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Short communication

Large-scale separation of resveratrol, anthraglycoside A and anthraglycoside B from *Polygonum cuspidatum* Sieb. et Zucc by high-speed counter-current chromatography

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Abstract

High-speed counter-current chromatography was successfully applied to the large-scale separation of resveratrol, anthraglycoside A and anthraglycoside B from the crude extract of *Polygonum cuspidatum* Sieb. et Zucc using a two-phase solvent system composed of chloroform, methanol and water. Resveratrol, anthraglycoside A and anthraglycoside B were separated from multigram quantities (5 g) of crude extract of *P. cuspidatum*. The separation yielded 200 mg to 1 g of these three compounds each at over 98% purity as determined by HPLC. The chemical structures of these components were identified by nuclear magnetic resonance (NMR) and MS. © 2001 Published by Elsevier Science B.V.

Keywords: *Polygonum cuspidatum*; Plant materials; Counter-current chromatography; Resveratrol; Anthraglycosides; Glycosides

1. Introduction

Polygonum cuspidatum Sieb. et Zucc (Huzhang in Chinese) is a kind of traditional Chinese medicinal herb commonly used for the treatment of dermatitis and abscess. The major components of *P. cuspidatum* including anthraglycoside A (emodin-6-methoxy-8-*O*-D-glucoside), anthraglycoside B (emodin-8-*O*-D-glucoside) and resveratrol have each specific pharmaceutical activities: anthraglycoside A and anthraglycoside B have been used for the treatment of acute hepatitis and symptoms of the reduction in leucocytes [1]. Resveratrol has been shown to have a cancer chemopreventive activity in assays that represent three major stages of carcinigenesis such as anti-initiation, anti-promotion and anti-progression activities. In addition, resveratrol inhibited the development of preneoplastic lesion in carcinogen-treated mouse mammary glands in culture as well as tumorigensis in a mouse skin cancer model. And resveratrol has been suggested to be used as a common constituent of the human diet and a potential cancer chemopreventive agent in humans [2,3]. Extract from *P. cuspidatum* is being developed into resveratrol-containing health food. Resveratrol, anthraglycosides A and B with high purity are

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needed for the quality control of any products or food from *P. cuspidatum* or other related products. So it is important to develop method to isolate and purify resveratrol, and anthraglycosides A and B.

On the other hands, these phenolic compounds produce some problems in isolation by conventional silica gel column chromatography because they are strongly adsorbed onto the solid support [4]. High-speed counter-current chromatography (HSCCC), being a kind of support-free liquid–liquid partition chromatography, eliminates irreversible adsorption of sample onto the solid support, and therefore is considered as a suitable alternative for the separation of phenolic compounds [5–8].

The present paper describes the large-scale separation of resveratrol, anthraglycoside A and anthraglycoside B from a crude extract of *P. cuspidatum*.

2. Experimental

2.1. Apparatus

The analytical HSCCC instrument employed in the present study is a Model GS 20 analytical high-speed counter-current chromatograph (designed and constructed at Beijing Institute of New Technology Application, Beijing, China) equipped with a PTFE multilayer coil separation column of 50 m×0.85 mm I.D. with a total capacity of 30 ml. The β value varied from 0.4 at the internal terminal to 0.7 at the external terminal ($\beta=r/R$ where r is the distance from the coil to the holder shaft, and R, the revolution radius or the distance between the holder axis and central axis of the centrifuge). The revolution speed of the apparatus could be regulated with a speed controller in the range between 0 and 2000 rpm, but an optimum speed of 1500 rpm was used in the present studies.

Preparative HSCCC was performed using a Model GS10A2 multilayer coil planet centrifuge (Beijing Institute of New Technology Application) equipped with a PTFE multilayer coil of 110 m×1.6 mm I.D. with a total capacity of 230 ml and a Model CCC-1000 (Pharma-Tech Research, MD, USA) equipped with three PTFE multilayer coils of 2.6 mm I.D. that were serially connected to make up a total capacity of 860 ml.

