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Short communication

Preparative isolation and purification of costunolide and dehydrocostuslactone from *Aucklandia lappa Decne* by high-speed counter-current chromatography

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Abstract

A preparative high-speed counter-current chromatography (HSCCC) method for isolation and purification of costunolide and dehydrocostuslactone from the Chinese medicinal plant *Aucklandia lappa Decne* (Muxiang in Chinese) was successfully established by using light petroleum–methanol–water (5:6.5:3.5, v/v/v) as the two-phase solvent system. The upper phase of light petroleum–methanol–water (5:6.5:3.5, v/v/v) was used as the stationary phase of HSCCC. 35.7 mg of costunolide and 43.6 mg of dehydrocostuslactone with the purity of 100% and 99.6%, respectively, were separated successfully in one-step separation from 110 mg of crude sample from *Aucklandia lappa Decne*. The structures of costunolide and dehydrocostuslactone were identified by ^{1}H NMR and ^{13}C NMR.

Keywords: High-speed counter-current chromatography; Costunolide; Dehydrocostuslactone; Sesquiterpene lactone; *Aucklandia lappa Decne*; Muxiang

1. Introduction

Muxiang, the roots of *Aucklandia lappa Decne* (Chinese traditional medicinal herb), officially listed in the Chinese Pharmacopoeia [1], has been widely used as traditional Chinese medicine for the treatment of various kinds of disorders such as asthma, cough [2], diarrhea, vomit, indigestion, colic, cholecystitis and hepatitis [3]. The effective components of this herb are sesquiterpene lactone. Costunolide and dehydrocostuslactone are the major components, whose chemical structures are given in Fig. 1. Pharmacological test revealed that costunolide and dehydrocostuslactone not only have the effect of antibacterial, antalgic and antivirus activities [4], but also could dilate bronchus, improve stomach function, depress blood pressure, and relieve the spasm of smooth muscle [5]. The content of costunolide and dehydrocostuslactone had been used as the standard index to appraise the quality of Muxiang and its products. Consequently, the isolation and purification of high-purity costunolide and dehydrocostuslactone becomes increasingly important.

High-speed counter-current chromatography (HSCCC), first invented by Ito [6], is a form of liquid–liquid partition chromatography. With the advantage of eliminating the use of solid support matrix, HSCCC is very suitable for the separation and purification of active components of traditional Chinese medicinal herbs and other natural products. In present study, costunolide and dehydrocostuslactone were purified successfully from crude extract of Muxiang in one-step separation. The purity of costunolide and dehydrocostuslactone was 100% and 99.6%, respectively, as determined by HPLC. The present work developed a successful HSCCC method for the separation and purification of costunolide and dehydrocostuslactone for the first time.

2. Experimental

2.1. Apparatus

The HSCCC instrument employed in the present study is TBE-300A high-speed counter-current chromatography (Tauto Biotechnique Company, Shanghai, China) with three

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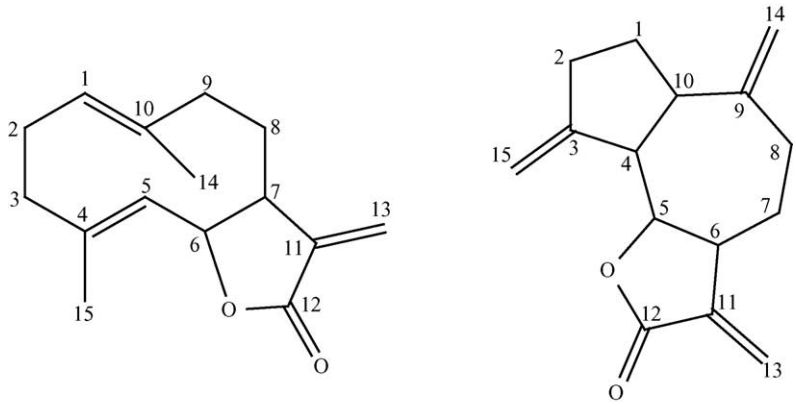

Fig. 1. Chemical structures of costunolide and dehydrocostuslactone.

