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RAPID AND EFFICIENT QUALITY CONTROL OF RADIX: METHOD OF ^{1}H NMR AND PCA

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Abstract

Radix Rheum is one of the most important herbal; and a serious problem of the adulteration or falsification has been discovered in the commercial market. In this study, 42 batches samples collected from different places were analyzed by ^{1}H NMR metabolite profiles together with principal component analysis (PCA). Results showed that the method could distinguish Radix Rheum samples from adulterants, as well as the different species identification. ^{1}H NMR-PCA was used in identification of Radix Rheum and its adulterants for the first time. The developed rapid and efficient method in this study can be used in quality control of Radix Rheum, and as a standard protocol for identification.

Key words: Radix Rheum, adulterants, ^{1}H NMR, PCA, Identification

Introduction

Radix Rheum is the dried root and rhizome of *Rheum tanguticum* Maxim. ex Balf., *Rheum palmatum* L. and *Rheum officinale* Baill. from the family *Polygonaceae* (The state pharmacopoeia commission of people's republic of China, 2015). Which was first recorded in *Shennong's Herbal*, and traditionally used as a folk medicine. In 17th and 18th centuries, several species were brought to Europe from Asia and used as food, named *R. rhaponticum* (Cullen & Alwxander, 1984; Clifford & Dale, 1991). Radix Rheum is widely distributed in Sichuan, Qinghai, Gansu and Xizang provinces of China. The constituents of Radix Rheum roots includes anthraquinone, anthrone, stilbene, polysaccharide, tannin and other compound (Matsuda *et al*., 2001; Babu *et al*., 2003; Komatsu *et al*., 2006; Lin *et al*., 2006; Nan *et al*., 2009). Radix Rheum exhibit a wide variety of pharmacological effects, such as purgative activity, cholagogic, liver-protective, hemostasis, detoxification, anti-microbial, anti-inflammatory, anti-hypertension, kidney improved function etc.(Yu *et al*., 2005; Agarwal *et al*., 2000; Chen *et al*.,2010; Her *et al*., 2010; Xiong *et al*., 2011; Mishra *et al*., 2014).

However, it is very difficult to use traditional organoleptic methods or base on several chemical markers to distinguish different species, due to complicated constituents. Previous researches explored the possibility of HPLC, UPLC, DNA-barcode and PCA on distinguishing different species (Jin *et al*, 2006; Chen *et al*, 2010; Li, H.*et al*, 2012; Li, M. *et al*., 2012; Wei *et al*., 2013; Lee *et al*., 2017; Liu *et al*., 2017) of smaller sample size. ^{1}H NMR fingerprint is an important method of identification and quality control for traditional Chinese medicine owing to its obvious characteristic and good reproducibility (Xie *at al*., 2006). Moreover, it has been reported that the combination of ^{1}H NMR fingerprint and PCA have been widely used in the analysis of biological metabolomics and food quality and safety control (Chen *et al*., 2006).

The plants' chemical constituents and qualities are influence by different cultivation areas and climatic conditions (Zhang *et al*., 2010; Zhang *et al*., 2011). Even if the plant morphology of the *Rumex* L. and *Rheum* L. are similar, the plant from *Rumex* L. is often used as medical genuine Rhei Radix et Rhizoma (Zhu *et al*., 2008). However, there is great difference in chemical constituents and clinical efficacy between adulterants and genuine Radix Rheum. Our previous work showed that the content of major active compounds were various in different species of Radix Rheum (Ren *et al*., 2014). Meanwhile, the adulteration or falsification of different species has been the serious problem in the commercial market recently. Therefore, application of Radix Rheum is growing steadily, development of a rapid, accurate quality control method is very important to detect and prevent adulteration or falsification.

In this study, we collected a large sample (>40) from 7 provinces around China (Fig. 1), which analyzed by ^{1}H NMR-PCA for the first time and allowed a thorough comparison of Radix Rheum in different species. The result is representative and potentially to guide authorities to regulate Radix Rheum markets.