The solvent was pumped into the column with a Model NS-1007 constant-flow pump (Beijing Institute of New Technology Application). A Model 8823A-UV Monitor (Beijing Institute of New Technology Application) was used to continuously monitor the effluent at 254 nm. A portable Yokogawa Model 3057 recorder (Sichuan Instrument Factory, Chongqin, China) was used to record the chromatogram. A manual sample injection valve with a 1.0-ml loop (for the analytical HSCCC) or a 20–50-ml loop (for the preparative HSCCC) (Tianjin High-New Science and Technology, Tianjin, China) was used to introduce the sample into the column.

The high-performance liquid chromatography (HPLC) equipment used was a Shimadzu LC-10A$_{VP}$ system including two LC-10AT$_{VP}$ solvent delivery units, an SPD-M10A$_{VP}$ UV–Vis photodiode array detector, an SCL-10A$_{VP}$ system controller, a CTO-10AS$_{VP}$ column oven, a DGU-12A degasser, a Model 7726 injection valve with a 20-μl loop, and a Class-VP-LC workstation (Shimadzu, Kyoto, Japan).

2.2. Reagents

All organic solvents used for HSCCC were of analytical grade and purchased from Beijing Chemical Factory, Beijing, China. Methanol used for HPLC analysis was of chromatographic grade and purchased from Tianjin Huaxi Special Reagent Factory, Tianjin, China.

2.3. Preparation of two-phase solvent system

The two-phase solvent system utilized in the present study was composed of chloroform–methanol –water (4:3:2, v/v). After thoroughly equilibrating the mixtures in a separatory funnel at room temperature, two phases were separated shortly before use where the aqueous phase was used as the stationary phase and the organic phase as the mobile phase.

2.4. Preparation of sample and sample solutions

About 1.0 kg of dried and powdered roots of *P. cuspidatum* was extracted with methanol (1.5 l×3) at room temperature. The extracts were combined and evaporated to dryness under reduced pressure

which yielded 110 g of dry powder. A 50-g amount of this dried extract was dissolved in 500 ml of hot water and after cooling extracted with 500 ml of diethyl ether for three times which yielded about 15 g of ether extract and 35 g of the remaining water-soluble extract. Both extracts were used in the present study. The sample solutions were prepared by dissolving 200 mg of each extract in 10 ml of a mixture of upper and lower phases for preparative separation, and 5 g of each extract in 50 ml of a mixture of upper and lower phases for large-scale preparative separation.

2.5. Separation procedure

The multiplayer coiled column was first entirely filled with the upper organic phase as the stationary phase. Then the lower aqueous phase was pumped into the head end of the column at a suitable flow-rate of 2.0 ml/min for Model GS02A2, and 5.0 ml/min for Model CCC1000 while the apparatus was rotated at an optimum speed of 800 rpm for Model GS02A2 and 1000 rpm for Model CCC1000. After hydrodynamic equilibrium was reached, as indicated by a clear mobile phase eluting from the tail outlet, the sample solution was injected through the injection valve. The effluent from the tail end of the column was continuously monitored with UV detection at 254 nm and peak fractions were each collected according to the chromatogram. The retention of the stationary phase relative to the total column capacity was computed from the volume of the stationary phase collected from the column after the separation was completed.

2.6. HPLC analyses and identification of CCC peak fractions

The crude methanol extract, the ether extract, the water-soluble extract and each purified peak fraction from the preparative HSCCC separation were analyzed by HPLC with a Shim-pack VP ODS column (150×4.6 mm I.D.) at 254 nm and column temperature of 40°C. Mobile phase: MeOH–1% HAc: 0.01 min (40% MeOH)–4 min (40% MeOH)–22 min (85% MeOH)–40 min (85% MeOH); flow-rate: 1.0 ml/min; UV wavelength: 254 nm.

Identification of HSCCC peak fractions was carried out by fast atom bombardment mass spectrometry (FAB-MS), UV and ^{1}H-nuclear magnetic resonance (NMR) and ^{13}C-NMR spectra.