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 a G1311A QuatPump, a G1315B UV–vis photodiode array detector, a Rheodyne 7725i injection valve with a 20 μl loop, a G1332 degasser and Agilent HPLC workstation.

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

The dried roots of *Aucklandia lappa Decne* 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 extract

The dried roots of *Aucklandia lappa Decne* were pulverized to about 30-mesh with a disintegrator. One hundred grams of the powder was dunked into 500 ml of light petroleum at 20 °C for 12 h and then extracted by sonication for 30 min. The ultrasonic extraction procedure was then repeated twice again (400 ml each time). The extracts were combined together and evaporated under reduced pressure. 5.7 g of thick cream was obtained and stored in a refrigerator (4 °C) for subsequent HSCCC separation.

2.4. Selection of the two-phase solvent systems

The two-phase solvent systems were selected according to the partition coefficient (K) of the target components of the crude sample extracted from Muxiang. The K values were determined by HPLC analysis as following: suitable amount of crude extract was added to the mixture of equal volume of the upper phase and the lower phase of the two-phase solvent system. The solution was then mixed thoroughly. After the equilibration was established, the upper phase and the lower phase were analyzed by HPLC, respectively. The peak area of the upper phase was recorded as A_U and that of the lower phase was recorded as A_L. The K value was calculated according to the following equation: $K = A_U/A_L$.

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

In the present study, light petroleum–methanol–water (5:6.5:3.5, v/v/v) was used as the two-phase solvent system for HSCCC separation. It was prepared by adding the solvents to a separation funnel according to the volume ratios

and thoroughly equilibrated by shaking repeatedly. Then, 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 110 mg of the crude extract in 5 ml of the upper phase of the two-phase solvent system.

2.6. HSCCC separation procedure

HSCCC separation was performed as follows: the upper phase (stationary phase) and the lower phase (mobile phase) of light petroleum–methanol–water (5:6.5:3.5, v/v/v) were pumped into the separation column simultaneously using ÄKTA prime system, according to the volume ratio of 50:50. After the column was totally filled with the two phases, the HSCCC apparatus was run at the revolution speed of 900 rmp. At the same time, the lower phase was pumped into the column at a flow rate of 2.0 ml min^{-1}. After hydrodynamic equilibrium was reached (about half an hour later), the sample solution (110 mg of crude sample dissolved in 5 ml of the upper phase) was injected into the separation column through the injection valve. The separation temperature was controlled at 25 °C all through the experiment. The effluent from the tail end of the separation column was continuously monitored at 254 nm. The data were collected immediately after the sample injection. Each fraction was collected manually according to the obtained chromatogram and then evaporated under reduced pressure. The residuals were dissolved in methanol for subsequent purity analysis by HPLC.

2.7. HPLC analysis and identification of HSCCC peak fractions

The HPLC analysis was performed with a YWG C_{18} column (200 mm × 4.6 mm I.D., 10 μm) at room temperature. The mobile phase was methanol–water (70:30, v/v). The effluent was monitored at 254 nm and the flow rate was kept at 1.5 ml min^{-1} constantly.

The structure 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

Selection of two-phase solvent system and purity analysis of HSCCC peak fractions were performed by HPLC. So in the first place, a good HPLC method should be developed. Acetonitrile–water and methanol–water with different volume ratios were used as the mobile phase to analyze crude extract from Muxiang by HPLC. When methanol–water (70:30, v/v) was used as the mobile phase of HPLC and the flow rate was 1.5 ml min^{-1}, two major peaks can be obtained, and each peak got baseline separation. The crude sample and peak fractions separated by HSCCC were analyzed by HPLC under the optimum conditions. The chromatograms are shown in Fig. 2.