Materials and Methods

Instruments and Reagents: Bruker Avance 600 nuclear magnetic resonance (NMR) (Bruker, from Germany), AB135-S electronic analytical balance (Mettler, from Switzerland), KQ-400KDE ultrasonic cleaner (Kunshan ultrasonic instruments Co., Ltd., from China), SIGMA1-14 centrifuge (Shanghai touching technology Co., Ltd., from China), Deuterated methanol (Norell, from American), WG-500 NMR tube (Wilmad, from American). We processed the data using the soft of SPSS 19.0.

Plant Materials: 42 samples from 7 provinces of Radix Rheum were collected, all of them were identified by Professor Linfang Huang (Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China). These samples include 9 batches *R. palmatum* L. (RPL), 15 batches *R. offcihale* Baill. (ROB), 9 batches *R. tanguticum* Maxim. ex Balf. (RTM) and 9 batches adulterants (ADU) (Fig. 2). A detailed sample list is specified in Table 1.

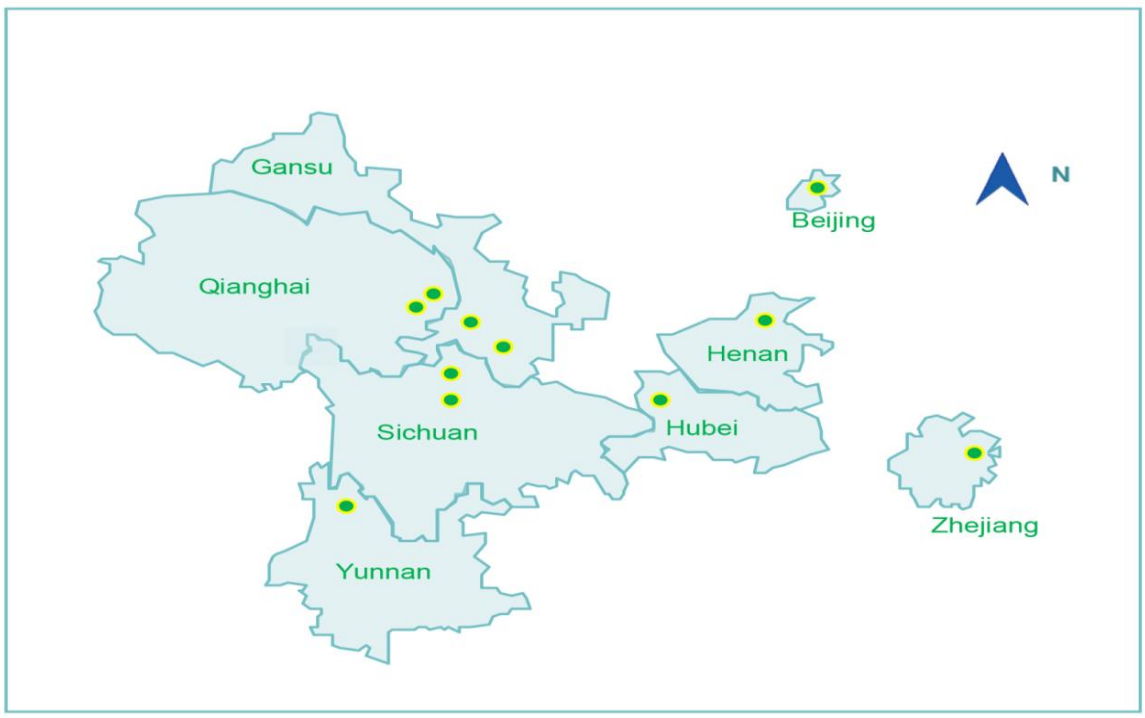

Fig. 1. The sampling points of Radix Rheum and its adulterants.

