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Identification of *Portulaca oleracea* L. from different sources using GC–MS and FT-IR spectroscopy

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ABSTRACT

A fingerprinting approach was developed by means of gas chromatography–mass spectrometry (GC–MS) and IR spectroscopy for quality control of *Portulaca oleracea* L., a traditional Chinese food and medicine that has been used for thousands of years in China. Eleven *P. oleracea* L. samples obtained from different sources were used to establish the fingerprints, and the similarity evaluation and hierarchical cluster analysis were performed to evaluate the similarity and variation of these samples. The results showed that the 11 samples all have the similarity of greater than 0.84, indicating that the samples from different sources were consistent to great extent. The cluster analysis results of GC–MS and IR spectra were similar, and 11 samples from five provinces of China were divided into two main clusters: one was from North China and another from South China. It proved that both GC–MS fingerprint and IR spectral fingerprint could be used for the identification and differentiation of *P. oleracea* L. from different sources.

1. Introduction

Quality assurance (QA) practice is now regarded as a core requirement for international trade especially for food and pharmaceuticals. In general, QA is applied to processes and products, which are well documented and characterized, and it is quite an exacting and involved task. However, there are many examples where popular products are not fully or exactly specified and QA becomes an even more challenging undertaking [1]. Important illustrations of such products are the many traditional medicines, which are in common worldwide use. For example, traditional Chinese medicine (TCM), which has a 5000-year history of application, is currently still attracting considerable attention worldwide because of its low toxicity and good therapeutical performance [2]. The quality control of TCM is an important concern for both the health authorities and the public [3–5].

Portulaca oleracea L. (*P. oleracea* L., purslane) is a common, herbaceous succulent annual plant, which is distributed extensively in temperate and tropical regions in worldwide [6,7]. It has been used as a kind of food and medicinal plant for thousands of years in China as well as many other nations. *P. oleracea* L. is of abundant nutrition with contents of proteins, carbohydrates and trace element. As a kind of Chinese traditional medicine, it has been used traditionally for the treatment of dysentery with bloody stools and externally for boils and sores, eczema, erysipelas, and insect and snake bites [8,9]. Among the effective ingredients of *P. oleracea* L., volatile oils are one of the most popular compounds in the plant kingdom.

Volatile oils are those plant-derived oils which are totally volatile or evaporating in nature. The volatile oils in plants generally consists of terpenoid, fatty group and aromatic series. Volatile oil in *P. oleracea* L. is widely used as medicine to prevent mosquito bites and to treatment prickly heat of infant and child, and it is also used as materials to produce various numerous skin care products and cosmetics [10]. Furthermore, it is also an important raw material for the perfume industry, food industry and chemical industry.

Since application of *P. oleracea* L. is growing steadily, development of a suitable quality control method for it was urgently required.

Chromatographic fingerprint, a more significant formulation for controlling the quality of herbal medicines and their products, has been accepted by many countries and organizations [11,12]. This technique emphasizes the systemic characterization of compositions of samples and focuses on the identification and assessment of the stability of components [13]. To facilitate the QA approaches for such complex products such as traditional Chinese medicine, the World Health Organization (WHO) has accepted chromatographic fingerprint as a strategy for the assessment of the substances [14–16].

Gas chromatography–mass spectrometry (GC–MS) is a central analytical technique that serves in a broad range of applications, and it is a commonly used method for characterization and identification of volatile organic compounds in complex mixtures [17,18].

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Table 1
Raw materials used in this experiment.

Sample No.	Location	Harvesting time	Medical parts
1	Jinan, Shandong Province, China	August 2008	Whole herb
2	Qufu, Shandong Province, China	August 2008	Whole herb
3	Taiyuan, Shanxi Province, China	August 2008	Whole herb
4	Linfen, Shanxi Province, China	August 2008	Whole herb
5	Zhenzhou, Henan Province, China	August 2008	Whole herb
6	Zhumadian, Henan Province, China	August 2008	Whole herb
7	Ganzhou, Jiangxi Province, China	August 2008	Whole herb
8	Nanchang, Jiangxi Province, China	August 2008	Whole herb
9	Changsha, Hunan Province, China	August 2008	Whole herb
10	Zhangjiajie, Hunan Province, China	August 2008	Whole herb
11	Shaoyang, Hunan Province, China	August 2008	Whole herb

Due to the powerful separation efficiency and the sensitive detection, GC–MS has become a popular and useful analytical tool in the research field of herbal medicines, especially in establishing the chromatographic fingerprint for the quantity control of traditional Chinese medicine [19,20].

