	Nd	Nd	tr	0.015	0.083
55	1394	γ Elemene	0.099	0.081	0.092	0.12	0.065	0.152

No.	RI	Components	Relative content (%)					
			PCR				11-17	12-15
			Gegingpi		Sihuaqingpi			
7-16	8-15	9-16	10-17	11-17	12-15			
56	1411	Dodecanal	0.03	0.082	0.03	Nd	Nd	0.087
57	1422	Caryophyllene	0.042	0.035	0.031	0.01	tr	0.024
58	1436	γ -Elemene	0.115	0.084	0.173	0.191	0.105	0.27
59	1456	α -Caryophyllene	0.061	0.091	0.053	0.03	0.025	0.04
60	1478	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	Nd	0.137	0.02	Nd	Nd	Nd
61	1483	α -Amorphene	Nd	0.081	Nd	Nd	Nd	Nd
62	1484	Germacrene D	0.12	tr	0.151	0.143	0.112	0.229
63	1489	β -Selinene	0.039	0.042	tr	Nd	Nd	Nd
64	1494	δ -Selinene	0.024	0.215	0.05	tr	Nd	tr
65	1498	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	0.044	0.128	0.022	Nd	Nd	tr
66	1499	Elixene	Nd	Nd	0.018	0.016	0.01	0.015
67	1510	α -Farnesene	0.1	0.16	0.042	0.01	Nd	0.066
68	1526	(+)- δ -Cadinene	0.1	0.249	0.08	0.033	0.027	0.05
69	1538	(+)-Valencene	Nd	0.047	Nd	Nd	Nd	Nd
70	1541	2,3,4,4a,5,6-Hexahydro-1,4a-dimethyl-7-(1-methylethyl)naphthalene	Nd	0.228	0.02	Nd	Nd	Nd
71	1553	Elemol	Nd	0.014	Nd	tr	Nd	0.03
72	1560	Germacrene B	0.033	0.021	0.05	0.04	0.03	0.072
73	1581	Spathulenol	0.08	Nd	Nd	Nd	Nd	Nd
74	1635	γ -Eudesmol	0.091	0.168	0.028	tr	Nd	tr
75	1654	β -Eudesmol	0.119	0.041	0.01	Nd	Nd	0.01
76	1657	α -Eudesmol	0.038	0.033	tr	Nd	Nd	tr
77	1658	Selina-6-en-4-ol	0.13	0.131	0.04	Nd	Nd	0.01
78	1758	α -Sinensal	1.23	0.922	0.241	0.136	0.06	0.483
79	1927	Hexadecanoic acid, methyl ester	0.153	0.025	Nd	Nd	Nd	Nd
80	1968	<i>n</i> -Hexadecanoic acid	3.9	0.885	Nd	Nd	Nd	Nd
81	2200	Docosane	0.51	0.03	Nd	Nd	Nd	Nd
82	2263	3-methyl-heneicosane	0.532	0.073	Nd	Nd	Nd	Nd
83	2300	Tricosane	1.043	0.159	0.024	Nd	Nd	Nd
84	2373	3-Methyltricosane	0.33	0.024	Nd	Nd	Nd	Nd
85	2400	Tetracosane	0.41	0.053	Nd	Nd	Nd	Nd
86	2463	5-butyl docosane	0.35	0.042	Nd	Nd	Nd	Nd
87	2500	Pentacosane	0.616	0.11	0.014	Nd	Nd	Nd
		Total compounds ^a	67(89.856)	77(92.151)	66(97.881)	51(97.738)	46(97.229)	59(97.897)
		Monoterpene hydrocarbons	17(74.015)	17(70.907)	17(88.631)	16(91.263)	16(90.348)	16(83.9)
		Sesquiterpene hydrocarbons	13(0.943)	17(1.771)	16(0.975)	12(0.83)	10(0.549)	13(1.259)
		Alkanes	7(3.791)	7(0.491)	2(0.038)	0(0)	0(0)	0(0)
		Total oxygenated compounds	30(11.107)	36(18.982)	31(8.237)	23(5.645)	20(6.332)	30(12.738)
		Alcohols	14(2.852)	19(11.021)	16(5.724)	9(3.848)	5(4.621)	12(8.916)
		Phenol	1(1.332)	1(2.616)	1(0.82)	1(0.559)	1(0.337)	1(0.783)
		Ether	1(0.966)	1(1.07)	1(0.648)	1(0.37)	1(0.383)	1(0.452)
		Carbonyl compounds	10(1.866)	10(2.595)	12(0.985)	10(0.868)	9(0.946)	12(2.349)
		Acid	1(3.9)	1(0.885)	0(0)	0(0)	0(0)	0(0)
		Esters	2(0.18)	1(0.025)	0(0)	2(tr)	3(0.032)	3(0.238)
		Epoxides	1(0.011)	3(0.77)	1(0.06)	0(0)	1(0.013)	1(tr)