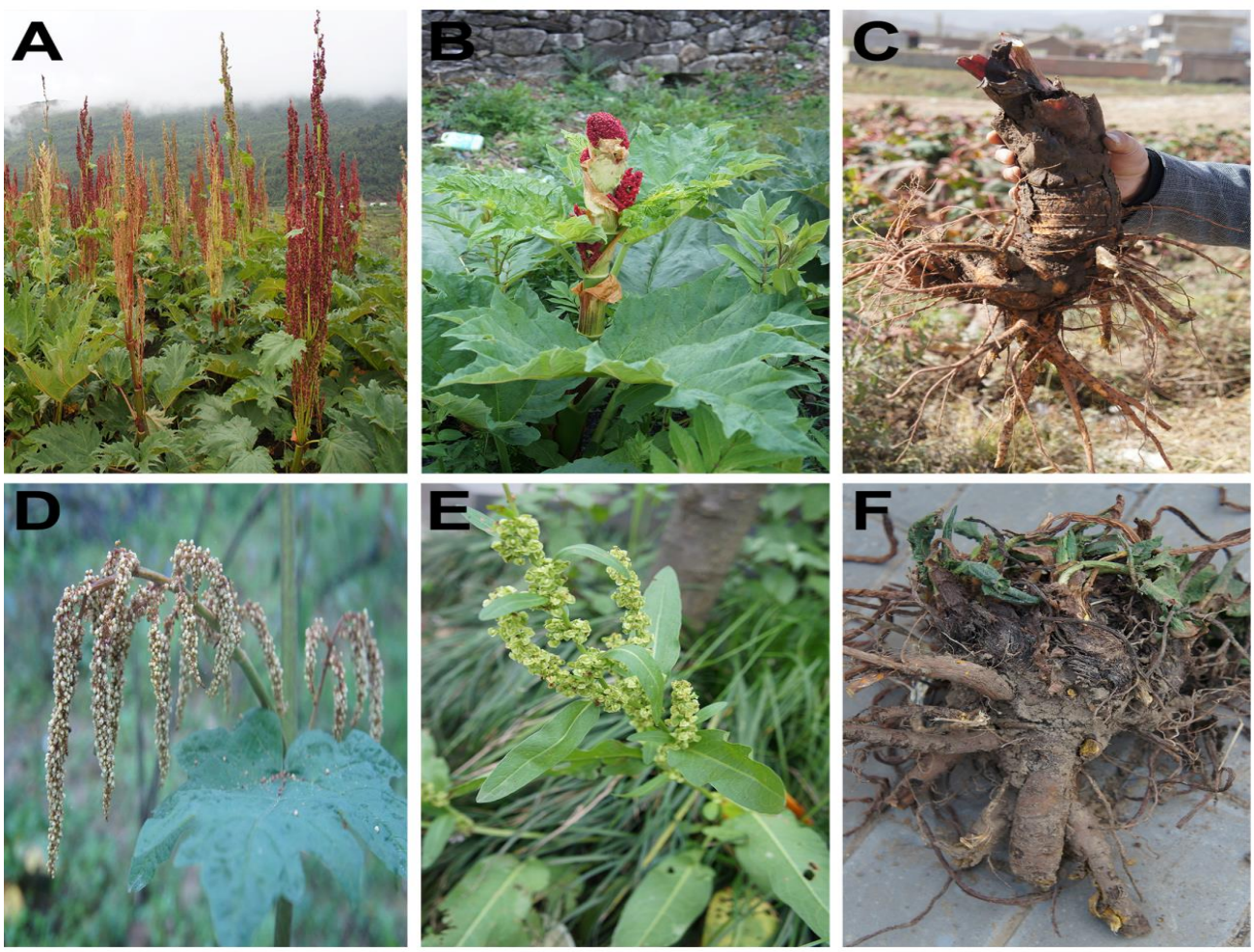

Fig. 2. The Radix Rheum of different species and its adulterants. A: The plant of *R. tanguticum* Maxim. ex Balf. B: The plant of *R. palmatum* L. C: The root of *R. palmatum* L. D: The plant of *R. offcihale* Baill. E: The plant of *R. japonicus* Houtt. F: The root of *R. japonicus* Houtt.