Fourier transform infrared spectroscopy (FT-IR) has been most widely used and is a good established tool for structure elucidation and quality control in various industries application [21]. This technique is usually considered to be non-destructive, simple and fast with a good repeatability and has been used for identifying the chemical constitutes in the Pharmacopoeia of many countries [22–26]. The IR spectrum of a specific chemical compound is unique, since it has the unique functional groups and bonding arrangements [27,28]. For a complex system, each substance has its unique "fingerprint" of IR spectrum. With the development of spectroscopic techniques and modern chemometrics, the FT-IR has been applied in the complex systems such as the traditional Chinese medicine [29–31].

In this study, a novel method, which developed fingerprints by means of gas chromatography–mass spectrometry (GC–MS) and IR spectra, was researched and it was firstly used for quality control of *P. oleracea* L. At the same time, we investigated the application of a suite of chemometric methods such as the well-known similarity analysis of chromatographic patterns and hierarchical clustering analysis [32–34], to broaden and improve the characterization potential of the fingerprints from *P. oleracea* L.

2. Experimental

2.1. Instruments and apparatus

GC–MS analysis was performed on a Thermo-Finnigan Trace 2000/Polaris Q GC/MS^n instrument (Thermo Finnigan, USA) coupled with a DB-1 fused silica capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, USA). IR spectra were registered on Spectrum One NTS (Perkin-Elmer, USA) equipped with a DTGS detector. A versatile plant pulverizer (FW-100, Beijing Yonggunagming Medical Treatment Instrument Factory, Beijing, China) was used to make the plant materials into powder. A volatile oil distillatory device (Tianjing Tianbo Glass Instrument Co., Ltd., Tianjin, China) and an Auto Science AS-2060B Ultrasonic Cleaner (Tianjin Automatic Science Instrument Co., Ltd., Tianjin, China) were used for extraction. A rotary evaporator (RE52CS, Shanghai YaRong Biochemical Instrument Factory, Shanghai, China) was used to concentrate the sample solution.

2.2. Materials

Eleven raw herbs of *P. oleracea* L. from five provinces of China were investigated and collected, as shown in Table 1. These herbal samples were authenticated by Professor Zhongdi Xia (Xiangya School of Medicine, Changsha, China). All reagents and chemicals used in this work were of analytical grade. Triple-distilled water was used throughout the study.

2.3. Sample preparation

Fresh samples were cleaned with water and then crushed into powder by versatile plant pulverizer after being dried at 50 °C (pass through 80 mesh sieve). Powders of samples were kept in a drying oven to prevent deterioration.

2.4. Extraction procedures

Powders of the samples (30 g) were accurately weighed and placed in a sealed vessel by adding 150 mL water and standing for 30 min. Then the vessel was placed into the ultrasonic bath, followed by sonication for 1 h. After ultrasonic-assisted extraction, the vessel was connected to volatile oil distillatory device, and 1.5 mL of *n*-hexane was added in the measuring tube of extraction device, and then boiling for 5 h. The extract was dried by anhydrous sodium sulfate, and then was diluted to 2.0 mL by *n*-hexane.

2.5. GC–MS analysis

One microlitre of the sample was injected into GC–MS using split mode (50:1). The purge flow was 3.0 mL/min. The column temperature was programmed as follows: initial temperature at 40 °C for 2 min; ramp to 80 °C at 5 °C/min; ramp to 160 °C at 7 °C/min; ramp to 200 °C at 9 °C/min; ramp to 280 °C at 20 °C/min, hold for 20 min; the maximum temperature was 325 °C. The mass spectrometer was used in the following conditions: ion source temperature, 200 °C; scan range, from m/z 40–500. Peak area was chosen as the analytical signal for quantification purposes.

2.6. FT-IR analysis

Each sample of volatile oil extracted from *P. oleracea* L. was coated on the KBr tablet. After that, the tablet was put into the sample pool and the IR spectra of all samples at room temperature was recorded. IR spectra were recorded from the accumulation of 32 scans in 400–4000 cm^{-1} range with a resolution of 4 cm^{-1}.

2.7. Similarity evaluation

The fingerprints of TCMs can be used for quality control. But the valid fingerprint method has not been accepted in general quality standards of TCMs owing to the shortage of an analytical method of scientifically evaluating the complex chromatograms of TCMs. In order to resolve the problem, State Food and Drug Administration (SFDA) of China suggested that the most commonly used standard for evaluation of similarity of the fingerprint, the correlation coefficient, r_{cor}, and the congruence coefficient, r_{con}, were adopted to

assess the consistency, and they are formulated respectively as follows:

$$r_{cor} = \frac{\sum_{i=1}^{num}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{(\sum_{i=1}^{num}(x_i - \bar{x})^2)(\sum_{i=1}^{num}(y_i - \bar{y})^2)}}$$

$$r_{con} = \frac{\sum_{i=1}^{num} x_i y_i}{\sqrt{(\sum_{i=1}^{num}(x_i)^2)(\sum_{i=1}^{num}(y_i)^2)}}$$

where x_i and y_i are the ith elements in two different fingerprints, say x and y, respectively, and 'num' is the number of elements in the fingerprints. $\bar{x}$ and $\bar{y}$ are the mean values of the n elements in fingerprints x and y, respectively, that is

$$\bar{x} = \left(\sum_{i=1}^{num} \frac{x_i}{n}\right), \qquad \bar{y} = \left(\sum_{i=1}^{num} \frac{y_i}{n}\right)$$