^aNumber of compounds. The value in brackets is total percentage; tr (trace), relative content < 0.01 %; Nd, not detected

qualitatively and quantitatively. Although some compounds presented in the oils of *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* are identical, their contents are varied. Moreover, some components could be found in *Pericarpium citri reticulatae viride* or *Pericarpium citri reticulatae* but not in another such as alkanes. Six samples have almost the equivalent qualitative constituents of monoterpenes, which represent the main compound family in all oils, except for 1,3,8-*p*-menthatriene, which was not detected in samples 10-17, 11-17 and 12-15, whilst varied in the quantitation from 70.907-91.263 %. Its major compounds were D-limonene

(55.111-77.659 %), γ -terpinene (6.579-7.841 %) and β -myrcene (1.336-2.862 %). Six specimens all differed qualitatively and quantitatively in sesquiterpenes to some extent which amounts were so low and only constituted 0.549-1.771 %. δ -Elemene (0.116-0.318 %) and γ -elemene (0.084-0.27 %) were the notable sesquiterpenes. Six samples all varied qualitatively and quantitatively in oxygenated compounds contents (5.645-18.982 %) were relatively poor. Alcohols (2.852-11.021 %) and carbonyl compounds (0.868-2.595 %) were the main groups and linalool (1.197-7.773 %), α -terpineol (0.4-5.979 %), α -sinensal (0.06-1.23 %), decanal (0.23-0.51 %) were the prominent compounds.

The content 5.979 % of α -terpineol in sample 8-15 is much larger than the other five samples' that makes the amounts of alcohols 11.021 % and oxygenated compounds 18.982 % are higher than those of the other five specimens, too.