Table 1. The sample list of Radix Rheum and its adulterants

No.	Botanical origin	Source	Collection time	Remark
1	*R. palmatum* L.	Xianglingfeng Village, Min County, Gansu Province	2012.10.22	Cultivate, 3 years
2		Gelong Village, Dangchang County, Gansu Province	2012.10.22	Cultivate, 3 years
3		Yugang Village, Hadapu Town, Dangchang County, Gansu Province	2012.10.22	Cultivate, 3 years
7		Ledu County, Qinghai Province	2013.10.12	Cultivate, 3 years
8		Ledu County, Qinghai Province	2013.10.12	Cultivate, 3 years
9		Ledu County, Qinghai Province	2013.10.12	Cultivate, 3 years
19		Heishui County, Sichuan Province	2013.10.20	Cultivate, 3 years
20		Heishui County, Sichuan Province	2013.10.20	Cultivate, 3 years
21		Kangding County, Sichuan Province	2013.10.20	Cultivate, 3 years
16	*R. Offcihale* Baill.	Haba Village, Xianggelila County, Yunnan Province	2013.10.2	Cultivate, 3 years
17		Haba Village, Xianggelila County, Yunnan Province	2013.10.2	Cultivate, 3 years
18		Haba Village, Xianggelila County, Yunnan Province	2013.10.2	Cultivate, 3 years
25		Baoxing County, Sichuan Province	2011.7.23	Wild, 3 years
26		Baoxing County, Sichuan Province	2011.7.23	Wild, 3 years
27		Baoxing County, Sichuan Province	2011.7.23	Wild, 3 years
13		Shennongjia scenic area, Hubei Province	2013.10.4	Wild, 3 years
14		Shennongjia scenic area, Hubei Province	2013.10.4	Wild, 3 years
15		Shennongjia scenic area, Hubei Province	2013.10.4	Wild, 3 years
4		Tai-hang Mountains, Hui County, Henan Province	2013.10.10	Wild, 5 years
5		Tai-hang Mountains, Hui County, Henan Province	2013.10.10	Wild, 5 years
6		Tai-hang Mountains, Hui County, Henan Province	2013.10.10	Wild, 5 years
31		Medicinal botanical garden, Institute of Medicinal Plant Development, Beijing	2013.11.2	Cultivate, 3 years
32		Medicinal botanical garden, Institute of Medicinal Plant Development, Beijing	2013.11.2	Cultivate, 3 years
33		Medicinal botanical garden, Institute of Medicinal Plant Development, Beijing	2013.11.2	Cultivate, 3 years
10	*R. tanguticum* Maxim. ex Balf.	Tongren County, Huangnan Tibetan Autonomous Region, Qinghai Province	2013.10.8	Wild, 3 years
11		Tongren County, Huangnan Tibetan Autonomous Region, Qinghai Province	2013.10.8	Wild, 3 years
12		Tongren County, Huangnan Tibetan Autonomous Region, Qinghai Province	2013.10.8	Wild, 3 years
22		Heishui, Sichuan Province	2013.10.20	Cultivate, 3 years
23		Heishui, Sichuan Province	2013.10.20	Cultivate, 3 years
24		Heishui, Sichuan Province	2013.10.20	Cultivate, 3 years
28		Medicinal botanical garden, Institute of Medicinal Plant Development, Beijing	2013.11.2	Cultivate, 3 years
29		Medicinal botanical garden, Institute of Medicinal Plant Development, Beijing	2013.11.2	Cultivate, 3 years
30		Medicinal botanical garden, Institute of Medicinal Plant Development, Beijing	2013.11.2	Cultivate, 3 years
34	*R. japonicus*Houtt.	Xianju County, Zhejiang Province	2013.11.2	Wild, 3 years
35		Xianju County, Zhejiang Province	2013.11.2	Wild, 3 years
36		Xianju County, Zhejiang Province	2013.11.2	Wild, 3 years
37	*R. patien-tis* L.	Medicinal botanical garden, Institute of Medicinal Plant Development, Beijing	2013.10.30	Cultivate, 3 years
38		Medicinal botanical garden, Institute of Medicinal Plant Development, Beijing	2013.10.30	Cultivate, 3 years
39		Medicinal botanical garden, Institute of Medicinal Plant Development, Beijing	2013.10.30	Cultivate, 3 years
40		Medicinal botanical garden, Institute of Medicinal Plant Development, Beijing	2013.4.16	Cultivate, 3 years
41		Medicinal botanical garden, Institute of Medicinal Plant Development, Beijing	2013.4.16	Cultivate, 3 years
42	*R. acetosa* L.	Medicinal botanical garden, Institute of Medicinal Plant Development, Beijing	2013.4.16	Cultivate, 3 years

