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Ecological Construction of Fingerprint of Radix Rehmannia by FT-IR

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Abstract

Objective: To set up the fingerprint of Huaqui Radix Rehmannia by FT-IR in terms of evolutionary ecology. **Methods:** FT-IR combined with comparative analysis software was used to analyze the 18 Radix Rehmannia samples of different variety, producing area and processing method**. Results:** (1) The fingerprint of Huaiqu Radix Rehmannia by FT-IR was established, and 13common characteristic peaks were obtained, laying the foundation for the identification of Radix Rehmanniain genuine producing environment. (2) The ecological similarity of Radix Rehmanniain Gansu and Sichuan had significant difference from that in Huaiqu, Indicating that the quality of Radix Rehmanni in Gansu and Sichuan was inferior to that in Huaiqu. (3) The cluster ecological analysis showed that the Radix Rehmannia in Huaiqu clustered, indicating that producing area can distinguish the similarity of Radix Rehmannia; No.3 (produced in Gansu), No.4and No.13, all of which adopted steam drying, clustered, indicating that processing method had influence on the quality of Radix Rehmannia. The Radix Rehmannia of same variety in Huaiqu clustered, but Jinjiu and Huaifeng also clustered, indicating that variety had certain influence on the quality of Radix Rehmannia. **Conclusion:** The constructed fingerprint of Huaiqu Radix Rehmannia by FT-IR had good stability and reproducibility in terms of evolutionary ecology, which was suitable for the quality control of Radix Rehmannia, thus providing theoretical and technical support for further rational development and utilization of Radix Rehmannia.

Keywords: evolutionary ecology, Radix Rehmannia, FT-IR, fingerprint

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INTRODUCTION

Evolutionary ecology merges the two scientific studies of evolution and ecology and focuses on the physical and genetic changes that occurred among organisms and how such modifications were affected by ecological factors. Basically, it also considers the effect of forces like competition, predation, parasitism, and mutualism in the evolution of individual species, in a population, or in the entire community.

Radix Rehmannia (di huang; the fresh or dry root of *Rehmanniaglutinosa* Libosch) (family Scrophulariaceae), has been widely used in traditional Chinese medicines (TCM), and earliest recorded in the Sheng Nong's Herbal Classic as being classified as top grade (Wen et al. 2002). As a traditional genuine medicinal material, it is mainly produced in Henan, Hebei, Shandong, and Shanxi, among which, Henan boasts of large output and good quality. The Radix Rehmannia produced largely in the ancient "Huaiqing Prefecture" (now Wenxian County, Wuzhi County, and Boai County in Henan province) is often referred to as "Rehmannia Glutinosa", one of the four major Huai Medicines of genuine medicinal materials (Jiang 2013, Li *et al.* 2012).

Fourier Transform Infrared Spectroscopy (FT-IR), a modern analytical technique, with advantages of rapid operation, simplicity and high sensitivity, has been widely used in various fields of pharmaceutical research (Li *et al*. 2006, Tian *et al*. 1995, Xu *et al*. 2009, Zhao *et al*. 2009). FT-IR has been used in a lot of behavioral ecological researches for the identification and quality control of traditional Chinese medicine, which not only has the advantages of nm characterization of overall

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sample characteristics and macro fingerprinting that are superior to traditional method, but also has the advantages of non-damage to sample and quickness (Chen *et al*. 2009, Jiang *et al*. 2010, Yang *et al*. 2009), thus, the fingerprint has become one of the important means of quality evaluation and quality control of traditional Chinese medicine. This ecological study initially established the analytical method of the fingerprint of Huaiqu Radix Rehmannia by FT-IR, and compared the fingerprint of Radix Rehmannia of different producing area by FT-IR, providing a scientific basis for the quality evaluation and quality control of Huaiqu Radix Rehmannia.

MATERIALS AND METHODS

Instruments and Test Samples

FT-IR/Rama Coupled Instrument (produced by American Nicolet company), HY-12 Infrared Tablet Press Machine (Tianjin Optical Instrument Factory), DHY-300 Ultra-Micro Pulverizer (Beijing Donghuayuan Medical Equipment Co., Ltd.); ZRD-A5110 Electric Constant Temperature Drying Oven (Shanghai Zhicheng Analytical Instrument Manufacturing Co., Ltd.); FA2204BElectronic Analytical Balance (Shanghai Jinghai Instrument Co., Ltd.).