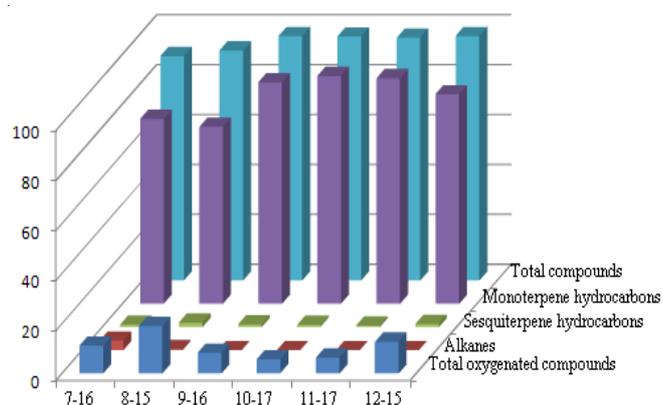


Fig. 5. Chemical variability of PCR and PCRV oils¹¹

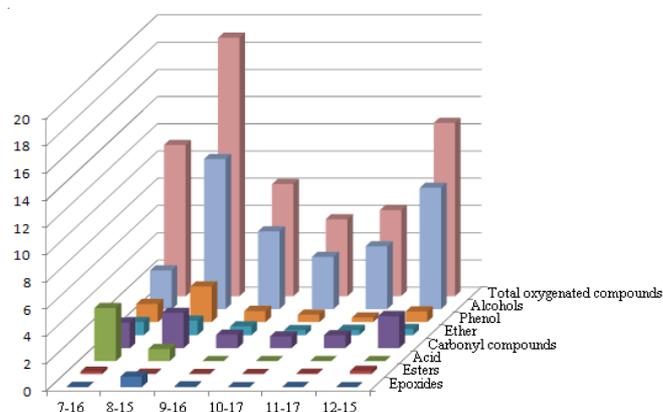


Fig. 6. Chemical changeability of the oxygenated compounds in oils¹¹

An interesting compound family alkane (0-3.791 %) including 4 *n*-alkanes (C₂₂-C₂₅) and 3 branched alkanes was found in samples 7-16 and 8-15, while only two of them were identified in sample 9-16 and none of them were detected in samples 10-17, 11-17 and 12-15. To our best of knowledge, those alkanes were never published as components in essential oils of *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* or the peels of *C. reticulata* or its cultivars beforehand^{5,9-11,14-16}. Alkanes were just found in *Geqingpi*, which imply such compounds maybe come from the sarcocarp. As the fruit growth, the ratio of sarcocarp should increase. Nevertheless, the amounts of alkanes sharply decrease as the fruit growth, 3.791, 0.491 and 0.038 % of the respective samples 7-16, 8-15 and 9-16, so more research is required.

Chemical variability of sample 7-16 or 8-15 severely differed with the other four samples. In the other four samples, the chemical changeability of specimen 12-15 is distinct from the other three samples to some extent. Chemical variability of the other three samples differed with each other a little. All these disparities can provide evidence for expounding the scientific essence of one material used as two kinds of Chinese materia medica such as *Pericarpium citri reticulatae* and *Pericarpium citri reticulatae viride*.

Chemotypes of essential oils in *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae*: Lota *et al.*¹⁴ differentiate the chemotypes of essential oils in peels of different cultivars of *C. reticulata* by the contents of limonene and γ -terpinene. In this way, they distinguish two chemotypes as limonene and limonene/ γ -terpinene. In fact, all oils consist of limonene as a primary component and most oils comprise γ -terpinene as the second high amount component^{5,9-11,14-16}. So, all oils can be classified as the chemotype of limonene. Then, according to the content of γ -terpinene, it can be continually sub classified as the chemotype of limonene or limonene/ γ -terpinene. Through analyzing Lota's report, it is presumed that the oil should be classified as limonene/ γ -terpinene if the content of γ -terpinene is not less than 3 %. And in this study, the volatile oils can be classified as the chemotype of limonene/ γ -terpinene. Whereas except for these two important components, we noticed another prominent component as linalool, so the oils of *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* from the cultivar of *C. Reticulata* Dahongpao in this case can be successively sub classified as the chemotype of limonene/ γ -terpinene/linalool, their contents varied from 55.111-77.659, 6.579-7.841 and 1.197-7.773 %, respectively. This conclusion can be confirmed by Wang *et al.*⁵ again. Following the same principle, the essential oils of *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* from the cultivar of *C. Reticulata* 'Chachi' produced in Guangdong can be classified as the chemotype of limonene/ γ -terpinene/benzoic acid, 2-(methylamino)-, methyl ester, which can be verified by reference⁵ and our research outcome too.

Conclusion

The various constituents in essential oils of *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* from the same origin were identified and quantified by GC-MS and AMDIS and then compared based on their qualitative and relatively quantitative results. Chemical variability of such oils differed, to some extent. The AMDIS could effectively improve the accuracy of quantitative and qualitative results along with PTRIs. The oils' chemotypes of *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* from different cultivars of *C. Reticulata* are first analyzed. The results acquired can be beneficial to elucidate the similarities and differences in bioactive components as volatile oils of *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* and have important significance to explain the scientific essence of "One material used as two sorts of Chinese materia medica" such as *Pericarpium citri reticulatae viride* and *Pericarpium citri reticulatae* in the end.

ACKNOWLEDGEMENTS

Financial supports to this work from the National Nature Foundation Committee of P.R. China (Grant No. 81072991) and the natural science key project of Ministry of Education of Sichuan province (No. 09ZA033) are highly appreciated. The authors thank the assistance of undergraduate Lijuan Liu and all employees for their supports to this research.

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