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Short communication

Preparative isolation and purification of psoralen and isopsoralen from *Psoralea corylifolia* by high-speed counter-current chromatography

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Abstract

Psoralen and isopsoralen were separated from *Psoralea corylifolia* by high-speed counter-current chromatography (HSCCC). A two-phase solvent system composed of *n*-hexane–ethyl acetate–methanol–water (5:5:4.5:5.5, v/v) was used for HSCCC separation, and yielded, from 100 mg of crude extract, 39.6 mg of psoralen and 50.8 mg of isopsoralen each at over 99% purity as determined by high performance liquid chromatography (HPLC). The identification of psoralen and isopsoralen were performed with ^{1}H NMR and ^{13}C NMR.

Keywords: High-speed counter-current chromatography; *Psoralea corylifolia*; Psoralen; Isopsoralen

1. Introduction

Psoralea corylifolia (Chinese traditional medicinal herb, Buguzhi in Chinese) has been widely used in traditional Chinese medicine for the treatment of various kinds of disorders such as asthma, cough, nephritis, vitiligo, and calvities [1]. The effective components of this herb are coumarins. Psoralen and isopsoralen are the major components. Pharmacological test revealed that they have antitumor [2], antibacterial and antivirus activities and can affect metabolism of some remedy [3]. Psoralen and isopsoralen are used as reference standards in the quality control of Buguzhi and its products. So the isolation and purification of psoralen and isopsoralen are of great interest.

High-speed counter-current chromatography (HSCCC) is a form of liquid–liquid partition chromatography, which was first invented by Ito [4]. No solid matrix was used in HSCCC. The liquid stationary phase is immobilized in the column by centrifugal force. When the mobile phase is pumped through the column, sample components are partitioned between the two phases and they are separated on the basis of difference in partition coefficients. Comparing with traditional liquid–solid column chromatography, HSCCC eliminates irreversible adsorption loss of samples and yields higher recovery and efficiency [5]. So it is very suitable for separation of active components from traditional Chinese medicinal herbs and other natural products. Many successful applications of HSCCC have been reported for the separation of various components such as alkaloids [6,7], flavonoids [8,9], polyphenols [10,11], terpenoids [12], antibiotics [13], dyes [14], and coumarin [15].

The present paper describes an HSCCC method for separation of psoralen and isopsoralen from a crude light petroleum extract of *P. corylifolia*. The optimum conditions were obtained, which led to the successful separation of psoralen and isopsoralen with the purity of each at over 99%.

2. Experimental

2.1. Apparatus

The HSCCC instrument employed in the present study is TBE-300A high-speed counter-current chromatography

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(Tauto Biotechnique Company, 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 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. The loop volume of the sample injection is 20 mL. The revolution speed of the apparatus can be regulated with a speed controller in the range between 0 and 1000 rpm. The experimental temperature was adjusted by HX 1050 constant temperature circulating implement (Beijing Boyikang Lab Implement, Beijing, China). An ÄKTA prime (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 can be used for gradient formation. The data were collected with Sepu 3000 chromatography workstation (Hangzhou Puhui Science Apparatus Co. Ltd., Hangzhou, China).

The HPLC equipment used was Agilent1100 system including a G1311A QuatPump, a G1315B DAD, a 7725i injection valve with a 20 μL loop, a G1332A degasser and Agilent HPLC workstation.

Nuclear magnetic resonance (NMR) spectrometer used here was Mercury Plus 400 NMR (Varian Inc., USA).

2.2. Reagents

All solvents used for preparation of crude sample and HSCCC separation were of analytical grade (Jinan Reagent Factory, Jinan, China). Methanol used for HPLC was chromatographic grade (Yucheng Chemical plant, Yucheng, China), and water used was distilled water.

The dried seeds of *P. corylifolia* were purchased from a local drug store and identified by Professor Yongqing Zhang (Shandong University of Traditional Chinese Medicine, Jinan, China).

