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HPLC fingerprinting and LC–TOF-MS analysis of the extract of *Pseudostellaria heterophylla* (Miq.) Pax root

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Abstract

High-performance liquid chromatographic (HPLC) was developed for fingerprint analysis of *Pseudostellaria heterophylla* (Miq.) Pax. Liquid chromatography–electrospray ionization-time-of-flight mass spectrometry (LC–TOF-MS) technique was first employed to identify the components of the fingerprint. Twelve major peaks in chromatographic fingerprint were analyzed by on-line LC–TOF-MS analysis; one cyclic peptide was unequivocally identified and five cyclic peptides were tentatively assigned based on their MS data. These cyclic peptides served as the marker peaks in the HPLC fingerprints. The chromatographic fingerprints have been analyzed by similarity index calculations and hierarchical clustering analysis (HCA). The result showed that the HPLC fingerprints could be used to determine the optimal harvest time for *P. heterophylla* (Miq.) Pax and to authenticate the species of the herb.

Keywords: Fingerprint chromatogram; *Pseudostellaria heterophylla* (Miq.) Pax; HPLC–TOF-MS; Hierarchical clustering analysis; Similarity analysis; Cyclic peptides

1. Introduction

Recently, chromatographic fingerprinting technique, as an approach to control the quality of herbal samples, has been attracting more and more attention because of its effectiveness and convenience in real world applications [1–6]. In 2000, SFDA (State Food and Drug Administration of China) has promulgated the regulation requiring all the injections made from herbal medicines or related materials to be standardized by chromatographic fingerprint [7]. However, despite these intense research efforts in recent years, there is still no general agreement on how to standardize the fingerprinting procedures. When chromatograms from the same batch of samples are compared for reproducibility evaluation, effective analytical techniq1ues are lacking to quantify the similarities among the complex chromatograms generally observed for herbal medicines. SFDA suggested that such similarity matching should be based on calculations of correlation coefficient and/or cosine value of the vectorial angle of the chromatograms after proper reduction of the dimensions of the original data [8,9]. The validity of the methods remains to be established, and our study was initiated because of this consideration.

Pseudostellaria heterophylla (Miq.) Pax or Taizishen (TZS), one of the most popular Traditional Chinese Medicine (TCM), is distributed widely in Fujian, Zhejiang, Jiangsu, Shandong and Anhui provinces. TZS is commonly used in China for the treatment of various diseases associated with the lung, and as a spleen tonic [10]. Because of its widespread use in Chinese medicinal practices, it is of importance to have a valid method for quality control.

In standardizing herbal medicines, the ideal method is to quantify directly those components responsible for bioactivities. However, this is often difficult to do because the active ingredients in herbal products are poorly understood in many cases. Take TZS as an example, the specified quality control technique in Chinese Pharmacopoeia (2005) is by TLC assay. However, the compositional information provided by the assay is too limited to reflect the true quality of the herb. Based on these reason, a

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new and reliable HPLC–TOF-MS method for the identification and quality evaluation of the extract from TZS was established at the same time.

Liquid chromatography–electrospray ionization-time-of-flight mass spectrometry (LC–TOF-MS) has grown into one of most powerful analytical technique currently available [11,12]. Among its advantages, LC–TOF-MS has provided a high level of sensitivity and resolution. It is widely used in the analysis of complex mixtures. In this work, LC–TOF-MS was first utilized to corroborate the structure of the main constituent in the TZS.

2. Experimental

2.1. Instrumentation and reagents

An Agilent Technology 1100 Series HPLC system equipped with a quaternary pump, a degasser, a thermostatic auto-sampler and a photodiode array detector (DAD), was used for analysis. A SK3200LH ultrasonic cleaning instrument (Shanghai Kudos Ultrasonic Instrument Co., Shanghai, China) was used for extraction. The vacuum concentrator system consisted of a rotary evaporator and a digital bath (EYELA, Japan). Acetonitrile is of chromatographically grade and purchased from Merck (Germany). The water used was treated with a Milli-Q water purification system (Millipore, Molsheim, France). The MS instrument used to perform the studies was a G1969A TOF-MS from Agilent. Pseudostellarin B (Fig. 1) standard was purified and identified in our laboratory [13].