A total of 18 Radix Rehmannia samples of different variety collected from different regions such as Huaiqu of Henan province, Shanxi, Sichuan and Gansu processed by different methods were identified as *Rehmanniaglutinosa* Libosch by associate professor Li Faqi at Henan Normal University. The test Radix Rehmannia samples were obtained by using the drying crushing method, and then sieved using a 80-mesh sieve; potassium bromide (purchased from Tianjin Botian Shengda Technology Development Co., Ltd.). Details were as shown in **Table 2**.

Experimental Method

Determination of index components: Determination of index components and content of ethanolie extractive of Huaiqu Radix Rehmannia samples referred to the Chinese Pharmacopoeia 2015 edition.

Sample preparation : Before sampling, the Radix Rehmannia samples were placed at 60℃ and dried for 30min. 3 mg of Radix Rehmannia samples and 200 mg of potassium bromide powder were placed in a mortar, and ground for 4-5 min under an infrared light, then, appropriate amount of comminuted powders were smoothly paved on an infrared compression mold, which was moved to a tablet press machine for vacuum pumping and pressurizing (20 MPa) for 2 min, and then the infrared compression mold was removed, and the Radix Rehmannia samples were viewed against a light, among which, the samples of homogeneity and semi-transparency were preferred. The sample holder was placed in a sample chamber of infrared spectrophotometer, and scanned in the 4,000～400cm^{-1} wavelength range.

Precision experiment: The same Radix Rehmannia sample was taken, and scanned and determined 5 times continuously, and the correlation coefficient between infrared spectrograms was 1. 0000, 0.9998, 0.9999, 0. 9999 and 0.9999, respectively, and the RSD of the wave number of common peak of the sample was 0.007%.

Stability experiment: The same Radix Rehmannia sample was taken and put in a dryer for preservation, and determined once every 1 h for 3 times, and the infrared spectrograms obtained were basically the same, and the correlation coefficient between infrared spectrograms was 0.9999, 1.0000, 0.9998, 0.9998 and 0.9999, respectively, and the RSD of the wave number of common peak of the sample was 0.008%.

Reproducibility experiment: The same Radix Rehmannia sample was weighed and went through tablet pressing 3 times, the infrared spectra obtained were basically the same, and the infrared spectrograms obtained were also basically the same, and the correlation coefficient between infrared spectrograms was 0.9999, 1.0000, 0.9996, 0.9998 and 0.9998, respectively, and the RSD of the wave number of common peak of the sample was 0.015%. The results showed good reproducibility.

Data processing: OMNIC 6.2 software was used to analyze the infrared spectra, and softwares such as Origin, Excel, and SPSS were used to conduct statistics, similarity evaluation and cluster analysis of the infrared spectra data of different Radix Rehmannia samples.

RESULTS

Construction of Fingerprint of Huaiqu Radix Rehmannia by FT-IR

According to 2.2 Sample Preparation, the test Radix Rehmannia samples went through tablet pressing respectively, and were detected according to the selected test conditions, and determined for the infrared spectrogram, as shown in **Fig. 1**. By determination, both the index components and content of ethanolie