Samples extraction method: For each batch of samples, several roots were sieved through a grinder, and filtered through a NO. 4 sieve. 50mg of the powder was extracted with 0.4mL deuterated methanol in 2 mL EP tube, sonicated for 30 min. After the materials were centrifuged, the supernatants were transferred separately into NMR tubes for NMR measurements.

NMR measurement: One dimensional ^{1}H NMR spectra were measured at a temperature of 298K on a Bruker Av 600 Spectrometer. 128 scans were kept a record of 301.9K data points over a spectral width of 6009.6 Hz using the zgcppr pulse length of the 30°. One — dimensional ^{1}H NMR spectra were recorded after the free induction decays going through fourier transformation. The chemical shifts for the samples were referenced to TMS (Tetramethylsilane) at 0.00 ppm.

Data analysis: The spectral ^{1}H NMR region from δ=0.2 to δ=10.0 was segmented into regions with widths of 0.04 ppm. Bucketing of spectra was performed by MestReNova software version 6.1.0. The regions were normalized to the whole spectrum for PCA and partial least squares discriminant analysis (PLS-DA). PCA, PLS-DA were performed with SPSS 19.0 software.

PCA is a multivariate data analysis method to summarize a lot of variables in a dataset into a few correlated variables (Dien, 2102; Linting & Kooij.2012). Intensities of selected chemical shifts were plotted based on the normalized data. PLS-DA extends a regression of PCA and uses class information to maximize the separation between groups of observations. This classification method is categorical and expresses the class membership of the statistical units (Eriksson *et al*., 2008; Andersen *et al*., 2012; Meskaldji *et al*., 2016). For our study, PLS-DA was performed using the ^{1}H NMR data of different species samples.

Results and Discussion

NMR spectra of different species: In Fig. 3, examples of NMR spectra of Radix Rheum shows a few differences between the spectra. The signal peaks of Radix Rheum from different species are mainly included in the region of 0.2-10.0 ppm. According to the characteristic signal peaks, saturated fat region is at 0.2-3.0 ppm, 3.0-5.5 ppm and 5.5-10.0 ppm are sugar anomeric protons and aromatic regions, respectively. Among all of them, the signal peak of sugar anomeric protons region from the polysaccharides is the strongest. In spite the signal of two other regions are weak, the spectral lines are clearly visible after enlarging. The saturated fat region signal is derived from terpenoids and fatty acid of Radix Rheum, while the aromatic region signal is from anthraquinone, anthrone, stilbene and phenolic acids compounds. It follows that the signal peaks of aromatic region major come from the active substance of the crude drug, and there are certain differences between species and producing area. For this reason, the signals can be used in fingerprint discriminant classification of Radix Rheum.

PCA, PLS-DA analysis of different samples: We excluded one outlier in the preliminary PCA, the accumulated contribution rate of two principal components (PC1/PC2) is 86.8%, and the PLS-DA was performed using 42 samples. As shown in Fig. 4, there was a separation of PLS-DA-derived score plots between the different species samples. The adulterants are one group only in the first quadrant, while the different species of Radix Rheum are in the other three quadrants. The results showed that the 3 species of Radix Rheum samples had a very high similarity, and there was a significant difference comparing with the data of adulterants. It is successfully distinguished the Radix Rheum samples and the adulterants, as well as the different species of Radix Rheum. Which validate that the Radix Rheum and adulterants are belong to different genus: *Rheum* L., *Rumex* L., respectively.

