Comparative Study on the Fingerprint of Asparagi Radix by HPLC

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Abstract: Objective: Asparagi Radix is a commonly used herbal material in Traditional Chinese Medicine. A positive phase liquid chromatography fingerprints method was developed to compare the differences between Asparagi Radix and its decoction pieces and counterfeits.

Methods: The chromatographic separation was performed on a GOLD HILIC column (250 mm x 4.6 mm, 5 μm) with a gradient elution program using a mixture of acetonitrile and 0.01% phosphoric acid water as mobile phase at 220 nm wavelength, the flow rate was 0.8mL/min and the column temperature was 30℃. Results: The fingerprints of Asparagi Radix from different sources and its decoction pieces and counterfeits were determined by HPLC, and the similarity was analyzed. Eight common peaks were identified of 5 batches of Asparagi Radix from different origins, with similarity of 0.503-0.812. One common peak was identified by fingerprints of 5 batches of Asparagi Radix and 9 batches of its decoction pieces, with similarity of 0.407-0.766. Five batches of Asparagi Radix and two batches of its counterfeit were identified three common peaks, the similarity was 0.511-0.727. Three common peaks were identified by fingerprint of 9 batches of Asparagi Radix decoction pieces, with similarity of 0.507-0.793. Conclusion: By comparing the similarity of different origins of Asparagi Radix, the similarity of Asparagi Radix and its decoction pieces, the similarity of Asparagi Radix and its counterfeits, it was found that the common chemical compounds of Asparagi Radix was less, and the difference was more significant. The results indicated that the developed method could be reflect the differences in chemical composition among different sources of Asparagi Radix, its decoction pieces and its counterfeits, and served for the quality identification.

1. Introduction

Asparagi Radix is derived from the dried roots of the *Asparagus cochinchinensis* (Lour.) Merr. Its possess various therapeutic activities, such as treating lung cough, phlegm sticky, lumbar and knee pain, ect [1]. The chemical components of Asparagi Radix including saponins, polysaccharide, amino acids, β-sitosterols, ect. The main active components are saponins [2-3]. Modern pharmacological studies have found that saponins have anti-tumor, anti-platelet coagulation, anti-oxidation and other effects [4-5]. Polysaccharide have anti-oxidation and anti-aging effects [6]. The chemical components of Asparagi Radix were complex and difficult to extract, it is not representative to study single chemical component for quality evaluation of Asparagi Radix. TCM fingerprint is a multi-index quality control mode, which can reflect the types and quantities of chemical components comprehensively, and reflect the integrity and comprehensive function of TCM components more...
effectively, could be readily utilized as a quality control method for TCM and its preparations [7]. In previous studies, reversed-phase high performance liquid chromatography (RP-HPLC) fingerprint of Asparagi Radix from different sources was established [8]. However, the polar of main chemical components in Asparagi Radix is large, and the positive phase chromatographic column was often used for the separation of large polar compounds. The present study was carried out on Asparagi Radix by positive phase HPLC fingerprint, and compared the differences between Asparagi Radix from different areas, Asparagi Radix and its counterfeits, Asparagi Radix and its decoction pieces, and Asparagi Radix decoction pieces by similarity, so as to provide reference basis for quality evaluation of Asparagi Radix.

2. Materials and methods

2.1 Instrument
1260 Agilent High Performance Liquid Chromatography (Agilent Technology Co., Ltd.); AB265-S Electronic Balance (Metler-Toledo Instrument Co., Ltd.); KQ-100E Ultrasound Cleaner (Kunshan Ultrasound Instrument Co., Ltd.); 800Y Multifunctional Crusher (Yongkang Platinum Ou Hardware Co., Ltd.); HH-4 Digital Display Hot Water Bath pot (Shanghai Pudong Physical Optical Instrument Factory).