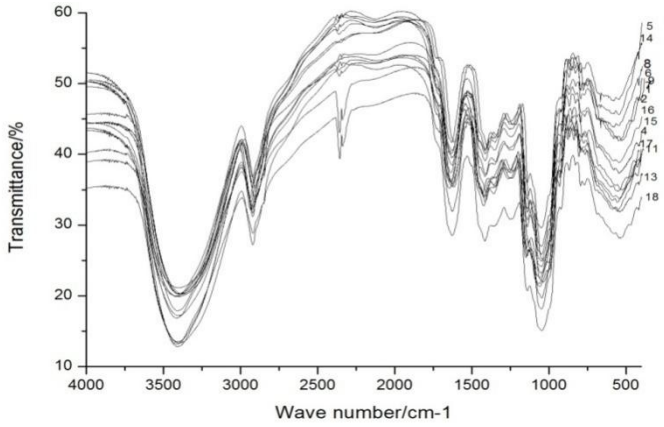

Fig. 1. Infrared superposition spectrum of Huaiqu Radix Rehmannia

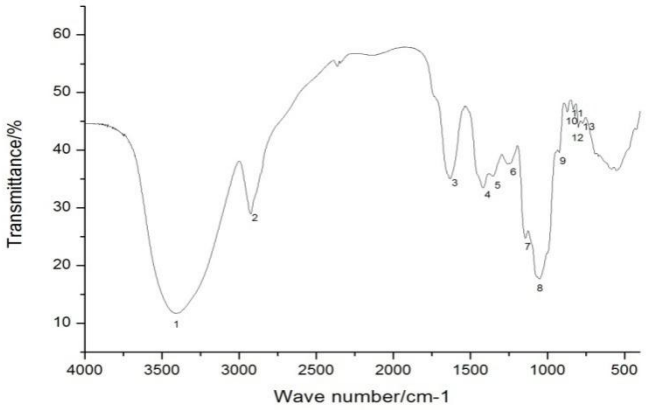

Fig. 2. Infrared fingerprint of Huaiqu Radix Rehmannia

extractive of Huaiqu Radix Rehmannia samples met the Chinese Pharmacopoeia 2015 edition, with the average of its absorption intensity as the absorption intensity of control fingerprint, finally the infrared fingerprint of Huaiqu Radix Rehmannia was obtained, as shown in **Fig. 2**.

By comparison and analysis of the infrared spectrograms of all Huaiqu Radix Rehmannia, 13 peaks were determined as the common characteristic peaks of the infrared fingerprint of Huaiqu Radix Rehmannia. From **Figs. 1** and **2**, it can be seen that the wave number of 1~13 peaks was: 3,396 cm-1, 2,923cm-1, 1,630 cm-1, 1,418cm-1, 1,347 cm-1, 1,248 cm-1, 1,140 cm-1, 1,051 cm-1, 927 cm-1, 869 cm-1, 825 cm-1, 797 cm-1 and 770 cm-1, respectively.

Attribution of Common Peaks

Identification and attribution of main absorption peaks of the infrared spectrogram of Huaiqu Radix Rehmannia were carried out according to the literature (Liu *et al.* 2010, Yao *et al.* 2010), as shown in **Table 1**.

From **Table 1**, it can be seen that the absorption peaks of 3,396cm-1 were the characteristic absorption peaks of carbohydrates and amino acids, the absorption peaks of 1,630 cm-1 were the characteristic absorption peaks of Dihuangcerebrosides andiridoids, and the absorption peaks of 1,248 cm-1 and 1,140cm-1 were the characteristic absorption peaks of iridoid glycosides, and the absorption peaks of 1,051 cm-1 were the characteristic absorption peaks of flavonoids, sugar and Dihuancerebrosides.

Analysis of Similarity of Infrared Fingerprint of Radix Rehmannia Samples

Correlation Coefficient Method and Vector Included Angle Cosine Method were commonly used as the calculation methods for the similarity of the fingerprint of traditional Chinese medicine (Nie *et al.* 2005, 2008, Zhan *et al.* 2010). Analysis of similarity of the infrared spectrum and infrared fingerprint of Huaiqu Radix Rehmannia samples were conducted, and calculation was conducted using the Correlation

Table 1. Attribution of main absorption peaks of the infrared spectrogram of Huaiqu Radix Rehmannia

No.	Wave/cm^{-1}	Attribution	
1	3396	ν(O-H),ν(N-H)	Saccharides,amino acids
2	2923	ν(CH_2)	
3	1630	δ(C=C) ,νrf (ar)	phenenethyalcoholglycosides,Iridoid glycosides
4,5	1418,1347	δ (C-H)	
6,7	1248,1140	ν (C-O-C)	Iridoid glycosides
8	1051	ν(C=O), ν (C-OH)	Flavonoids,saccharides,phenenethyalcohol glycosides
9,10,11,12,13	927,869,825,797,770	ν_f(C-C)	

Table 2. Correlation coefficient and vector included angle cosine values of the infrared spectrum of Huaiqu Radix Rehmannia samples