2.3. Preparation of crude sample [16]

The dried seeds of *P. corylifolia* were ground to powder (about 30 mesh). The powder (1000 g) was marinated with 2000 mL of 50% ethanol for 24 h and then filled in a glass column and eluted with 8000 mL of 50% ethanol for 8 days at room temperature. The extract was evaporated under reduced pressure to about 3500 mL, and the concentrate was stored in a refrigerator (4 °C) for 24 h. The deposition (500 g) generated in the solution was extracted with 400 mL of light petroleum (boiling range 60–90 °C) at 85 °C for 2 h. The extraction was repeated five times. Then the light petroleum extracts were combined and evaporated under reduced pressure. 10.3 g of crude extract powder was obtained. It was stored in a refrigerator (4 °C) for the subsequent HSCCC separation.

2.4. Selection of two-phase solvent system

Selecting the solvent system for HSCCC means simultaneously choosing the stationary phase and the mobile phase. The solvent system was selected according to the partition coefficients (K) of the target compounds. The K-values were determined by HPLC as following: suitable amount of crude extract powder was dissolved in the upper phase of the solvent system and analyzed by HPLC. The peak areas of the peaks were recorded as A_1. Then equal volume of the lower phase was added to the solution and mixed thoroughly to reach partition equilibrium. The upper phase was then analyzed by HPLC again. The latter peak areas were recorded as A_2. The K-values were calculated according to the following equation: $K = A_2/(A_1–A_2)$.

2.5. Preparation of two-phase solvent system and sample solution

In the present study, the two-phase solvent system composed of *n*-hexane–ethyl acetate–methanol–water was used for HSCCC separation. Each solvent was added to a separatory funnel and thoroughly equilibrated at room temperature. The upper phase and the lower phase were separated and degassed by sonication for 30 min shortly before use.

The sample solution for HSCCC separation was prepared by dissolving suitable amount of crude extract in the upper phase of the solvent system.

2.6. HSCCC separation procedure

HSCCC was performed with a TBE-300A HSCCC instrument. The upper phase (stationary phase) and the lower phase (mobile phase) of the two-phase solvent system were pumped into the column simultaneously by using ÄKTA prime system, with the volume ratio of 60:40. When the column was totally filled with the two phases, the lower phase was pumped at a flow rate of 2.0 mL min^{-1}, and at the same time, the HSCCC apparatus was run at a revolution speed of 900 rmp. After hydrodynamic equilibrium was reached (about half an hour later), 5 ml of the sample solution containing 100 mg of the crude extract was injected into the separation coil tube through the injection valve. Chromatogram was recorded 100 min after sample injection. All through the experiment, the separation temperature was controlled at 20 °C. The effluent from the tail end of the column was continuously monitored with a UV absorbance detector at 254 nm. Each peak fraction was collected according to the chromatogram and evaporated under reduced pressure. The residual was dissolved in methanol for subsequent study.

2.7. HPLC analysis and identification of HSCCC peak fractions

The crude extract of *P. corylifolia* and each peak fraction from HSCCC separation were analyzed by HPLC.

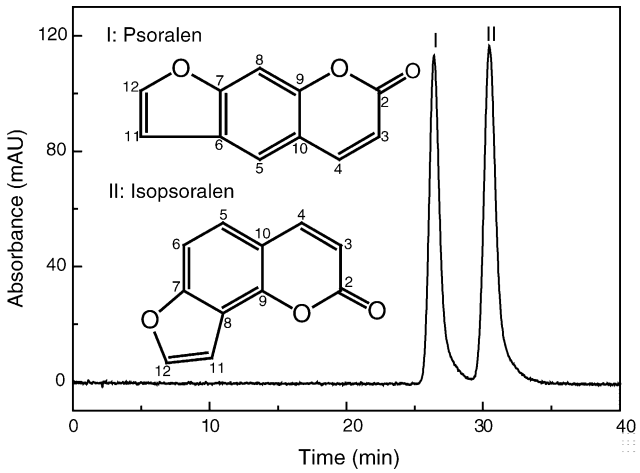

Fig. 1. HPLC chromatograms of crude extract from *P. corylifolia*. Conditions: column, reversed-phase YWG C_{18} column (200 mm × 4.6 mm i.d., 10 μm); mobile phase, methanol–water (40:60, v/v); flow rate, 1.0 ml min^{-1}; detection wavelength, 254 nm.

The column used for HPLC analysis was YWG C_{18} column (200 mm × 4.6 mm i.d. 10 μm). The mobile phase was methanol–water (40:60, v/v) and the flow rate was 1.0 ml min^{-1}. The effluent was monitored at 254 nm.