The two indices r_{cor} and r_{con} will produce exactly the same result only when the fingerprints have been standardized. However, neither was shown to be always better than the other. The value r (r_{con} or r_{cor}) is in the range $0 < r \leq 1$. The larger the value of r is, the higher the quality of target samples is. It is considered as the best, the better or the worst drug with a corresponding correlation coefficient above 0.9, between 0.8 and 0.9, or below 0.8, respectively. When r equals 1, it is identical [35,36].

2.8. Hierarchical clustering analysis

Hierarchical clustering is a method of cluster analysis which seeks to build a hierarchy of clusters. Hierarchical clustering has the distinct advantage that any valid measure of distance can be used. Hierarchical clustering creates a hierarchy of clusters which may be represented in a tree structure called a dendrogram. The root of the tree consists of a single cluster containing all observations, and the leaves correspond to individual observations. Any valid metric may be used as a measure of similarity between pairs of observations. The choice of which clusters to merge or split is determined by a linkage criteria, which is a function of the pairwise distances between observations [37].

Given a set of n items to be clustered, and an $n \times n$ distance matrix, the basic process of hierarchical clustering is this: Start by assigning each item to its own cluster, so that if there are n items, there are n clusters, each containing just one item. Let the distances between the clusters equal the distances between the items they contain. Find the closest pair of clusters and merge them into a single cluster, so that now there is one less cluster. Compute distances between the new cluster and each of the old clusters. Repeat steps 2 and 3 until all items are clustered into a single cluster of size n [38].

3. Results and discussion

3.1. Selection of extraction method

Three different extraction methods were investigated, including Soxhlet extraction, steam distillation and volatile oil distillatory. The results showed that both steam distillation and volatile oil distillatory could obtain better fingerprint information of the components in the volatile oil of *P. oleracea* L. Compared with steam distillation, volatile oil distillatory extraction was simple and rapid, so the optimal extraction method of volatile oil from *P. oleracea* L. was determined (as the description in Section 2.4).

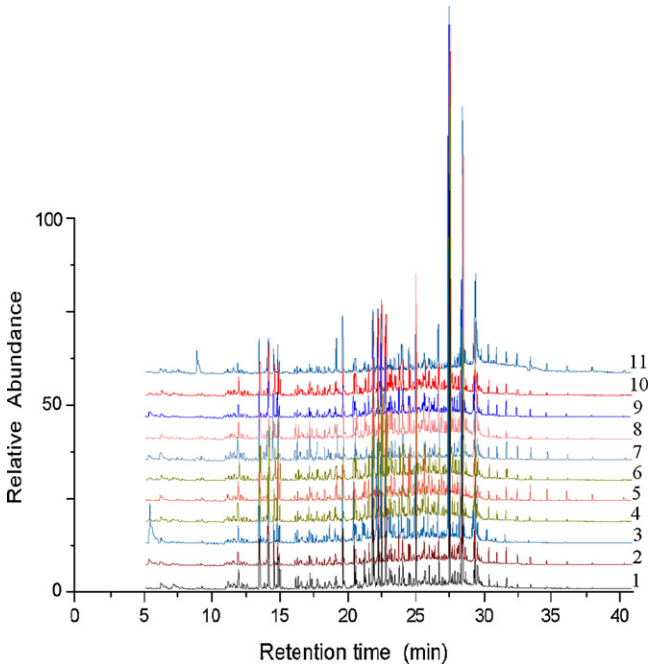

Fig. 1. The fingerprints of *P. oleracea* L. from different locations.

