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Preparative isolation and purification of five compounds from the Chinese medicinal herb *Polygonum cuspidatum* Sieb. et Zucc by high-speed counter-current chromatography

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Abstract

High-speed counter-current chromatography (HSCCC) was applied to the separation and purification of five compounds from the Chinese medicinal herb *Polygonum cuspidatum* Sieb. et Zucc. The crude extracts from *P. cuspidatum* Sieb. et Zucc were treated with light petroleum–ethyl acetate–methanol–water (2:5:4:6, v/v). Sample 1 was obtained from the lower phase and sample 2 from the upper phase. The sample 1 was separated with light petroleum–ethyl acetate–water (1:5:5, v/v) and yielded 19.3 mg of piceid, 17.6 mg of anthraglycoside B from 200 mg of sample 1. The sample 2 was separated with light petroleum–ethyl acetate–methanol–water (3:5:4:6, v/v) and light petroleum–ethyl acetate–methanol–water (3:5:7:3, v/v) in a gradient elution and yielded 18.5 mg of resveratrol, 35.3 mg of emodin and 8.2 mg of physcion from 220 mg of sample 2. The purity of each compound is over 95% as determined by HPLC. The chemical structures of these components were identified by ^{1}H NMR and ^{13}C NMR.

Keywords: *Polygonum cuspidatum* Sieb. et Zucc; HSCCC; Piceid; Anthraglycoside B; Resveratrol; Emodin; Physcion

1. Introduction

Polygonum cuspidatum Sieb. et Zucc, a well-known traditional Chinese medicine and officially listed in the Chinese Pharmacopoeia [1], has been traditionally used for treatment of various inflammatory diseases, hepatitis, tumors, and diarrhea in East Asian countries such as China, Korea, Taiwan, and Japan [2]. Extracts of *P. cuspidatum* has also been mentioned to inhibit several kinds of virus [3]. Moreover, it possessed the antiviral activity against HBV [4]. The major components of *P. cuspidatum*, including piceid, resveratrol, anthraglycoside B, emodin and physcion, each have specific pharmaceutical activity. Resveratrol and piceid have effects of inhibiting the copper-catalyzed oxidation of low-density lipoprotein [5], inhibiting platelet clotting and arachidonate metabolism, reducing liver injury from peroxidized oil [6], and having cancer-chemopreventive activities [7]. Anthraglycoside B has been used for the treatment of acute hepatitis and symptoms of the reduction of leucocytes [8]. Some findings indicated that emodin is phytoestrogen with an affinity to human estrogen receptor [9]. Piceid, resveratrol and anthroglycosides B with high purity are needed for the quality control of products from *P. cuspidatum* or other related products. So it is important to develop the method for isolation and purification of all these compounds. The chemical structures of them are shown in Fig. 1.

High-speed counter-current chromatography (HSCCC) is a kind of support-free all-liquid partition chromatography which was first invented by Ito [10]. Successful application of HSCCC for the separation and purification of resveratrol, piceid and anthraglycoside B from the Chinese herb *P. cuspidatum* has been reported previously [11,12]. The sepa-

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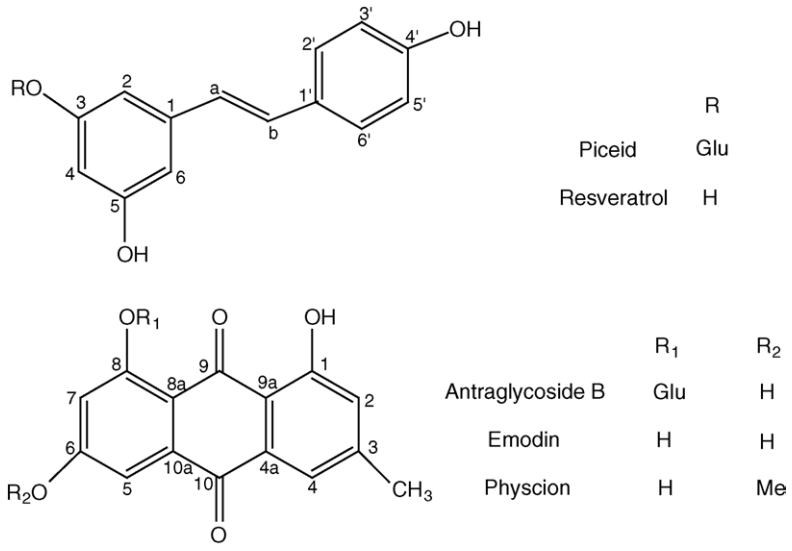