The identification of HSCCC peak fractions was carried out by ^{1}H NMR and ^{13}C NMR. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Mercury Plus 400 NMR with TMS (for ^{1}H NMR) and $C^{2}HCl_3$ (for ^{13}C NMR) as internal standards.

3. Results and discussion

3.1. Optimization of HPLC method

The partition coefficient of each component in crude extract was determined by HPLC analysis. So in the first place, a good HPLC method should be developed for analysis of crude extract. Different mobile phases (methanol–water, acetonitrile–water) were used in HPLC to separate psoralen and isopsoralen from the crude extract of *P. corylifolia*. The results indicated that when methanol–water (40:60, v/v) was used as the mobile phase, psoralen and isopsoralen could obtain baseline separation. Analyzed by Agilent 1100 workstation, the purity factor of each peak was within the calculated threshold limit. The HPLC chromatogram of crude extract from *P. corylifolia* was given in Fig. 1.

3.2. Optimization of HSCCC conditions

According to the HPLC analysis results (Fig. 1), there are two major compounds present in the crude extract from *P. corylifolia*. In order to achieve efficient resolution of target compounds in HSCCC separation, the *K*-values of these two compounds in different solvent systems were determined by HPLC as the procedure shown in Section 2.4. The results are given in Table 1. A two-solvent system of ethyl acetate–water was tested first. The *K*-values of the target compounds in it are very big. By adding methanol to ethyl acetate–water, the *K*-values could be reduced. But the phase separation could not be achieved when the amount of methanol is big. The addition of *n*-hexane to ethyl acetate–methanol–water system could improve the phase separation. According to the *K*-values, *n*-hexane–ethyl acetate–methanol–water solvent systems with different volume ratios were tested in HSCCC separation. The results of HSCCC tests indicated that when (5:5:4:6, v/v) was used as the solvent system, the separation time was too long. When *n*-hexane–ethyl acetate–methanol–water (5:5:5:5, v/v) was used, the two compounds could not get satisfactory separation. When *n*-hexane–ethyl acetate–methanol–water (5:5:4.5:5.5, v/v) was used as the solvent system, good separation results and acceptable separation time could be obtained.

Table 1
The *K*-values of psoralen and isopsoralen

Solvent system	*K*[a]	
	Psoralen	Isopsoralen
Ethyl acetate–water (5:5, v/v)	145	147
Ethyl acetate–methanol–water (5:1:5, v/v)	69	46
Ethyl acetate–methanol–water (5:2:5, v/v)	17	16
Ethyl acetate–methanol–water (5:3:5, v/v)	3.4	3.5
Ethyl acetate–methanol–water (5:4:5, v/v)	No phase separation	
n-Hexane–ethyl acetate–methanol–water (5:5:4:6, v/v)	4.42	6.38
n-Hexane–ethyl acetate–methanol–water (5:5:4.5:5.5, v/v)	1.73	3.07
n-Hexane–ethyl acetate–methanol–water (5:5:5:5, v/v)	1.23	1.80
n-Hexane–ethyl acetate–methanol–water (5:5:6:4, v/v)	0.79	0.86

[a] *K* is expressed as the solute concentration in the upper stationary phase divided by that in the lower mobile phase.

The flow rate of the mobile phase, the separation temperature, the retention percentage of the stationary phase and the revolution speed of the separation coil were also optimized. The HSCCC chromatogram of crude extract from *P. corylifolia* obtained under the optimum conditions was shown in Fig. 2. 39.6 mg of psoralen (peak I) and 50.8 mg of isopsoralen (peak II) could be obtained from 100 mg of crude extract in one-step separation.

3.3. HPLC analysis and identification of HSCCC peak fractions

The purity of each peak fraction of HSCCC was determined by HPLC. The HPLC chromatogram and the UV spectra of each fraction were shown in Fig. 2. The purity of psoralen (peak I in Fig. 2) and isopsoralen (peak II in Fig. 2) was 99.4 and 99.6%, respectively.