3. Results and discussion

The crude methanol extracts of *P. cuspidatum* containing resveratrol, anthraglycoside A and anthraglycoside B was first separated into the ether and the water-soluble extracts by two-phase solvent extraction. As shown in Fig. 1, HPLC analyses of the these extracts from *P. cuspidatum* indicated that relatively hydrophobic resveratrol was concentrated in the ether extract while polar anthraglycosides A and B were found in the water-soluble extract.

In order to achieve an effective separation by HSCCC, one must first determine a suitable solvent system which provides an optimum range of partition coefficient ($2>K>0.5$) for the target compounds.

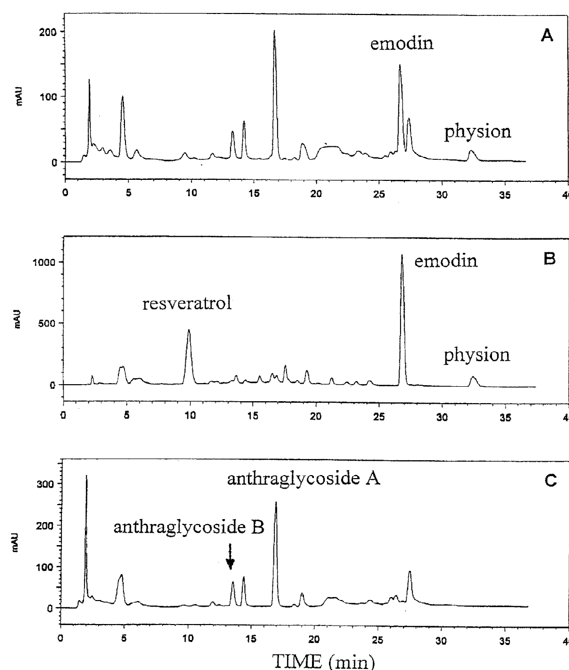

Fig. 1. HPLC analyses of crude methanol extract (A), ether extract (B) and water-soluble extract (C) from *P. cuspidatum*. Column: Shim-pack VP ODS column (150×4.6 mm I.D.); mobile phase: MeOH–1% HAc: 0.01 min (40% MeOH)–4 min (40% MeOH)–22 min (85% MeOH)–40 min (85% MeOH); flow-rate: 1.0 ml/min; column temperature: 40°C; UV wavelength: 254 nm.

This can be done according to the following two methods: a simple and most effective method is to find a previous report on CCC experiments for similar compounds. If such data are not available, one may conduct a systematic search for the solvent system as described elsewhere [9,10] although this trial and error procedure may require several hours. In the present study, we found previous partition data for similar flavonoid compounds using a solvent system composed of chloroform–methanol–water at various volume ratios [4,7].

After selecting the two-phase solvent system for the separation of the three target compounds by using analytical HSCCC, we found that both samples can be separated by the same solvent system composed of chloroform–methanol–water (4:3:2, v/v).