Fig. 1. Chemical structures of target compounds from *P. cuspidatum*.

ration was used with a two-phase solvent system composed of chloroform–methanol–water (4:3:2, v/v) and piceid was separated by a two-step HSCCC separation. Chloroform is a kind of deleterious organic solvent, which polluted the environment seriously and is not suitable for the separation of the Chinese traditional medicine in industry. In this paper, the two-phase solvent system composed of light petroleum–ethyl acetate–water (1:5:5, v/v) was applied to the separation and purification of piceid (I) and anthraglycoside B (II); a gradient elution with a pair of two-phase solvent system composed of light petroleum–ethyl acetate–methanol–water at volume ratios of 3:5:4:6 and 3:5:7:3 was used for the separation and purification of resveratrol (III), emodin (IV) and physcion (V). The solvents used in this experiment were propitious to the protection of the environment. The purity of piceid, anthraglycoside B, resveratrol, emodin and physcion was 97.5%, 97.1%, 98.7%, 99.3% and 98.5%, respectively, as determined by HPLC.

2. Experimental

2.1. Apparatus

The HSCCC instrument employed in the present study is TBE-300A high-speed counter-current chromatography (Shanghai Tauto Biotech Co. Ltd., Shanghai, China) with three multilayer coil separation column connected in series (I.D. of the tubing = 1.6 mm, total volume = 260 ml) and a 20 ml sample loop. The revolution radius or the distance between the holder axis and central axis of the centrifuge (R) was 5 cm, and the β values of the multilayer coil varied from 0.5 at internal terminal to 0.8 at the external terminal ($\beta = r/R$, where r is the distance from the coil to the holder shaft). The revolution speed of the apparatus can be regulated with a speed controller in the range between 0 and 1000 rpm. An HX 1050 constant-temperature circulating implement (Beijing Boyikang Lab Instrument Company, Beijing, China) was used to control the separation temperature. A ÄKTA prime system (Amersham Pharmacia Biotechnique Group, Sweden) was used to pump the two-phase solvent system and perform the UV absorbance measurement. It contains a switch valve and a mixer, which were used for gradient formation. The data were collected with Sepu 3000 chromatography workstation (Hangzhou Puhui Science Apparatus Company, Hangzhou, China).

The HPLC equipment used was Agilent 1100 HPLC system including G1311A QuatPump, G1315B UV–vis photodiode array detector, Rheodyne 7725i injection valve with a 20 μl loop, G1332 degasser and Agilent HPLC workstation (Agilent Technologies, Germany).

The nuclear magnetic resonance (NMR) spectrometer used here was a Mercury Plus 400 NMR system (Varian Inc., USA).

A FZ102 plant disintegrator (Taisite Instrument Company, Tianjin, China) was used for disintegration of the sample.

2.2. Reagents and materials

All solvents used for preparation of crude sample and HSCCC separation were of analytical grade (Jinan Reagent Factory, Jinan, China). The boiling point range of the light petroleum used for all experiments was 60–90 °C). Methanol used for HPLC was of chromatographic grade (Yucheng Chemical Factory, Yucheng, China), and water used was distilled water.

P. cuspidatum was purchased from a local drug store and was identified as the dried root of *P. cuspidatum* Sieb. et Zucc by Professor Yongqing Zhang (Shandong University of Traditional Chinese Medicine, Jinan, China).