3.2. Optimization of HSCCC conditions

The selection of the two-phase solvent system is the most important step in performing HSCCC method. In the present study, different solvent systems such as ethyl acetate–methanol–water, light petroleum–ethyl acetate–methanol–water and light petroleum–methanol–water were used as the two-phase solvent system to optimize the HSCCC separation condition. The partition coefficient (K) of costunolide and dehydrocostuslactone in these solvent systems were determined by HPLC as described in Section 2.4 and the results are shown in Table 1. It can be seen that when ethyl acetate–methanol–water systems were used as the two-phase solvent system, the target compounds mainly partitioned in the organic phase. When light petroleum–ethyl acetate–methanol–water systems were used as the two-phase solvent system, the target compounds could not be well separated and the purity of the compounds was not satisfactory. So ethyl acetate–methanol–water and light petroleum–ethyl acetate–methanol–water were unsuitable for HSCCC separation of the compounds present in Muxiang. Another kind of two-phase solvent system tested in present study was light petroleum–methanol–water. Suitable K values could be obtained with it. So light petroleum–methanol–water systems were tested for HSCCC separation. When light petroleum–methanol–water (5:6:4, v/v/v) was used for HSCCC separation, it required a long time to elute the target compounds. When light petroleum–methanol–water (5:7:3, v/v/v) was used, costunolide and dehydrocostuslactone could not get baseline separation. When light petroleum–methanol–water (5:6.5:3.5, v/v/v) was used, costunolide and dehydrocostuslactone were well separated and the separation time was also acceptable. So light petroleum–methanol–water (5:6.5:3.5, v/v/v) was used as the two-phase solvent in present study.

The influence of revolution speed, flow rate of the mobile phase, and temperature on HSCCC peak resolution were also investigated. When the flow rate was 2.0 ml min^{-1}, revolution speed was 900 rpm, and separation temperature was 25 °C, the retention percentage of the stationary phase was 50%, good separation results could be obtained. The crude samples from Muxiang were separated and purified under the optimum HSCCC conditions. The HSCCC chromatogram is shown in Fig. 3. 35.7 mg of costunolide (peak I, collected during 135–165 min) and 43.6 mg of dehydrocostuslactone (peak II, collected during 210–253 min) could be obtained from 110 mg of crude sample by one-step HSCCC separation. The purity of costunolide and dehydrocostuslactone was 100% and 99.6%, respectively, as determined by HPLC. The