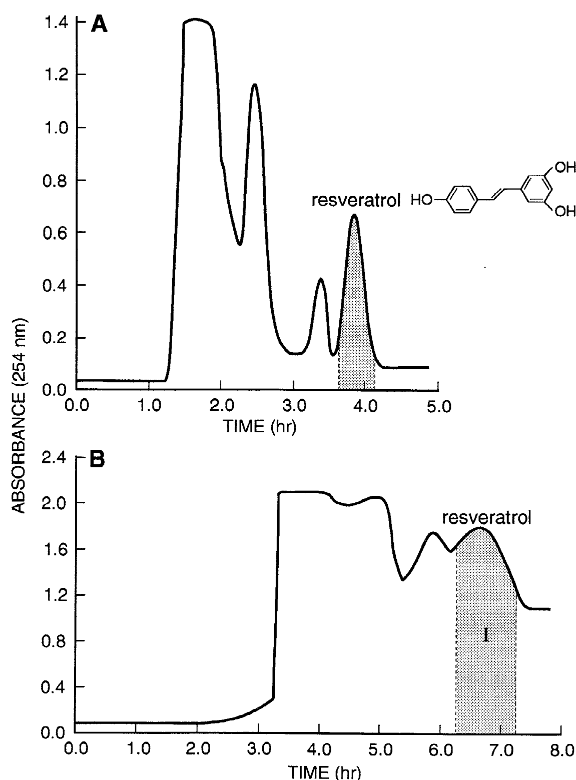

Fig. 2. HSCCC separations of ether extract from *P. cuspidatum* by Model GS10A2 (A) and Model CCC-1000 (B). Two-phase solvent system: chloroform–methanol–water (4:3:2, v/v); stationary phase: upper aqueous phase; mobile phase: lower organic phase; flow-rate: 2.0 ml/min (A) and 5.0 ml/min (B); revolution speed: 800 rpm (A) and 1030 rpm (B); sample size: 200 mg (A) and 5.0 g (B); retention of stationary phase: 65% (A) and 60% (B); UV wavelength: 254 nm.

Fig. 2 shows the results of the preparative separation of 0.2 g (Fig. 2A) and 5 g (Fig. 2B) of the ether extract from *P. cuspidatum* by HSCCC which yielded, respectively, 30 mg and 600 mg of resveratrol at a purity of over 98% determined by HPLC.

Fig. 3 shows the results of preparative HSCCC separation of 0.2 g (Fig. 3A) and 5 g (Fig. 3B) of the water-soluble extract from *P. cuspidatum*: the separation shown in Fig. 3A yielded about 50 mg of anthraglycoside A and 20 mg of anthraglycoside B while the separation in Fig. 3B yielded about 1.0 g of anthraglycoside A and 200 mg of anthraglycoside B all at a high purity of over 98% determined by HPLC.

Fig. 4 shows the results of HPLC analyses of the three target compounds purified by HSCCC. The structural identification of HSCCC peak fractions was carried out by MS, UV, ^{1}H-NMR and ^{13}C-NMR.

The results of our studies clearly demonstrate that

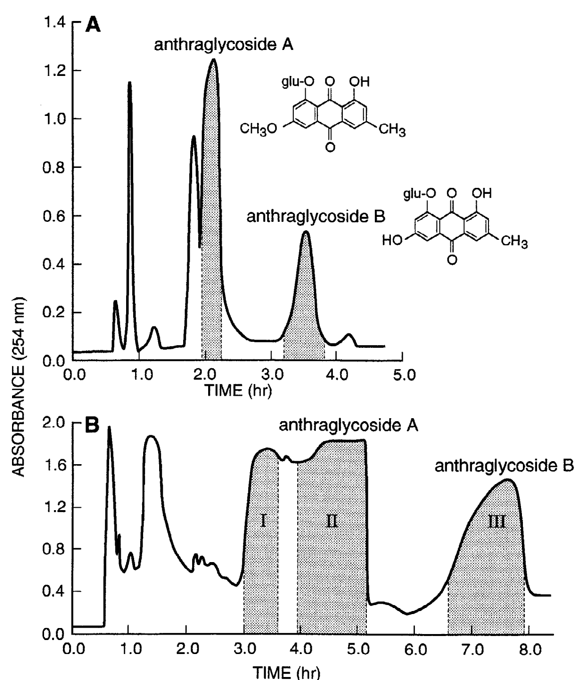

Fig. 3. HSCCC separations of water-soluble extract from *P. cuspidatum* by Model GS10A2 (A) and Model CCC-1000 (B). Two-phase solvent system: chloroform–methanol–water (4:3:2, v/v); stationary phase: upper aqueous phase; mobile phase: lower organic phase; flow-rate: 2.0 ml/min (A) and 5.0 ml/min (B); revolution speed: 800 rpm (A) and 1030 rpm (B); sample size: 200 mg (A)and 5.0 g (B); retention of stationary phase: 76% (A) and 75% (B); UV wavelength: 254 nm.