3.2. GC–MS fingerprint of P. oleracea L.

Volatile oils extracted from 11 batches of the samples were analyzed by GC–MS, respectively. About 50–60 components were found in each batch of sample and their total ion chromatograms were shown in Fig. 1. As shown from Fig. 1, we could find that there were a lot of peaks and their contents varied greatly in the profiles. Among these compounds, 38 components were identified compared with the standard mass spectra in National Institute of Standards and Technology (NIST) library. Peak area was chosen as the analytical signal for the relative amount. These identified compounds were listed in Table 2. From Table 2, we could find that there were higher content of *n*-hexadecanoic acid (Rt: 28.52), 9-Eicosyne (Rt: 27.53) and Cedrol (Rt: 25.07) in the volatile oil of *P. oleracea* L. according to the relative peak area. And some components such as palmitic acid (*n*-hexadecanoic acid) and myristic acid (Rt: 26.78) which were usually used as natural ingredients for skin care products and cosmetics were found.

3.3. Similarity analysis of GC–MS fingerprint of P. oleracea L.

As shown from Fig. 1, all the samples have high similarity in retention time while their peak abundances are different. The relationship within a set of chromatographic fingerprints could be currently analyzed through comparison in terms of similarity or dissimilarity of the objects with a certain reference. Correlation coefficient and congruence coefficient were used as similarity measure in our work to examine the similarity of the samples. The similarity analysis of each chromatogram to their common chromatogram was listed in Table 3. As shown from Table 3, the correlation coefficients and the congruence coefficients of the nine samples were more than 0.90 and the other two samples were more than 0.84, which indicated that their chromatographic fingerprints of volatile oil in *P. oleracea* L. samples from different regions were stable and generally consistent.

3.4. Standardization of GC–MS fingerprint of P. oleracea L.

The chromatogram which is able to demonstrate the chemical characteristics of *P. oleracea* L. was regarded as the standardized

Table 2
The main compounds identified in volatile oil extracted from *P. oleracea* L. and the identified components of the 24 common peaks.

Compound	The 24 common peaks	Rt (min)	*M*	Formula	Relative peak area (%)
1,3,5,7-Cyclooctatetraene	–	8.86	104	C_8H_8	0.37–1.36
2-Pentyl-furan	1	11.90	138	$C_9H_{14}O$	0.69–0.87
Benzeneacetaldehyde	2	13.47	120	C_8H_8O	1.18–2.75
cis-Linalool oxide	3	14.18	170	$C_{10}H_{18}O_2$	1.03–2.58
Cyclopropanemethanol	4	14.55	126	$C_8H_{14}O$	0.70–1.50
Santolina triene	5	14.84	136	$C_{10}H_{16}$	1.35–1.99
7-Methyl-3,4-octadiene	–	14.96	124	C_9H_{16}	0.97–1.27
2-Nonyn-1-ol	–	16.31	122	$C_9H_{16}O$	0.33–0.56
3-Methyl-6-(1-methylethylidene)-cyclohexene	6	17.22	136	$C_{10}H_{16}$	0.19–0.45
Myrcene	–	18.30	136	$C_{10}H_{16}$	0.68–0.89
Santolina triene	–	19.10	136	$C_{10}H_{16}$	0.91–1.21
Carvacrol	–	19.17	150	$C_{10}H_{14}O$	1.73–2.01
2-Methoxy-4-vinylphenol	7	19.70	150	$C_9H_{10}O_2$	1.34–2.92
Isoeugenol	8	20.47	164	$C_{10}H_{12}O_2$	0.36–1.38
2-(1,3-Butadienyl)-1,3,5-trimethyl-benzene	–	20.58	172	$C_{13}H_{16}$	0.93–1.43
(−)-Alloaromadendrene	9	21.84	204	$C_{15}H_{24}$	3.72–4.86
4,11,11-Trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-bicyclo[7.2.0]undec-4-ene	10	22.27	204	$C_{15}H_{24}$	0.64–2.82
Humulene	11	22.53	204	$C_{15}H_{24}$	0.26–2.38
(1,2,6,7)-Tricyclo[5.3.1.1(2,6)]dodecane-11,12-dione	12	22.85	174	$C_{12}H_{16}O_2$	1.37–3.06
Patchoulane	–	23.22	206	$C_{15}H_{26}$	0.56–0.63
Decahydro-1,6-bis(methylene)-4-(1-methylethyl)-naphthalene	13	23.51	204	$C_{15}H_{24}$	0.74–2.20
cis-Lanceol	14	24.63	202	$C_{15}H_{24}O$	0.44–1.45
Cedrol	15	25.07	222	$C_{15}H_{26}O$	0.54–3.47
1-Chloro-tetradecane chloride	–	26.02	232	$C_{14}H_{29}Cl$	0.55–1.70
2-Methyl-3,5-dodecadiene	–	26.27	180	$C_{13}H_{24}$	0.26–0.85
1,1,2-Trimethyl-cyclohexane	–	26.45	126	C_9H_{18}	0.23–0.43
Myristic acid	16	26.78	228	$C_{14}H_{28}O_2$	0.98–1.37
9-Eicosyne	17	27.54	278	$C_{20}H_{38}$	11.91–23.32
n-Hexadecanoic acid	18	28.53	256	$C_{16}H_{32}O_2$	8.63–11.56
Hexanoic acid, 9-decen-1-yl ester	–	29.42	254	$C_{16}H_{30}O_2$	0.96–2.32
1-Mono-linolenin	19	29.59	352	$C_{21}H_{36}O_4$	2.67–4.71
Cyclopropanebutanoic acid	–	29.71	374	$C_{25}H_{42}O_2$	1.17–1.71
Heptacosane	20	30.45	380	$C_{27}H_{56}$	0.22–1.06
Docosane	21	31.05	310	$C_{22}H_{46}$	0.06–1.13
Pentatriacontane	22	31.76	492	$C_{35}H_{72}$	0.18–1.27
Dotriacontane	23	32.58	450	$C_{32}H_{66}$	0.11–0.93
Nonacosane	24	36.25	408	$C_{29}H_{60}$	0.06–0.93
Tetratetracontane	–	38.10	618	$C_{44}H_{90}$	0.02–0.44