The identification of peak fractions in Fig. 2 was performed with ^{1}H NMR and ^{13}C NMR. ^{1}H NMR and ^{13}C NMR data of each fraction were given as follows:

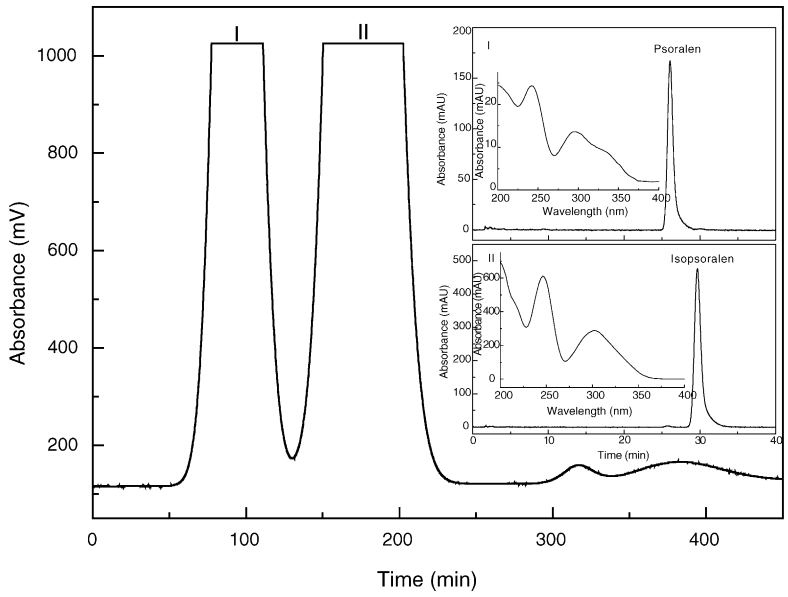

Fig. 2. HSCCC chromatogram of crude extract from *P. corylifolia*. Conditions: two-phase solvent system, *n*-hexane–ethyl acetate–methanol–water (5:5:4.5:5.5, v/v); mobile phase, the lower phase; flow rate, 2.0 ml min^{-1}; revolution speed, 900 rpm; detection wavelength, 254 nm; separation temperature, 20 °C; sample size, 100 mg of crude sample dissolved in 5 ml of the upper phase; retention percentage of the stationary phase, 60%. I: psoralen (collected during 78–112 min); II: isopsoralen (collected during 150–202 min).

HSCCC peak I in Fig. 2: ^{1}H NMR (400 MHz, C^2HCl_3): δ ppm: 7.81 (1H, d, J = 10 Hz, C_4–H), 7.70 (1H, d, J = 2.4 Hz, C_{12}–H), 7.69 (1H, s, C_5–H), 7.49 (1H, s, C_8–H), 6.84 (1H, d, J = 2.4 Hz, C_{11}–H), 6.39 (1H, d, J = 10 Hz, C_3–H); ^{13}C NMR (400 MHz, C^2HCl_3): δ ppm: 161.10 (C-2), 156.37 (C-7), 151.96 (C-9), 146.91 (C-12), 144.12 (C-4), 124.86 (C-6), 119.82 (C-5), 115.37 (C-10), 114.60 (C-3), 106.35 (C-11), 99.87 (C-8).

HSCCC peak II in Fig. 2: ^{1}H NMR (400 MHz, C^2HCl_3): δ ppm: 7.83 (1H, d, J = 9.6 Hz, C_4–H), 7.71 (1H, d, J = 2.4 Hz, C_{12}–H), 7.45 (1H, d, J = 8.8 Hz, C_6–H), 7.40 (1H, d, J = 8.8 Hz, C_5–H), 7.15 (1H, d, J = 2.4 Hz, C_{11}–H), 6.41 (1H, d, J = 9.6 Hz, C_3–H); ^{13}C NMR (400 MHz, C^2HCl_3): δ ppm: 160.66 (C-2), 157.33 (C-7), 148.93 (C-9), 145.90 (C-12), 144.59 (C-4), 141.25 (C-3), 123.82 (C-5), 117.04 (C-8), 114.10 (C-10), 108.84 (C-6), 104.11 (C-11).

According to the literature [17], peaks I and II in Fig. 2 were identified as psoralen and isopsoralen, respectively.

In conclusion, HSCCC was successfully used for the isolation and purification of psoralen and isopsoralen from *P. corylifolia*. 39.6 mg of psoralen and 50.8 mg of isopsoralen each at over 98% purity can be obtained from 100 mg of the crude extract in one-step separation.

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