No.	Sample	Producing area	Processing method		Correlation coefficient	Similarity
1	Jinjiu	Wenxian	Coal drying		0.9534	0.9792
2	Huaifeng	Wenxian	Electric drying		0.9886	0.9989
3	---	Gansu	Steam drying		0.9009	0.9899
4	A	WuzhiBailiao	Steam drying		0.9936	0.9984
5	Huaifeng	WuzhiBailiao	Steam drying		0.9892	0.9941
6	85-5	Wenxian	Electric drying		0.9745	0.9774
7	Beijing No.3	Wenxian	Coal drying		0.9824	0.9953
8	Beijing No.3	Wuzhi	Coal drying		0.9581	0.9855
9	Huaidi 81	Wuzhibailiao	Steam drying		0.7390	0.9784
10	---	Shanxi	Coal drying		0.9727	0.9951
11	Huaidi 81	Wuzhi	Electric drying		0.8854	0.9881
12	---	Sichuan	Coal drying		0.8545	0.9873
13	B	WuzhiBailiao	Steam drying		0.9673	0.9940
14	Tuodu85-5	Wenxian	Electric drying		0.9643	0.9825
15	Huaidi Jin8	Wenxian	Electric drying		0.9534	0.9792
16	Beijing No.3	Wenxian	Coal drying		0.9900	0.9990
17	Jinjiu	Wenxian	Coal drying		0.9938	0.9982
18	Beijing No.3	Mengzhou	Coal drying		0.9896	0.9978

Coefficient Method and Vector Included Angle Cosine Method respectively, and the results were as shown in **Table 2**.

As shown in **Table 2**, except for Huaidi 81, the correlation coefficients of other Huaidi samples were higher than 0.95, and the correlation coefficient of Huaid 81 was lower than 0.9, which may be due to the influence by the space mutation. The correlation coefficients of Huaiqu Radix Rehmannia samples in Gansu were 0.9009, and 0.8545 in Sichuan, displaying significant difference from that of Huaiqu Radix Rehmannia samples, indicating that the quality of Radix Rehmannia in Gansu and Sichuan was inferior to that of Huaiqu Radix Rehmanni. The correlation coefficients of Radix Rehmannia samples in Shanxi were 0.9727, which may be due to the fact that the producing area of such samples was near from Huaiqu or they were of the same provenance. The calculation results by using the Vector Included Angle Cosine Method showed that the included angle cosine values of all Radix Rehmannia samples were greater than 0.97.

When the similarity of the infrared fingerprint of Radix Rehmannia samples was calculated by using the Vector Included Angle Cosine Method, the calculation results of different Radix Rehmannia samples were similar, which however displayed significant difference when calculated by using the Correlation Coefficient Method, which was consistent with the results of study by Wang et al. (2011).

Cluster Analysis of FT-IR Data of Radix Rehmannia Samples of Different Producing Area

Comparison was conducted for the infrared spectrum of Radix Rehmannia samples of different producing area using the SPSS software, with the absorbance value at 4,000～400cm-1 being selected as the absorbance matrix, cluster analysis was conducted for the 18 Radix Rehmannia samples by using the Interblock Coupling Clustering Method, as shown in **Fig. 3**.

By analysis from **Fig. 3**, it can be seen that No. 1 Jinjiu, No. 17 Jinjiu, No. 7 Beijing No. 3, No. 16 Beijing No. 3, No. 18 Beijing No. 3 and No. 8 Beijing No. 3 clustered; No. 6.85-5, No. 14 Tuodu85-5, No. 15Huaidi and Jin 8 clustered; No. 12 (produced in Sichuan, coal drying) clustered separately; No. 4A, No. 13B and No. 3 (produced in Gansu, steam drying) clustered; No. 9 Huaidi81, No. 11 Huaidi81, No. 2 Huaifeng, No. 5 Huaifeng and No. 10 (produced in Shanxi, coal drying) clustered. The above indicated that Jinjiu and Huaifeng had the similar provenance, and A,