characteristic fingerprint of *P. oleracea* L. Peaks existing in all chromatograms of the samples were assigned as "common peaks". The 24 common peaks which were well isolated and identified could be found in all the 11 studied *P. oleracea* L. samples. The fingerprint of Sample No. 2 was used as the standardized fingerprint because the similarity value of Sample No. 2 was the highest, and the standardized fingerprint of *P. oleracea* L. marked with the 24 common peaks was shown in Fig. 2. The main common components of the 24 common peaks were identified and were listed in Table 2. In this case, the fingerprint pattern of the different samples was different. However, using the standardized fingerprint, it would be helpful to distinguish them by comparing some of the unique characteristic peaks.

Table 3
The similarity analysis of GC–MS fingerprint of *P. oleracea* L. from different habitats ($n = 5$).

	Correlation coefficient[a]	Congruence coefficient[a]
1	0.9056 ± 0.023	0.8974 ± 0.011
2	0.9733 ± 0.017	0.9603 ± 0.019
3	0.9564 ± 0.016	0.9511 ± 0.021
4	0.9569 ± 0.011	0.9421 ± 0.031
5	0.9132 ± 0.031	0.9082 ± 0.033
6	0.9237 ± 0.015	0.9189 ± 0.023
7	0.9233 ± 0.018	0.9177 ± 0.011
8	0.8567 ± 0.027	0.8401 ± 0.031
9	0.9152 ± 0.026	0.9013 ± 0.029
10	0.9186 ± 0.021	0.9097 ± 0.033
11	0.8758 ± 0.021	0.8702 ± 0.025

[a] The value is mean ± standard deviation.

3.5. Cluster analysis of GC–MS fingerprint of P. oleracea L.

Hierarchical cluster analysis based on peak's characteristics from the chromatographic fingerprints profiles of the tested 11

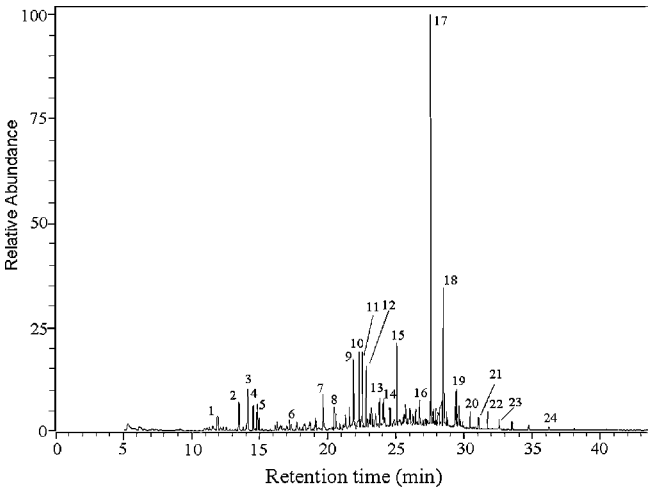

Fig. 2. Characteristic fingerprint of *P. oleracea* L. containing main common components.