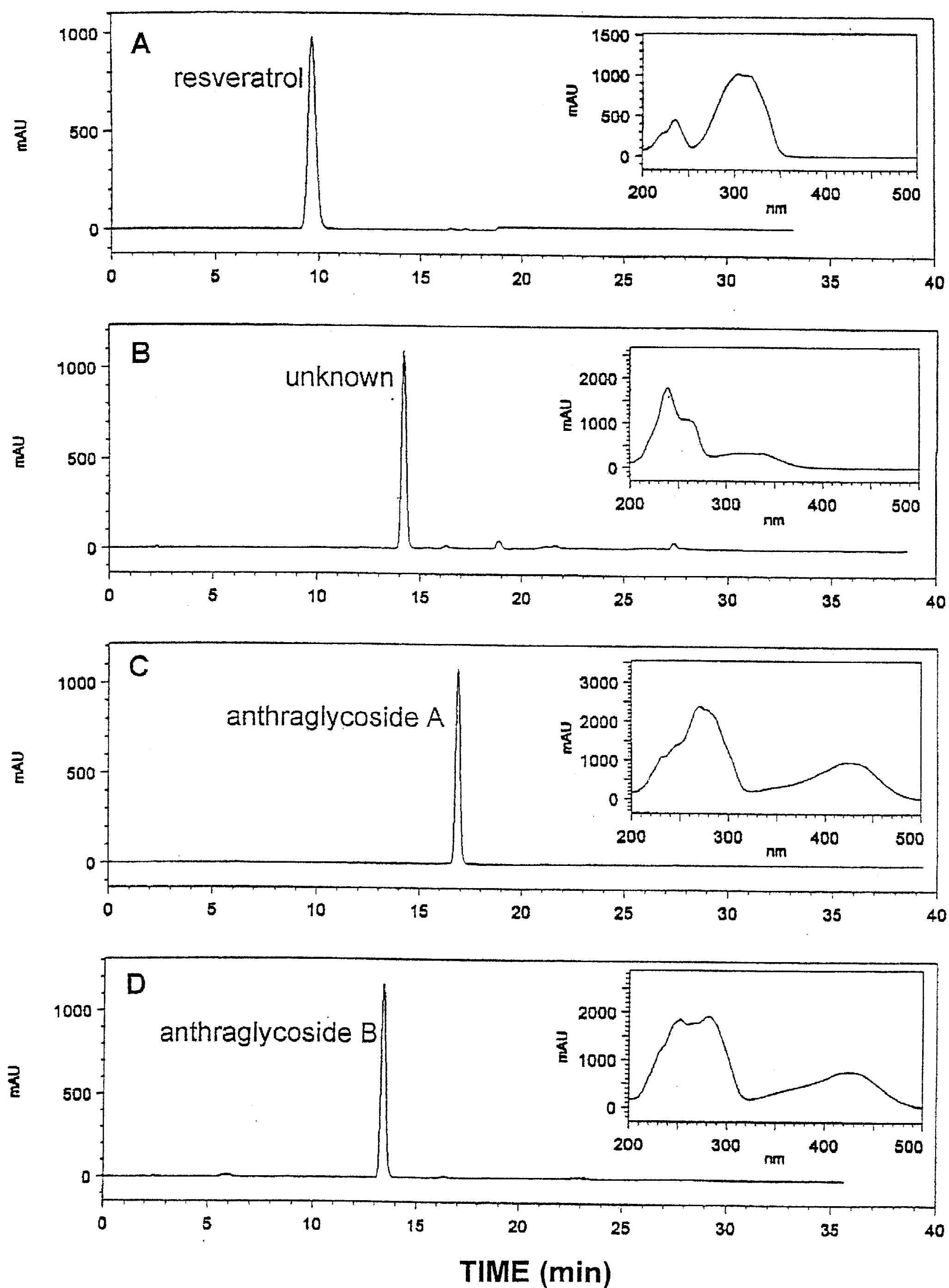

Fig. 4. HPLC analyses and UV spectra of each peak fractions from the preparative HSCCC separations (Fig. 2B and Fig. 3B). Column: Shim-pack VP ODS column (150×4.6 mm I.D.); mobile phase: MeOH–1% HAc: 0.01 min (40% MeOH)–4 min (40% MeOH)–22 min (85% MeOH)–40 min (85% MeOH); flow-rate: 1.0 ml/min; column temperature: 40°C; UV wavelength: 254 nm.

HSCCC was successfully used for the large-scale separation of resveratrol, anthraglycoside A and anthraglycoside B from multigram quantities (5 g) of crude extract of *P. cuspidatum*.

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