2.2. Materials

Two sets of samples labeled respectively as Set 1 and Set 2 were collected for analysis. The two sets were collected from different sub-species of TZS. A total of 21 samples were collected—10 samples in Set 1 and 11 samples in Set 2. They were sampled during the growing period of the herb at a 5-day interval in May, June, and July (25/05/2005, 30/05/2005, 05/06/2005, 10/06/2005, 15/06/2005, 20/06/2005, 25/06/2005, 30/06/2005, 05/07/2005, 10/07/2005 for Set 1 samples; 20/05/2005, 25/05/2005, 30/05/2005, 05/06/2005, 10/06/2005, 15/06/2005, 20/06/2005, 25/06/2005, 30/06/2005, 05/07/2005, 10/07/2005 for Set 2 samples). The herb species were authenticated and prepared by Mr. Wenshen Wang (B&C Technology Inc., Xiamen, China) according to morphological characteristics.

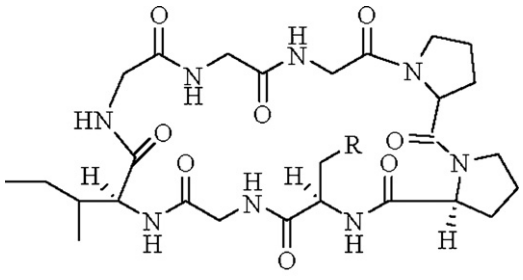

Fig. 1. The structure of Pseudostellarin B.

2.3. Sample preparation

0.5 g sample of the fine-grinded powder was accurately weighted and extracted with 50 ml of methanol in ultrasonic bath for 30 min and filtered. This extraction was repeated two additional times. The combined filtrate was evaporated to dryness in vacuo. The residue was then dissolved and diluted to 10 ml volumetric flask and filtered through a 0.45 μm filter membrane before analysis. Twenty microliters of the sample solution was injected to HPLC column and separated under below chromatographic conditions.

2.4. HPLC procedures

Chromatographic separations were carried out on a C_{18} analytical column (SinoChrom ODS-BP 4.6 mm × 200 mm, 5 μm) supplied by Dalian Elite Analytical Instruments, Dalian, China. The mobile phase consisted of water–acetic acid (A; 100:0.1, v/v) –acetonitrile (B); A:B was as follows: 0 min, 98:2; 10 min, 90:10; 30 min, 55:45; 40 min, 45:55; 60 min, 10:90; 65 min, 0:100; 75 min, 0:100; the flow-rate was 1.0 ml/min and the column temperature was maintained at 30 °C. All solvents were filtered through a 0.45 μm filter and were then degassed by sonication in an ultrasonic bath before use.

2.5. TOF-MS parameter

This HPLC system was interfaced to a time-of-flight mass spectrometer Agilent G1969A TOF-MS (Agilent Technologies) equipped with an electrospray interface operating in positive ion mode, using the following operation parameters: Capillary voltage: 3500 V; nebulizer pressure: 40 psi; drying gas: 12.0 l/min; gas temperature: 350 °C; fragmentor voltage: 200 V; skimmer voltage: 60 V. LC–TOF-MS accurate mass spectra were recorded across the range from 100 to 2000 *m/z*. The data recorded was processed with the Applied Biosystems/MDS-SCIEX Analyst QS software (Frankfurt, Germany) with accurate mass application specific additions from Agilent MSD TOF software. The mass axis was calibrated using the mixture provided by the manufacturer over the *m/z* 100–3000 range. A second orthogonal sprayer with a reference solution was used as a continuous calibration using the following reference masses: 121.0509 and 922.0098 *m/z* (resolution: 9500 ± 500 at 922.0098 *m/z*). Spectra were acquired over the *m/z* 100–2000 range at a scan rate of one second per spectrum.

2.6. Data analysis

Similarity analysis was performed by a professional software named Similarity Evaluation System for Chromatographic Fingerprint, which was recommended by SFDA of China. The software quantifies the similarity indexes among different chromatograms by calculating the correlative coefficient and/or