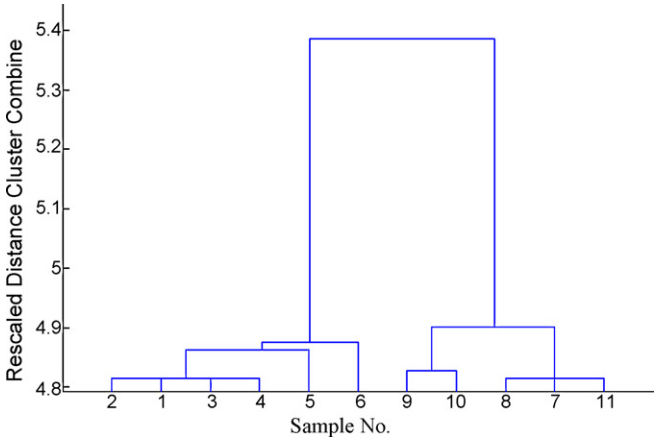

Fig. 3. Cluster dendrogram constructed from the GC–MS data for 11 samples.

samples and the nearest neighbor and cosine, which is a pattern similarity measure, were selected as measurement for hierarchical cluster analysis. The dendrogram was shown in Fig. 3. As shown from Fig. 3, samples are divided into two main clusters, and the samples from North China (1–6, Shandong, Shanxi, and Henan) are different from the samples from South China (7–11, Jiangxi and Hunan). Through hierarchical cluster analysis, the variations of different batches from different regions were obvious.

3.6. FT-IR fingerprints of P. oleracea L.

Volatile oils extracted from 11 batches of the sample were also analyzed by infrared spectrometer, and their FT-IR spectra were shown in Fig. 4. Volatile oil is a complex mixture system, and their IR spectra show a total overlap of each absorption spectrum of various components. As shown in Fig. 4, each band represented an overall overlap of some characteristic absorption peaks of functional groups in the sample. The IR characteristic fingerprint peaks of *P. oleracea* L. are mostly in the range of 1600–700 cm^{-1}, and the spectra of all the samples are rather similar. The 15 common peaks could be found in all the IR spectra of 11 studied *P. oleracea* L. samples, and the typical spectra of *P. oleracea* L. was shown in Fig. 5. It can be seen from Fig. 5, as a kind of macro-fingerprinting of natural product complex, several characters can be extracted, such as, the strongest peak at 3305 cm^{-1} belonged to the stretching vibration of O–H groups, the peaks at 2948 cm^{-1}, 2900 cm^{-1} and 2975 cm^{-1} assigned to the stretching vibration of $-CH_2-$ groups, the peaks at 1458 cm^{-1} and 1381 cm^{-1} assigned to C–H deformation vibration, the peaks at 880 cm^{-1} and 712 cm^{-1} assigned to C–H deformation vibration of aromatic substance, and the stronger peaks in the range of 1088–1049 cm^{-1} mainly attributed to the stretching vibration of C–O, which displayed the characteristic absorptions of alcohols, phenols and esters in the volatile oils.

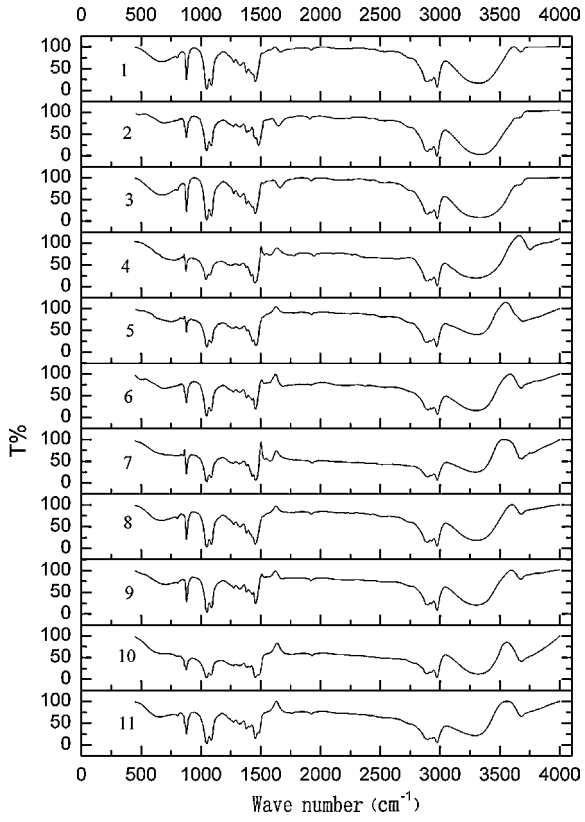

Fig. 4. IR spectra of volatile oils in *P. oleracea* L. from different regions.

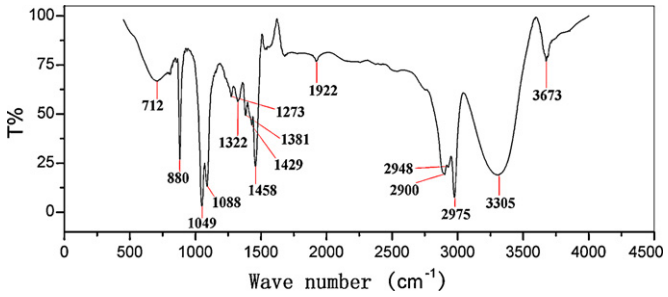

Fig. 5. The typical IR spectra of *P. oleracea* L.