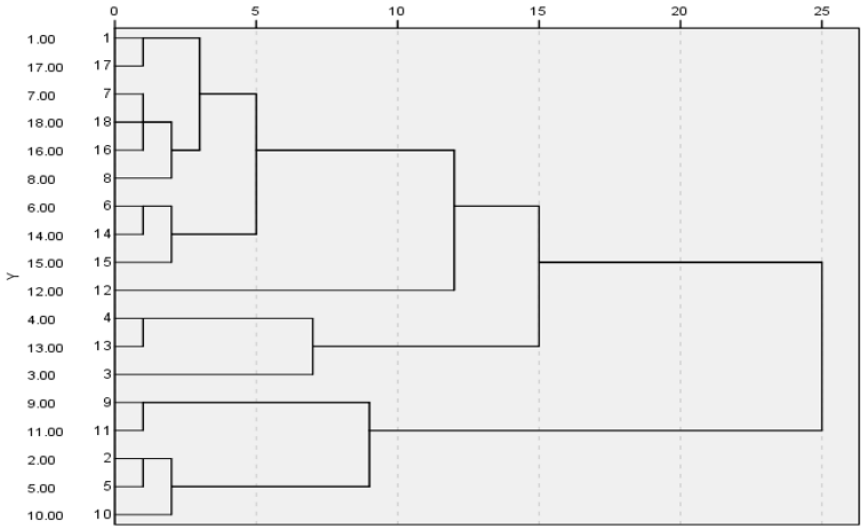

Fig. 3. Diagram of results of cluster analysis of Radix Rehmannia samples

B and Radix Rehmannia in Gansu had the similar provenance, and Huaifeng and Huaidi 81 had the similar provenance.

DISCUSSION

The results of cluster analysis showed that all the Huaiqu Radix Rehmannia samples can cluster, indicating that producing area can distinguish the similarity of Radix Rehmannia; No. 10 (produced in Shanxi)was classified wrongly, which may be due to the fact that Shanxi was near from Henan or they were of the same provenance; No. 12 (produced in Sichuan)clustered separately, No.3 (produced in Gansu), No.4 and No.13, all of which adopted steam drying, clustered, indicating that processing method had influence on the quality of Radix Rehmannia. The Huaiqu Radix Rehmannia samples of same variety clustered, but Jinjiu and Huaifeng also clustered, indicating that variety had certain influence on the quality of Radix Rehmannia.

The results of fingerprint analysis showed that the Huaiqu Radix Rehmannia of different producing area, different variety and different processing method had both correlation and difference. The corresponding common peaks were reflected in the infrared spectrogram, displaying good similarity of fingerprint. In this experiment, the infrared fingerprint of Huaiqu Radix Rehmannia by FT-IR was established, which can distinguish the Radix Rehmannia variety in genuine producing area quickly and effectively. The similarity analysis can identify the quality of Radix Rehmanniae, which was suitable for the quality control of Radix Rehmannia. The cluster analysis showed that the Radix Rehmannia of different producing area, different variety and different processing method had influence on the quality of Radix Rehmannia, thus providing theoretical and technical support for further rational development and utilization of Radix Rehmannia.

CONCLUSION

In this ecological study, the fingerprint of Huaiqu Radix Rehmannia by FT-IR was established, and 13 common characteristic peaks were obtained, laying the foundation for the identification of Radix Rehmannia in genuine producing area; the results of analysis of the full-spectrum similarity of the infrared fingerprint of Radix Rehmannia samples conducted using the Correlation Coefficient Method can accurately reflect the actual situation of the quality of Radix Rehmannia samples, so it can be used for the evaluation of the quality of Radix Rehmannia (Wang *et al.* 2011). By analysis of the similarity of the infrared spectrum and infrared fingerprint of Huaiqu Radix Rehmannia samples, except for Huaidi 81, the correlation coefficients of other Huaidi samples were higher than 0.95, and the correlation coefficient of Huaidi 81 was lower than 0.9, which may be due to the influence by the space mutation. The correlation coefficients of Huaiqu Radix Rehmannia samples in Gansu were 0.9009, and 0.8545 in Sichuan, displaying significant difference from that of Huaiqu Radix Rehmannia samples, indicating that the quality of Radix Rehmannia in Gansu and Sichuan was inferior to that of Huaiqu Radix Rehmannia. The correlation coefficients of Radix Rehmannia samples in Shanxi were 0.9727, which may be due to the fact that the producing area of such samples was near from Huai Zone or they were of the same provenance.

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