2.2 Reagents and Plant materials
Methanol and acetonitrile are chromatographic purity (TEDIA Company, USA), phosphoric acid is analytical purity (Chongqing Chuandong Co., Ltd., Cina), and purified water (Hangzhou Wahaha Co., Ltd., Cina). The Asparagi Radix samples were identified by professor xiangpei Wang of Guizhou University of Traditional Chinese Medicine, and samples were sealed and stored in a cool and dry place. The specific sources of Asparagi Radix, its decoction pieces and counterfeit are listed in Table 1.

| The serial number | source | The samples name |
|-------------------|--------|------------------|
| S1                | Longli County, Guizhou Province | Asparagi Radix |
| S2                | Longli County, Guizhou Province | Asparagi Radix |
| S3                | Huaxi District, Guiyang City | Asparagi Radix |
| S4                | Huaxi District, Guiyang City | Asparagi Radix |
| S5                | Huaxi District, Guiyang City | Asparagi Radix |
| S6                | Sichuan Province | Asparagi Radix Decoction Pieces |
| S7                | Sichuan Province | Asparagi Radix Decoction Pieces |
| S8                | Sichuan Province | Asparagi Radix Decoction Pieces |
| S9                | Sichuan Province | Asparagi Radix Decoction Pieces |
| S10               | Guangxi Province | Asparagi Radix Decoction Pieces |
| S11               | Guangxi Province | Asparagi Radix Decoction Pieces |
| S12               | Guiyang City, Guizhou Province | Asparagi Radix Decoction Pieces |
| S13               | Guiyang City, Guizhou Province | Asparagi Radix Decoction Pieces |
| S14               | Yunnan Province | Asparagi Radix Decoction Pieces |
| S15               | Longli County, Guizhou Province | Asparagus lycopodineus (Baker) Wang et Tang |
| S16               | Zunyi City, Guizhou Province | Asparagus filicinus Buch.-Ham.ex D.Don |

2.3 Chromatographic conditions
A GOLD HILIC(250mm×4.6mm, 5μm) column was used, the detection wavelength was set at 225 nm and column temperature was 30°C. The injection volume was 10 μL, the flow rate was 0.8 mL/min. The separation was carried out with gradient elution procedure and mobile phase acetonitrile (B) and 0.01% phosphoric acid (D) ratios linear changed as follows: 0-8 min, 97% - 96% B; 8-16 min, 96% -
92% B; 16-20min, 92% - 88% B; 20-25 min, 88-85% B; 25-45 min, 85-68% B; 45-55 min, 68-100% B.

2.4 Preparation of sample solutions
1.0g fine powder was accurately weighed and extracted with 50mL of methanol in ultrasonic processor for 30min and filtered. The filtrate was collected and heated to 10 mL in a constant temperature water bath. The concentrated filtrate was filtered by 0.45 μm membrane for reserve.

2.5 Data analysis
The data were processed and analyzed by the "Similarity Evaluation System Software for Chromatographic Fingerprints of Traditional Chinese Medicine" (2004 edition), and the control fingerprints were generated.

3. Results
3.1 Precision
Injection precision was assessed by repetitive injections of the same sample solution for six time. The RSD of retention time and peak area of each common peak were lower than 3.0%, which indicated that the instrument had good precision.

3.2 Sample stability
Stability was evaluated by analysis of the same sample solution at 0, 2, 4, 8, 12 and 24h, respectively. The RSD of relative retention time and relative peak area of each common peak were calculated to be lower than 3.0%, indicated that the sample solution remained stable for 24h.

3.3 Repeatability
Repeatability was determined by analyzing six independently prepared samples of Asparagi Radix using the same method, and RSD of the relative retention time and relative peak area of each common peak were calculated to be less than 3.0%, which showed that the method had good repeatability.

3.4 Sample determination
According to the results of each batch of samples, fingerprints were established by using the similarity evaluation system of chromatographic fingerprints of traditional Chinese medicine (National Pharmacopoeia Commission, 2004A edition). S1 was set as reference spectra, matched automatically, and the control spectra were generated by median method. The common pattern maps of Asparagi Radix, Asparagi Radix with counterfeit, Asparagi Radix with its decoction pieces, and Asparagi Radix decoction pieces were obtained.