3.7. Similarity analysis of FT-IR fingerprint of P. oleracea L.

In order to examine the similarity among the samples, the data of IR spectra, correlation coefficient and congruence coefficient were used as a similarity measure in our work. The similarity analysis of the IR spectra of 11 studied *P. oleracea* L. samples was listed in Table 4. As shown from Table 4, the correlation coefficients and the congruence coefficients of the 11 samples were more than 0.84, which indicated that their IR spectra of volatile oil in *P. oleracea* L. samples from different regions were stable and generally consistent.

3.8. Cluster analysis of FT-IR fingerprint of P. oleracea L.

The data of the IR spectra of the tested 11 samples and the nearest neighbor and cosine were selected as measurement for hierarchical cluster analysis. The dendrogram was shown in Fig. 6. As shown from Fig. 6, samples are divided into two main clus-

Table 4
The similarity analysis of IR spectra of *P. oleracea* L. from different habitats ($n = 5$).

Sample No.	Correlation coefficient[a]	Congruence coefficient[a]
1	0.9052 ± 0.031	0.9001 ± 0.031
2	0.9394 ± 0.023	0.9311 ± 0.014
3	0.9003 ± 0.027	0.8971 ± 0.027
4	0.9308 ± 0.029	0.9263 ± 0.016
5	0.9574 ± 0.023	0.9467 ± 0.017
6	0.8731 ± 0.018	0.8626 ± 0.033
7	0.8864 ± 0.021	0.8751 ± 0.018
8	0.9311 ± 0.027	0.9223 ± 0.022
9	0.9028 ± 0.033	0.8986 ± 0.031
10	0.8502 ± 0.027	0.8413 ± 0.032
11	0.8986 ± 0.031	0.8903 ± 0.028

[a] The value is mean ± standard deviation.

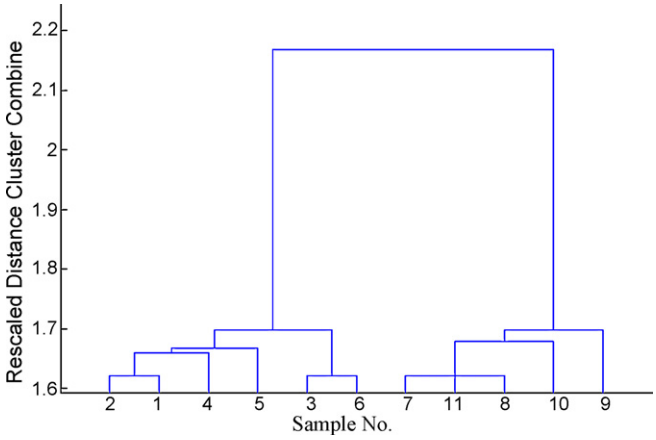

Fig. 6. Cluster dendrogram constructed from the IR spectra for 11 samples.

ters, and the same results were achieved as the hierarchical cluster analysis of GC–MS. The samples from North China (1–6, Shandong, Shanxi, and Henan) are different from the samples from South China (7–11, Jiangxi and Hunan).

3.9. The second derivative IR spectra of P. oleracea L.

The FT-IR spectra of 11 studied *P. oleracea* L. samples were rather similar, but they also had some small differences in shape or intensity. Generally, the second derivative spectrum can enhance the apparent resolution of IR spectrum. It can even separate the previously overlapped peaks. By using this technique, some overlapped absorption peaks can be resolved. Fig. 7 shows the second derivative spectra in the range from 1200 cm^{-1} to 780 cm^{-1} of *P. oleracea* L. samples, which enhance the apparent resolution and amplify tiny differences of IR spectrum. As shown from Fig. 7, Sample No. 3 and Sample No. 7 were from different clusters according to the hierarchical cluster analysis. Stark differences could be found between the second derivative spectra of the two samples in the range from 1200 cm^{-1} to 780 cm^{-1}, especially around the peak of 858 cm^{-1}, 886 cm^{-1}, 872 cm^{-1} and 889 cm^{-1}. Sample No. 3, which is from North China, has a strong absorption peak in 886 cm^{-1}, and other absorption peaks in 849 cm^{-1} and 889 cm^{-1}. However, Sample No. 7, which is from South China, has strong absorption peaks in 872 cm^{-1} and 858 cm^{-1}. According to these differences, it can be speculated that, the results of cluster analysis may be caused by different components in volatile oils of *P. oleracea* L. from different regions.