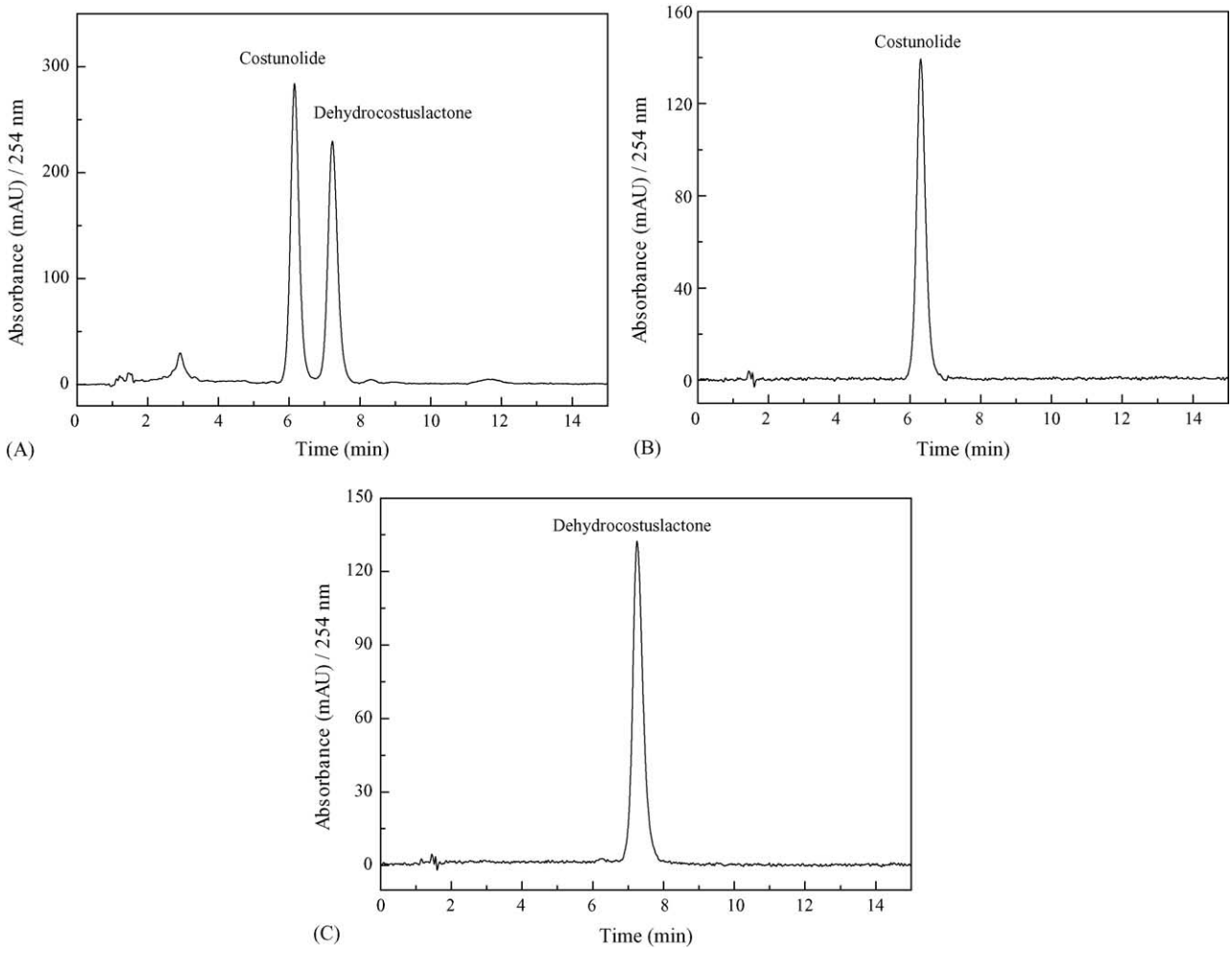

Fig. 2. HPLC chromatograms of crude extract from Muxiang and HSCCC peak fractions. Conditions: column, reversed phase YWG C_{18} column (200 mm × 4.6 mm I.D., 10 μm); mobile phase, methanol–water (70:30, v/v); flow rate, 1.5 ml min^{-1}; detection wavelength, 254 nm. (A) Crude extract from Muxiang; (B) HSCCC peak fraction I in Fig. 3; (C) HSCCC peak fraction II in Fig. 3.

chromatograms of HPLC of these compounds are shown in Fig. 2.

3.3. Identification of HSCCC peak fractions

Identification of each HSCCC fraction was carried out according to its ^{1}H NMR and ^{13}C NMR data.

Peak I. ^{1}H NMR (400 MHz, C^2HCl_3), δ (ppm): 6.27 (1H, d, J = 3.6 Hz, H-13a), 5.53 (1H, d, J = 3.6 Hz, H-13b), 4.85 (1H, dd, J = 10.5, 4.2 Hz, H-1), 4.74 (1H, d, J = 9.6 Hz, H-5), 4.58 (1H, t, J = 9.3 Hz, H-6), 1.70 (3H, s, H-15), 1.42 (3H, s, H-14). ^{13}C NMR (400 MHz, C^2HCl_3), δ (ppm): 170.51 (C-12), 141.53 (C-11), 139.99 (C-4), 136.97 (C-10), 127.18 (C-5), 127.00 (C-1), 119.73 (C-13), 81.89 (C-6), 50.34 (C-

Table 1
The K values of costunolide and dehydrocostuslactone in some two-phase solvent systems