Among them, five batches of Asparagi Radix from different origins were identified eight common peaks, with similarities of 0.637, 0.632, 0.503, 0.748 and 0.812, respectively. The fingerprints of Asparagi Radix and its counterfeits were identified three common peaks with similarities of 0.647, 0.531, 0.663, 0.550, 0.511, 0.671 and 0.727, respectively. One common peak was identified by fingerprint between Asparagi Radix and its decoction pieces, with similarities of 0.577, 0.464, 0.407, 0.692, 0.693, 0.736, 0.755, 0.766, 0.726, 0.638, 0.695, 0.409, 0.512 and 0.509. Three common peaks were identified by fingerprint of Asparagi Radix decoction pieces, with similarities of 0.666, 0.531, 0.507, 0.738, 0.710, 0.764, 0.793 and 0784 respectively. By comparison, the results showed that there were significant differences among the medicinal materials of Asparagi Radix from different origins, different batches of Asparagi Radix, Asparagi Radix and its decoction pieces, Asparagi Radix and its counterfeits, and the chemical composition were different. Show in Fig. 1, 2, 3 and 4.
(S1 Huaxi District, Guiyang City; S2 Huaxi District, Guiyang City; S3 Huaxi District, Guiyang City; S4 Longli County, Guizhou Province; S5 Longli County, Guizhou Province)

Figure 1  Fingerprint of Asparagi Radix

(S1 Guangxi Province; S2 Guangxi Province; S3 Guiyang City, Guizhou Province; S4 Guiyang City, Guizhou Province; S5 Sichuan Province; S6 Sichuan Province; S7 Sichuan Province; S8 Sichuan Province; S9 Yunnan Province)

Figure 2  Fingerprint of Asparagi Radix decoction pieces

(S1 Huaxi District, Guiyang City; S2 Huaxi District, Guiyang City; S3 Huaxi District, Guiyang City; S4 Longli County, Guizhou Province; S5 Longli County, Guizhou Province; S6 Asparagus lycopodineus (Baker) Wang et Tang; S7 Asparagus filicinus Buch.-Ham.ex D.Don)

Figure 3  Fingerprint of Asparagi Radix and its counterfeit
4. Discussion

It is reported that environmental conditions such as temperature, light and humidity can not only affect the photosynthesis, respiration and transpiration of Asparagi Radix, but also have a certain influence on the formation and accumulation of chemical components. Moreover, different growing months have different changing rules on the content of saponins, amino acids and soluble sugars in Asparagi Radix [9]. As a commonly used Chinese medicinal herb in clinic, Asparagi Radix is often processed by washing, steaming or drying after boiling [10]. Different processing methods have different effects on the content of its chemical components. For example, the chemical composition of amino acids in Asparagi Radix was significantly increased after soy boil [11].

4.1 Similarity analysis of Asparagi Radix, Asparagi Radix decoction pieces and its counterfeits from different sources

The similarity of five batches of Asparagi Radix from different sources in this study ranged from 0.503 to 0.812, with eight common peaks. Two samples from Longli and three samples from Guiyang had low similarity with each other. The reasons for the low similarity may be due to the different geographical environment, growth years of Asparagi Radix and the different formation and accumulation of chemical components in the body of Asparagi Radix. The similarity between the five batches of Asparagi Radix from different sources and their counterfeits is between 0.511 and 0.727, with only three common peaks.

4.2 Similarity analysis of Asparagi Radix from different sources, Asparagi Radix and its decoction pieces

The fingerprints of nine batches of Asparagi Radix showed that there were three common peaks, the similarity were between 0.507 and 0.793, and the similarity were low. The fingerprints of five batches of Asparagi Radix and nine batches of Asparagi Radix showed that there was only one common peak, the similarity was between 0.407 and 0.766, and the similarity was low. The number of common peaks and the similarity evaluation results showed that there is a big difference between the Asparagi Radix decoction pieces, the components decoction pieces and Asparagi Radix. The difference in the Asparagi Radix decoction pieces may be related to the harvesting season, the growth period, and the
environmental factors of the production area. The cause of the difference between the Asparagi Radix and its decoction pieces may be related to the processing method, so that the chemical components of Asparagi Radix decoction pieces changes after processing.

5. Conclusion
The quality of Asparagi Radix was influenced by growing years, harvesting season, producing area and processing methods. Therefore, it is necessary to establish a quality control method to improve the quality of Asparagi Radix and ensure the correct use of genuine Asparagi Radix. The HPLC fingerprint established in this study can identify the authenticity of Asparagi Radix, reflect the difference between Asparagi Radix and its fake products and decoction pieces and to ensure the correct use of Asparagi Radix.

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