The differences between the second derivative spectra of samples from the same cluster were also shown in Fig. 7. As shown from Fig. 7, two samples are from the same cluster (North China), and the second derivative spectra of the two samples have differences (783–806 cm^{-1}) as well. However, there is no obvious difference of the peaks between the samples in the same cluster. In Fig. 7, two samples are both from another cluster (South China), and we can get the same result that there is no obvious difference of the peaks between the samples in the same cluster.

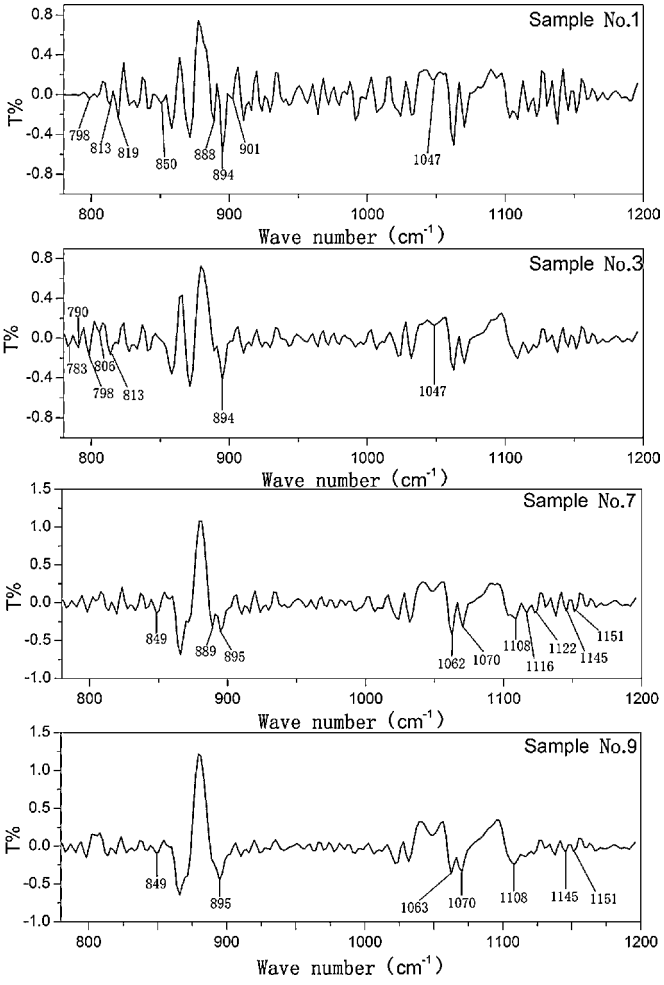

Fig. 7. Second derivative IR spectra of Sample No. 1, No. 3, No. 7 and No. 9.

4. Conclusions

In this study, GC–MS fingerprint analysis and FT-IR fingerprint analysis with chemometric methods were used for the purpose of species differentiation and the consistency check of *P. oleracea* L. collected from different sources. The results showed that both GC–MS and FT-IR fingerprint analytical method were practical and reliable for above purpose.

In the GC–MS fingerprint study, 24 characteristic peaks in the common pattern were identified to further characterize the chromatographic fingerprint and contribute to the quality control of *P. oleracea* L. The present GC–MS study in this paper also demonstrated that the GC–MS can be used to select control substances and to construct fingerprint of *P. oleracea* L.

In the FT-IR fingerprint study, 15 common peaks were found in all the FT-IR spectra of 11 studied *P. oleracea* L. samples and several characters were extracted to characterize the FT-IR fingerprint. In the second derivative IR spectra, the relative intensity of peak in the range from 1200 cm^{-1} to 780 cm^{-1} enhances gradually, and the main differences of the samples from different sources could be clearly found. The present FT-IR study in this paper demonstrated that the FT-IR spectra and the second derivative IR spectra can be used to identify and to construct the fingerprint of *P. oleracea* L.

As two kinds of advanced instrumental analysis methods, both chromatography and IR methods could be used for the quality control of TCM, and they both have their advantages. The information of the compounds and the information of the relative content of the compounds could be obtained through chromatography mass spectrometry analysis, and this chromatography method is considered as the most appropriate approach for QA because both qualitative and quantitative research could be investigated. The IR spectrum shows a total overlap of each absorption spectrum of all components, therefore, infrared spectrometry can provide semi-qualitative information only. However, the IR method is simple and manageable, and is more suitable for on-line analysis. Therefore, the IR method can be used as the important supplement for chromatographic fingerprint.

The resulting GC–MS and FT-IR fingerprints can be used for original identification and quality evaluation of related herbal medicines, and will become a key technique for Chinese herbal quality control and a powerful support for the progress of Chinese herbal medical prescription.

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