Solvent systems	K (costunolide)	K (dehydrocostuslactone)
Ethyl acetate–methanol–water (5:0:5, v/v/v)	218	275
Ethyl acetate–methanol–water (5:1:5, v/v/v)	115	131
Ethyl acetate–methanol–water (5:2:5, v/v/v)	71	81
Ethyl acetate–methanol–water (5:3:5, v/v/v)	13	15
Light petroleum–ethyl acetate–methanol–water (5:5:5:5, v/v/v/v)	7.65	10.42
Light petroleum–ethyl acetate–methanol–water (5:5:6:4, v/v/v/v)	2.63	3.25
Light petroleum–ethyl acetate–methanol–water (5:5:7:3, v/v/v/v)	1.52	1.97
Light petroleum–methanol–water (5:5:5, v/v/v)	5.24	9.92
Light petroleum–methanol–water (5:6:4, v/v/v)	2.34	4.26
Light petroleum–methanol–water (5:6.5:3.5, v/v/v)	1.21	2.23
Light petroleum–methanol–water (5:7:3, v/v/v)	0.98	1.26

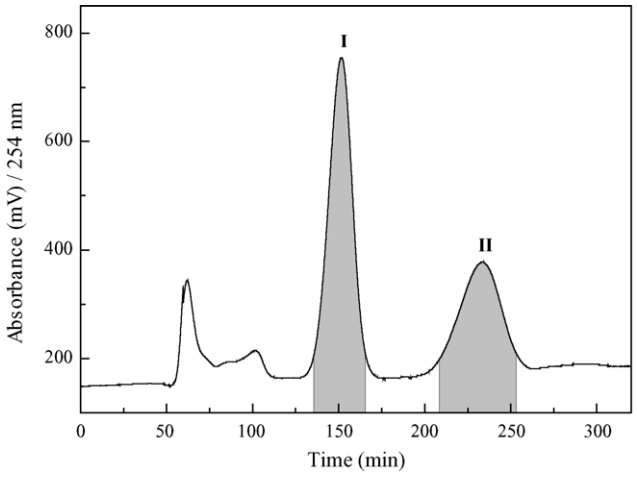

Fig. 3. HSCCC chromatogram of crude extract from Muxiang. Two-phase solvent system, light petroleum–methanol–water (5:6.5:3.5, v/v/v); mobile phase, the lower phase; flow rate, 2.0 ml min^{-1}; revolution speed, 900 rpm; detection wavelength, 254 nm; separation temperature, 25 °C; sample size, 110 mg of crude sample dissolved in 5.0 ml of the upper phase; retention percentage of the stationary phase, 50%. (I) costunolide (collected during 135–165 min); (II) dehydrocostuslactone (collected during 210–253 min).

7), 40.93 (C-3), 39.41 (C-9), 27.97 (C-2), 26.17 (C-8), 17.35 (C-15), 16.11 (C-14). Comparing the data with the literature [7], peak I was identified as costunolide.

Peak II. ^{1}H NMR (400 MHz, C^2HCl_3), δ (ppm): 6.22 (1H, d, J = 3.2 Hz, H-13a), 5.50 (1H, d, J = 3.2 Hz, H-13b), 5.27 (1H, brs, H-15a), 5.07 (1H, brs, H-15b), 4.90 (1H, brs, H-14a), 4.82 (1H, brs, H-14b), 3.97 (1H, t, J = 9.2 Hz, H-5). ^{13}C NMR (400 MHz, C^2HCl_3), δ (ppm): 170.24 (C-12), 151.22 (C-11), 149.17 (C-3), 139.66 (C-9), 120.18 (C-13), 112.55 (C-15), 109.53 (C-14), 85.22 (C-5), 51.95 (C-4), 47.52 (C-6), 45.04 (C-10), 36.24 (C-2), 32.54 (C-8), 30.88 (C-7), 30.24 (C-1). Comparing the data with the literature [7], peak II was identified as dehydrocostuslactone.

4. Conclusion

HSCCC was successfully used for the separation and purification of two main bioactive sesquiterpene lactones (costunolide and dehydrocostuslactone) from the roots of *Aucklandia lappa Decne*, with light petroleum–methanol–water (5:6.5:3.5, v/v/v) as the two-phase solvent system. 35.7 mg of costunolide and 43.6 mg of dehydrocostuslactone with the purity of 100% and 99.6%, respectively, were obtained from 110 mg of crude in one-step separation.

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