The combination of this result and clinical pharmacodynamic could be as quality judging criterion of Radix Rheum. At the same time, the fingerprint showed the relative content of the major compounds, which reflected the quality of Radix Rheum. Moreover, the results suggested that ^{1}H NMR fingerprint analysis method in this study can be used as a secondary research method, and may guide development of a standard protocol for quality control of Radix Rheum. ^{1}H NMR is easy and simple to handle, which detected rapidly with low cost, good reproducibility and large amount of information. Most importantly, the NMR technique can be used in every growth stage of the plant, especially seeding stage which is difficult to identify by other method. It is no doubt that the identification and quality control of the species by ^{1}H NMR is more authentic and can reflect the intrinsic quality of the medicinal materials more realistically.

Conclusions

This is the first report on the identification of Radix Rheum and adulterants using ^{1}H NMR-PCA. The 3 species of Radix Rheum and the 3 species of adulterants are well separated in the PLS-DA scores plot. This study demonstrated the effectiveness of using ^{1}H NMR with PCA on identification, and could guide the application in the future.

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Competing interest: Authors declared that they have no competing of interest.

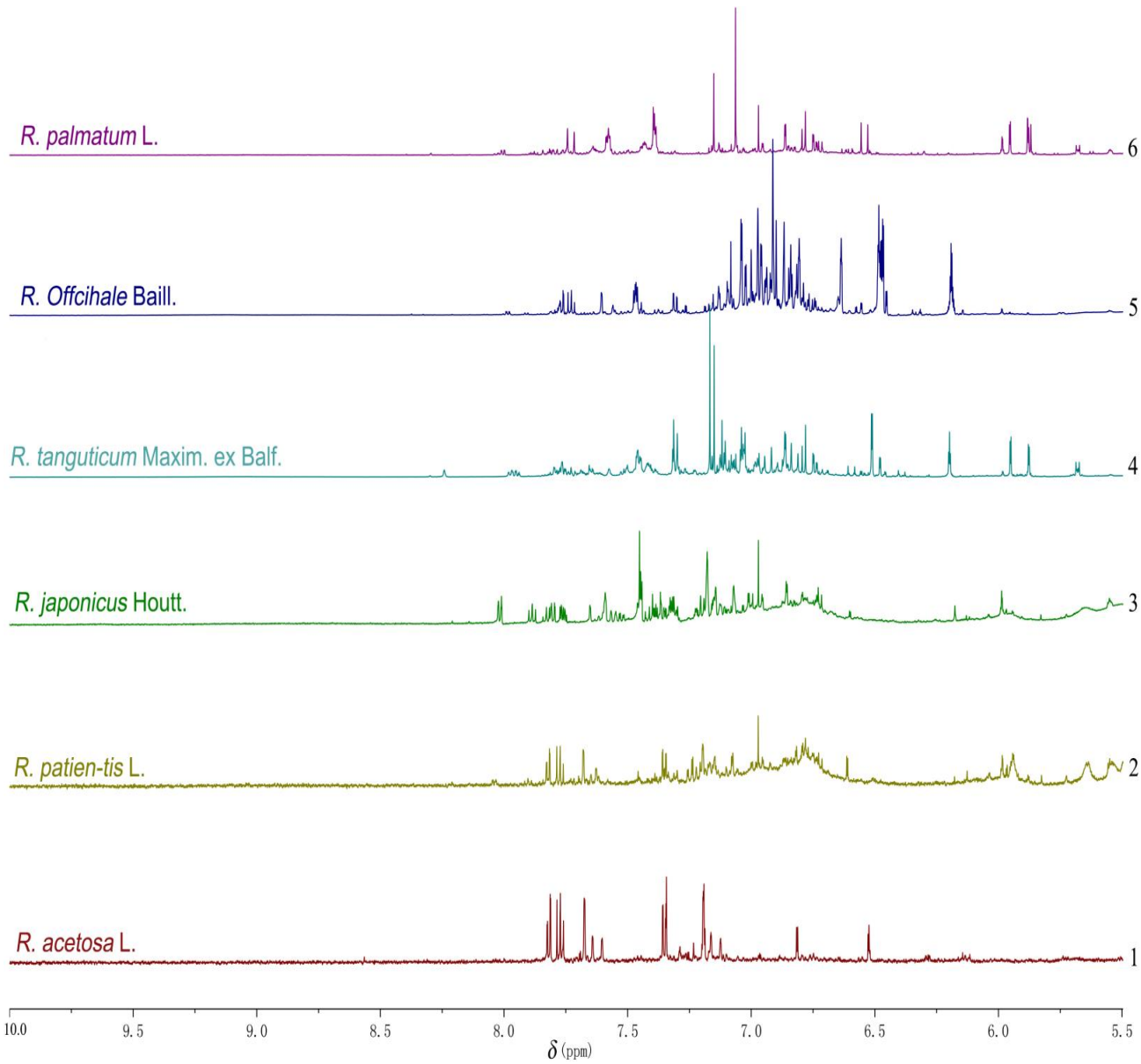

Fig. 3. Examples of ^{1}H NMR spectra before data pretreatment.

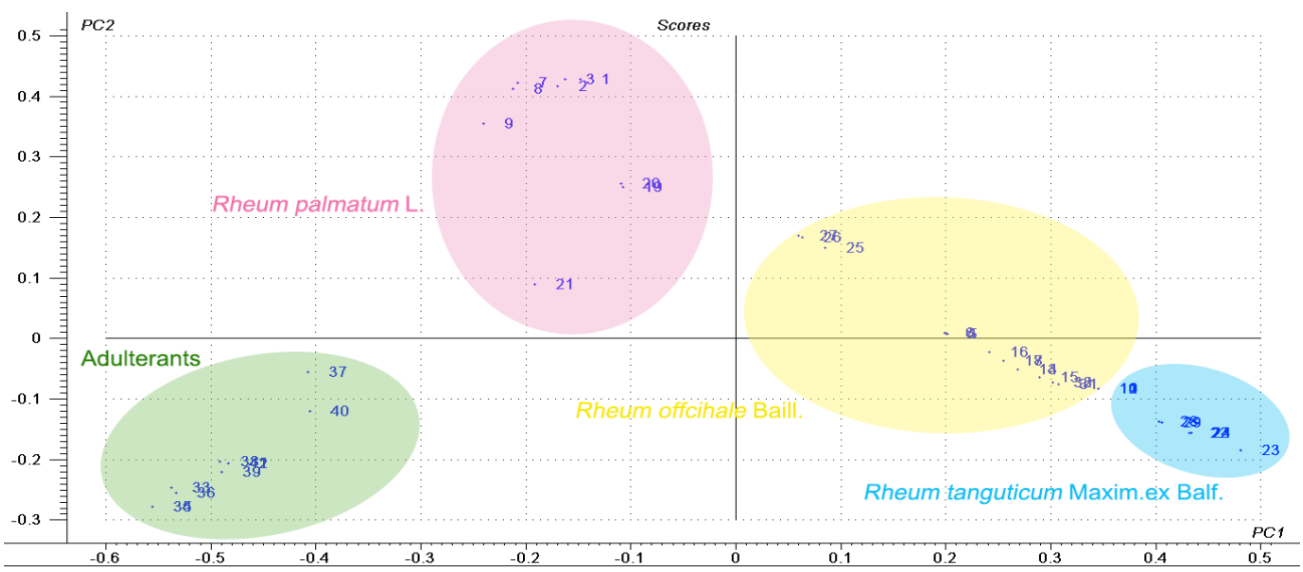

Fig. 4. Scores plot of PLS-DA obtained from ^{1}H-NMR of Radix Rheum and adulterants.

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